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Plasticity of hippocampal synaptic transmission and intrinsic excitability in a mouse model of Alzheimer's disease

Nan Jiang

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THÈSE PRÉSENTÉE

POUR OBTENIR LE GRADE DE

DOCTEUR DE L'UNIVERSITÉ DE BORDEAUX

Mention : Sciences de la Vie et de la Santé

Option : Neurosciences

Par **Nan JIANG**

Sous la direction de : Thierry AMEEDÉ

**Plasticity of hippocampal synaptic transmission and
intrinsic excitability in a mouse model
of Alzheimer's disease**

Soutenue publiquement le 17 septembre 2019

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Abstract

Alzheimer's disease (AD) is a neurodegenerative disease that is linked in its early stage to synaptic dysfunction and loss of synapses. Numerous clinical data obtained from patients but also experimental data obtained on mouse models of AD show that there is a sexual dimorphism evidenced by a higher amyloid plaque deposition and an early onset of memory disorders in female mice compared to male mice.

In this work, we investigated the molecular and cellular alterations of AD as well as the associated cognitive deficits in female APP/PS1 mice, a double transgenic murine model of AD. In parallel we studied the alterations of hippocampal synaptic transmission and plasticity in the *stratum moleculare*, a layer in the vicinity of the *dentate gyrus* (DG) which specifically displayed a high density of amyloid plaques. We showed the presence of numerous amyloid plaques in the DG in a larger amount in 6 month old females compared to age-matched males as well as a strong activation of astrocyte and microglia glial cells. These molecular and cellular alterations are accompanied by hippocampo-dependent memory deficits (contextual fear conditioning and novel object place recognition task) from the age of 4 months in females whereas males have no deficit until the age of 12 months. We then studied the electrical properties of DG neurons, the transmission and the plasticity of the perforant pathway - DG neurons (PP-DG synapse) in 6 month old females mouse by comparing the two genotypes APP/PS1 vs wild type (WT).

In both genotypes, DG neurons displayed two distinct populations in terms of input resistance and action potentials (APs) discharge pattern. In contrast, the resting membrane potential, the input resistance, the activation threshold and the amplitude of APs were not modified in APP/PS1 vs WT. The frequency of discharge of APs was increased in APP/PS1 without shift of E-S curve which relates EPSP-slopes to the associated AP firing probability.

Basal transmission at the PP-DG synapse was altered in the APP/PS1 mouse vs WT without alterations in the AMPA/NMDA ratio or the AMPA rectification index. The frequency of the NMDA miniature currents was increased in APP/PS1 DG neurons vs WT which suggests an increased number of silent synapses expressing NMDA receptors but devoid of AMPA receptors. The long term potentiation (LTP) of population spike amplitude was decreased by approximately 50% in APP/PS1 mice. The decrease in LTP observed in APP/PS1 was partly related to alterations in the intrinsic properties of DG neurons as

evidenced by LTP-induced shifts of E-S curves, which reflects an increased excitability for APP/PS1 mice.

In conclusion our results show a prominent sexual dimorphism with much earlier amyloid plaque deposition, neuroinflammatory glial activation in female *vs* male APP/PS1. In parallel, significant deficits in hippocampal-dependent memory are observed as well as alterations of synaptic transmission and plasticity at the PP-DG synapse, a key synapse of the integration of mnemonic informations originated from the entorhinal cortex

Key words: Alzheimer's disease, A β plaques, Lateral perforant path, PGE₂, Population spikes, Action potential, mEPSCs

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ABBREVIATIONS

AD: Alzheimer's disease

MCI: Mild cognitive impairment

A β : β -amyloid

SALAs: Selective A β ₄₂ - lowering agents

APP: Amyloid precursor protein

CNS: Central nervous system

AICD: APP intracellular domain

NFTs: Neurofibrillary tangles

BBB: Blood brain barrier

3APS: 3-Amino-1-propanesulfonic acid

MT: Microtubule

ROS: reactive oxygen species

NMDARs: N-Methyl-D-Aspartate receptors

NSAIDs: Non-steroidal anti-inflammatory drugs

PPAR γ : Peroxisome proliferator-activated receptor γ

APO ϵ 4: Apolipoprotein ϵ 4

AChEIs: Acetylcholinesterase inhibitors

GABA-A: Gamma-Aminobutyric acid-A

5-HT: 5-hydroxytryptamine

PDE4: The phosphodiesterase 4

PKA: Protein kinase A

CREB: cAMP response element binding

TNF: Tumor necrosis factor

LTP: Long term potentiation

GFAP: Glial fibrillary acidic protein

mPGES : Microsomal prostaglandin-E2 synthase
PGG₂: Prostaglandin G₂
PGH₂: Prostaglandin H₂
PGE₂: Prostaglandin E₂
PGF_{2a}: Prostaglandin F_{2a}
AC: Adenylyl cyclase
GPCRs: G protein-coupled E-prostanoid receptors
TNF- α : Tumor necrosis factor alpha
IL-1 β : Interleukin-1 β
IL-6 : Interleukin 6
COX: Cyclooxygenase
RMP: Resting membrane potential
R_{in}: Membrane resistance
AP: Action potential
CaN: Calcineurin
VDCC: Voltage dependent calcium channels
RyR: Ryanodine receptor
CICR: Ca²⁺ induced Ca²⁺ release
PLC: Phospholipase C
IP3: Inositol triphosphate
STIM2: Stromal interaction molecule 2
TRPC: Transient receptor potential cation
LTD: Long term depression
AMPARs: α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors
PS1: Presenilin-1
STP: Short-term plasticity
PTP: Post tetanic potentiation
PPR: Paired pulse ratio
PPF: Paired pulse facilitation

PPD: Paired pulse depression
PS: Population spike
EPSP: Excitatory postsynaptic potential
fEPSP: field EPSP
mEPSCs: miniature excitatory postsynaptic currents
EPSCs: Excitatory postsynaptic currents
mGluR: Metabotropic glutamate receptors
DG: Dentate gyrus
PP: Perforant pathway
EC: Entorhinal cortex
LPP: Lateral perforant pathway
MPP: Medial perforant pathway
L-AP4: L-2-amino-4-phosphonobutyric acid
D-APV: D- (-)-2-amino-5-phosphonovaleric acid
CPP: 3-[(+)-2-carboxypiperazin-4-yl]-propyl-1-phosphonic acid
Mf: Mossy fibres
CFC: Contextual fear conditioning
SC: Schaffer collaterals
FC: Frontal cortex
FV: Fiber volley
PCs: Pyramidal cells
GCs: Granule cells
TTX: Tetrodotoxin
PFA: Paraformaldehyde
ACSF: Artificial cerebro-spinal fluid
ISIs: Interspike intervals

I. Introduction

A. Alzheimer's disease

Alzheimer's disease (AD), which was first reported and described in 1906 by a clinical psychiatrist and neuroanatomist, Alois Alzheimer, is a progressive neurodegenerative disease that first display light cognitive problems such as forgetfulness. The age dependent prevalence of AD doubles every 5 years after aged 60 and increasing from a prevalence up to 40% of those aged 85 years and older (Strauss *et al.*, 1999). The disease is more frequent among women than men with a ratio of 1.2 to 1.5 (Gao *et al.*, 1998). In 2018, about 50 million people worldwide are suffering of dementia and 60 to 70% are due to AD (World Alzheimer Report 2018). Each year there are roughly 7.7 million new reported dementia cases (WHO, 2016). The number of worldwide cases of dementia will likely rise up to 82 million by 2030 and could possibly reach 152 million by 2050 (World Alzheimer Report 2018). The total estimated worldwide cost of dementia in 2018 is US \$ 1 trillion and could rise up to US \$ 2 trillion by 2030 (World Alzheimer Report 2018). Of paramount importance is the understanding of the pathogenesis and the developing of early diagnosis and effective therapy. At present, there are no possible therapeutics available for the prevention or alleviation of the disease progression. There are continuous efforts set up by researchers worldwide to discover new therapies for AD.

a. Progression of AD

The progression rate of AD varies considerably. Usually, patients with Alzheimer's could live up to 4-8 years after diagnosis, but some of them can live as long as 20 years, depending on multiple factors. The progression of the disease depends in part on age at diagnosis but also on the general health status of the person. The early stage of AD generally lasts from 2-8 years and goes through mild cognitive impairment stage (MCI) (Petersen *et al.*, 2009) before evolving to dementia (Figure 1).

A large percentage of people diagnosed with MCI have AD as an underlying cause for their cognitive impairment, although other disorders also can cause MCI. The moderate stage lasts 2-4 years and is characterized by a more serious memory deficit including long term memory and the loss of the ability to operate typical activities of daily life (for example bathing, dressing and grooming). In the late stage of AD, individuals lose the ability to respond advisably and are unable to talk with others. They will also be unable to control

actions like sitting, standing and walking. In advanced disease, patients often become mute, aggressive, anxious (David *et al.*, 2012). To study the disease, we dissertate here intricate cellular alterations during the disease developing, and how initial responses finally turn into detrimental, leading to an irreversible dysfunction of the brain.

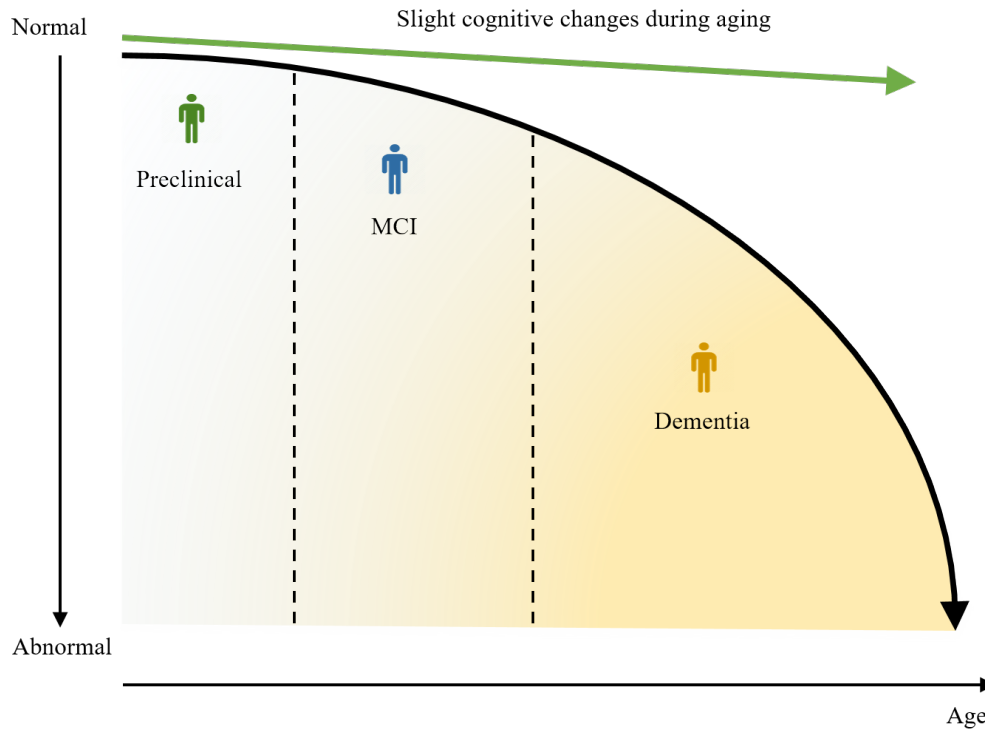


Figure 1. The progression of AD

The figure represents a sum of the progression of AD signs from normal cognitive to MCI and dementia.

b. Pathologic features of AD

The crucial neuropathological features of AD were described by Alois Alzheimer in 1906 (Alzheimer *et al.*, 1906). Memory deficits are the first clinical symptoms of AD (Jacobs *et al.*, 1995) and have been studied using different techniques of neuroscience: at the macro level, there is a considerable loss of brain tissue. At the micro level, pathological hallmarks of AD include amyloid β accumulation, tau phosphorylation, and synaptic neuronal loss.

1. Amyloid Hypothesis: APP Proteolytic Process

The finding of aggregates of β -amyloid ($A\beta$) in the extracellular space of the brain in post-mortem pieces led to the formulation of the ‘Amyloid Hypothesis’ (Hardy *et al.*, 1992). The hypothesis put forward was that the formation of $A\beta$ plaques is the preliminary event in AD, eventually leading to cell death and dementia.

However, there are evidences that are difficult to reconcile with this hypothesis. The primary component of plaques is the $A\beta$ peptide, a 36– to 43–amino acid peptide derived from amyloid precursor protein (APP), which is cleaved by β secretase and γ secretase (Hamley *et al.*, 2012) (Figure 2). $A\beta$ peptides can be present in aggregated insoluble forms including fibrils as well as soluble oligomers (Kayed *et al.*, 2003; Koffie *et al.*, 2009). When $A\beta$ is in soluble oligomer, it is generally considered as the most toxic form.

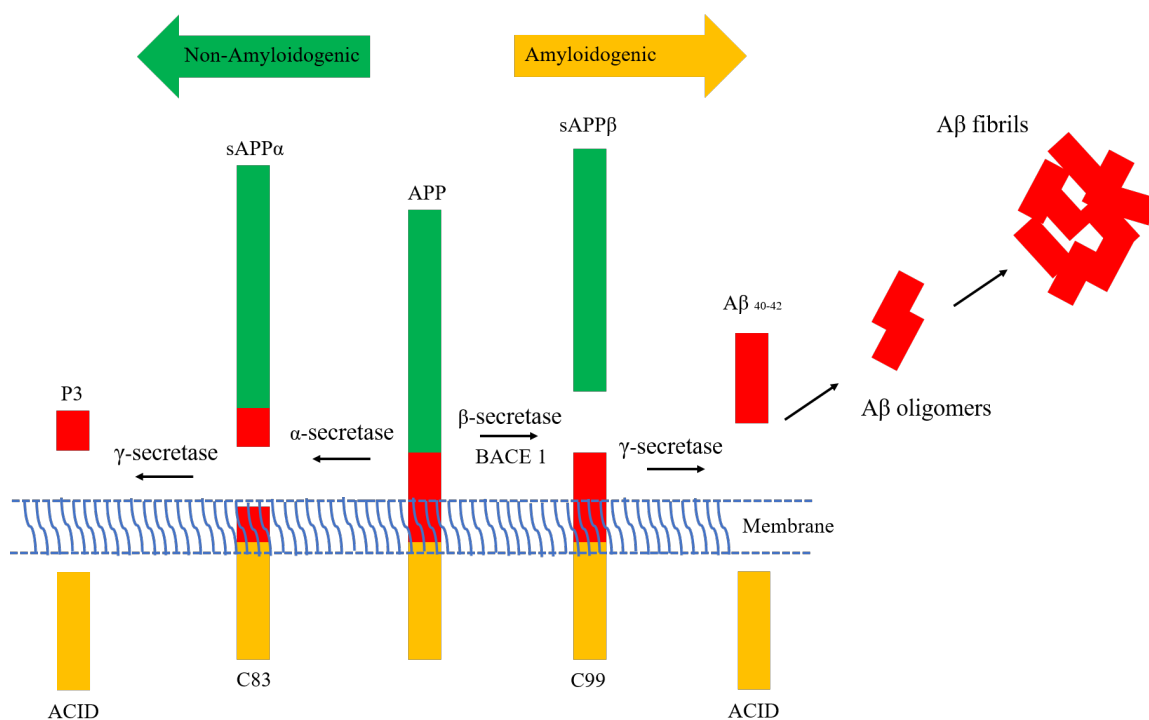


Figure 2. $A\beta$ fragments derived from APP processing

APP processes two distinct pathways: amyloidogenic and non-amyloidogenic pathway. Serial actions of APP by α -secretase and γ -secretase produces sAPP α , C83, APP intracellular domain (AICD) and P3. There is no $A\beta$ formed through this pathway. Serial actions of APP by β -secretase (BACE1) and γ -secretase produces sAPP β , C99, AICD and $A\beta$ ₄₀₋₄₂ (Thathiah and De Strooper, 2011).

When A β is aggregated in the extracellular space to form A β plaques, these plaques are surrounded by abnormal neuronal processes known as degenerating fibers or (Masliah *et al.*, 1993) but also by activated glial cells driving an inflammatory response (Meda *et al.*, 1995; Giulian *et al.*, 1998). It is highly likely that this inflammatory response contributes to neuronal damage, although there are numerous evidences that glial cells are playing a protective role (McGeer *et al.*, 1995; Lucin *et al.*, 2009).

2. Hyperphosphorylation of intracellular Tau protein

Tau is a 50- to 75-kDa protein displaying six different splice forms (Lace *et al.*, 2007). It was first characterized as a microtubule-associated protein in the brain, not only synthesized and produced in neuron cell bodies but also expressed by glial cells.

Under physiological conditions the function of tau is to bind to tubulin to stabilize microtubules (Figure 3). It is proposed that neurofibrillary pathology could contribute to neuronal dysfunction and strongly correlates with the clinical progression of AD (Ballatore *et al.*, 2007; Noble *et al.*, 2013). In AD, tau protein is hyperphosphorylated, leading to its dissociation from microtubules and has a trend to self-aggregate, forming neurofibrillary tangles (NFTs) in cell bodies (Grundke *et al.*, 1986; Goedert *et al.*, 1988; Wischik *et al.*, 1988; Wischik *et al.*, 1988) which finally lead to neurodegeneration (Avila *et al.*, 2004) (Figure 3). The abnormal phosphorylation also leads to the formation of both soluble and insoluble tau filaments (Braak *et al.*, 1994).

It is widely acknowledged that neuroinflammation plays a pivotal role in AD pathology where both A β plaques and NFTs co-exist with microglia and astrocytes (Heneka *et al.*, 2010; Serrano *et al.*, 2011). Finally a more general picture has emerged proposing that AD is the combination of A β and hyperphosphorylation of tau (Coello *et al.*, 2013; Schnabel *et al.*, 2013).

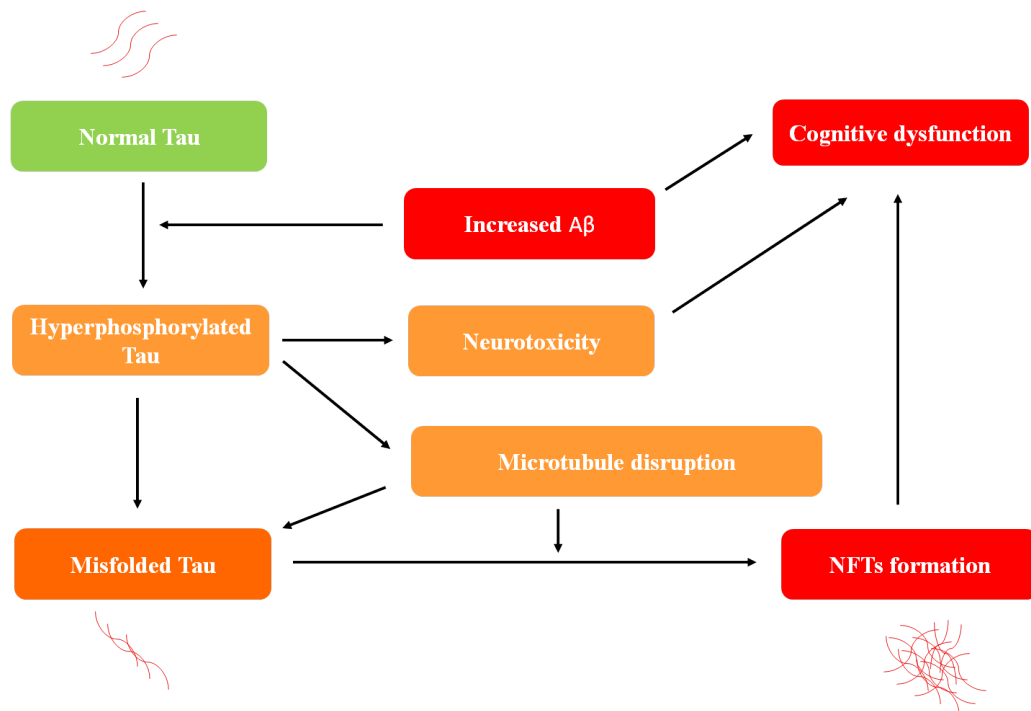


Figure 3. NFTs accumulation in AD

Mechanism of NFTs formation resulting in cognitive dysfunction by hyperphosphorylated Tau.

3. Loss of synapses and selective neuronal cell death

It is widely known that the neurons density is reduced in AD. The loss of neurons is detected by common microscopic study (Terry *et al.*, 1981). As AD is developing, cognitive dysfunction becomes worse together with synapse loss and neuronal cell death. Although it is generally acknowledged that neuronal death happens during neurodegenerative diseases, the mechanism of neuronal death is often unclear. There are different mechanisms which contribute to neuronal death, such as necrosis, apoptosis, autophagic cell death and excitotoxicity (Gorman *et al.*, 2008).

Within the neuritic plaques, synapse loss observed in AD may occurs in still living neurons and leads to the neuronal death (Terry *et al.*, 1991), which might be due to toxic roles of the aggregated amyloid or the soluble A β peptide (Podlisny *et al.*, 1995; Selkoe *et al.*, 2000) (Figure 4). Moreover, there is a regionally specific loss of neurons in AD and this specific neuronal loss in AD differs from what is found in normal aging (West *et al.*, 1994).

These results suggest that AD cannot be considered as a further step in the continuum of normal aging though age is certainly one of risk factors for AD.

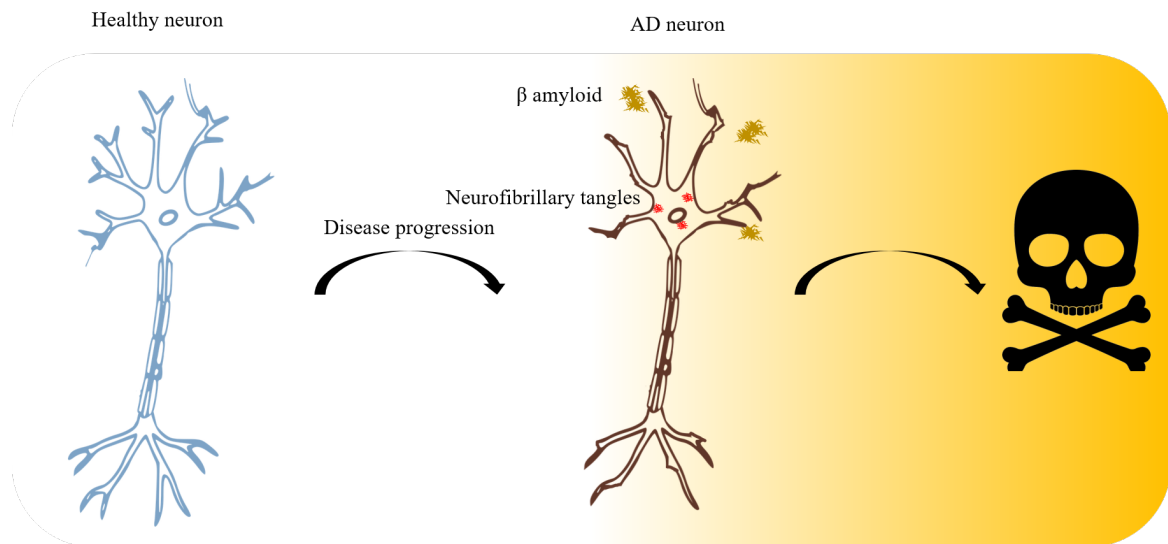


Figure 4. A β and NFTs in synapses loss and neuronal death

The appearance of AD is highlighted by the A β formation in the brain. Along the progression of the disease, tau becomes hyperphosphorylated, a procedure thought to be determined by A β and increasing dendritic tau concentrations. In AD, high concentrations of tau in the dendritic compartment are involved in enhanced toxic actions of A β at postsynaptic terminals and therefore could be part of a toxic pathway for synapses loss and neuronal death.

4. Activated glial cells

Activated glial cell is a prominent feature of AD neuropathological features, both with extracellular fibrillar A β and intracellular neurofibrillary tangles (Selkoe *et al.*, 1996), which are closely associated with reactive microglia and astrocytes (Figure 5) (Sheng *et al.*, 1995; Pozo *et al.*, 2011). The microglia are usually inside and close to the central core of A β plaques, while the astrocytes generally wall-off the plaques with some of their processes in the vicinity of the amyloid central core (Bamberger *et al.*, 2001). The kinetics to develop A β plaques are not known for sure, but it is plausible that such alterations develop over several years. The dialogue between microglia and astrocytes play a key role in the immune response surrounding the A β plaques. As a consequence, A β and activated glial cells could set up a harmful circle, fostering neuroinflammation and the pathology.

Hence, chronically activated microglia and astrocytes would become neurotoxic by releasing inflammatory cytokines, proteolytic enzymes, complement factors and reactive intermediaries. Regulation of glial activation can be impaired under sustained pro-inflammatory conditions as found in the aged brain (Bernhardi *et al.*, 2007).

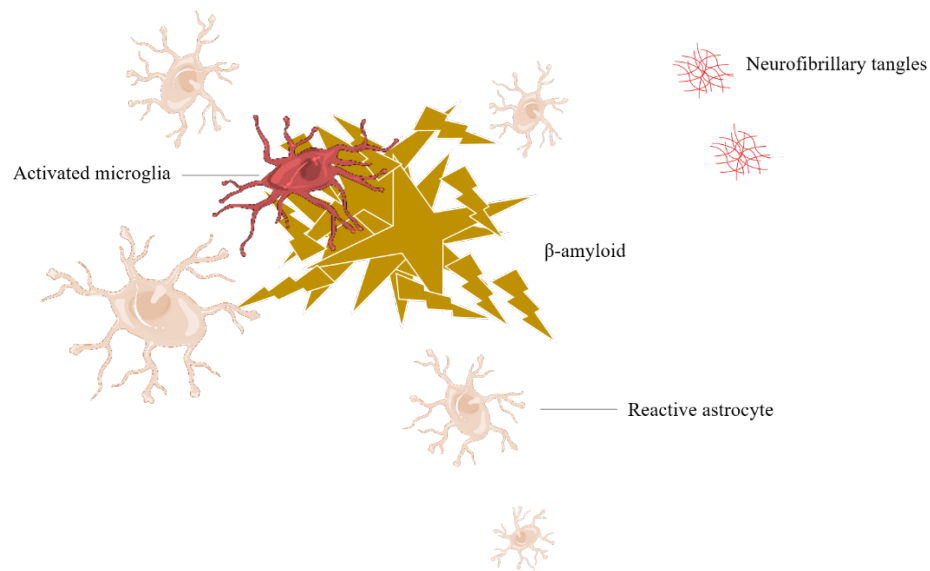


Figure 5. Activated glia cells with A β and NFTs

AD are characterized by the accumulation of A β and tau proteins which are closely associated with microglial and astrocytic activation. The activation of microglia and astrocytes promotes a neuroinflammatory environment which likely fosters the development of AD.

c. Clinical Strategies

Clinical strategies have been focussed on four aspects of the pathology: A β plaques, tau tangles, neurodegeneration, and dementia. In spite of a considerable knowledge of genetics, epidemiology, neuropathological and molecular mechanisms involved in the pathology, there is still no effective cure for AD (Table 1).

1. β -Amyloid-Based Therapeutics

The existing drug development treatments specifically designed multiple A β based therapeutics. With a strong evidence supporting the amyloid hypothesis, the most likely targets would be the successful alteration of A β processing through β and γ -secretase. BACE1

has appeared as a likely target for the treatment of AD since it is required for the formation of A β . Few BACE1 inhibitors (Lanabecestat, LY-2811376 and Verubecestat) (Stamford *et al.*, 2013; Oehlrich *et al.*, 2014; Yan *et al.*, 2016) were tested in clinical trials, but failed to demonstrate positive effects.

Other studies have shown that non-steroidal anti-inflammatory drugs (NSAIDs) lowering A β_{42} without obvious totally inhibition of γ -secretase activity, and it will be important to explain the potential molecular mechanism of this action to increase the relative proportion of shorter A β species in vitro, such as A β_{37} and A β_{38} , reduced prevalence of AD (Weggen *et al.*, 2001).

Removal of A β have been proposed and may provide an alternative method to decrease A β oligomers for the treatment of AD (Tanzi *et al.*, 2004; Pangalos *et al.*, 2005). A β aggregation inhibitors also have been tested with the aim to reduce A β load. For example, 3-Amino-1-propanesulfonic acid (3APS) which interacts with A β monomers reduces A β deposition both in transgenic mice and in humans (Aisen *et al.*, 2006; Gervais *et al.*, 2007).

Antibodies raised against A β (A β -antibodies) have also been tested and some studies have shown that they were alleviating plaque burden and cognitive deficits (Bard *et al.*, 2000; Dodart *et al.*, 2002; Lannfelt *et al.*, 2014). Different mechanisms could contribute to these results, such as the binding of A β -antibodies to A β forms (monomeric, oligomeric, and fibrillar) which prevent oligomerization, and increased clearance of fibrillar A β (Pangalos *et al.*, 2005). However, clinical trials using different anti-A β strategies that affect the formation, aggregation and clearance of A β have all failed and be stopped in phase II of phase III. Therefore, substitute hypotheses are urgently needed.

2. Tau-Based Therapeutics

On the contrary to strategies focused on β -amyloid, tau-related strategies have received few attentions until recently, despite the fact that a widespread tau pathology is crucial to the disease. Hence different strategies targeting the tau-dependent pathogenesis are actively studied carried, some of them being under clinical trials (Medina *et al.*, 2011, 2014; Li *et al.*, 2017).

Attenuating hyperphosphorylation of tau through the inhibition of kinases may seem a tempting strategy (Churcher *et al.*, 2006; Mazanetz *et al.*, 2007) but the task is complicated by the existence of numerous tau phosphorylation sites, and the ability of multiple kinases to phosphorylate individual sites (Mi *et al.*, 2006). Taking into consideration that

hyperphosphorylation of tau may be achieved *in vivo* by multiple protein kinase activities (Wang *et al.*, 2007; Hanger *et al.*, 2009; Medina *et al.*, 2015) few protein kinases, including GSK-3 β , MARK and CDK5, have been proposed as potential therapeutic targets (Schneider *et al.*, 2008). However, the development of protein kinase inhibitors faced toxicity and specificity, which resulted in a "dead end" during preclinical studies.

The idea that tau dispersed from microtubules result in abnormal microtubule (MT)-stabilizing function has led to MT-stabilizing molecules as therapeutic target. In those MT-stabilizing molecules, some anti-mitotic drugs such as paclitaxel or epothilone D have been tested in tau-overexpressing mouse models (Brunden *et al.*, 2010).

Tau aggregation and the various tau species formed (monomers, oligomers, fibrils) along the aggregation process have also been a great of interest for potential treatment. As such, various anti-tau immunotherapy strategies to clean pathological proteins have also been tested in animal models suggesting that cleaning toxic protein species could be useful to treat tauopathies in AD (Pedersen *et al.*, 2015).

3. Approaches to Inhibit Neurodegeneration

N-Methyl-D-Aspartate receptors (NMDARs) have always get the interest of researchers as potential drug targets (Kemp *et al.*, 2002; Olivares *et al.*, 2012; Zhang *et al.*, 2016). Accordingly, developed NMDA receptor antagonists must partially block the receptor and complete the normal functions to minimize the side effects.

The current therapy of AD includes two types: 1. the cholinesterase inhibitors (ChEIs), galantamine and rivastigmine (mild to moderate AD), donepezil (mild to severe AD); 2. non-competitive NMDA receptor antagonist memantine (moderate to severe AD). Both ChEIs and memantine could present beneficial effects on behavior and cognition (Patel *et al.*, 2011; Deardorff *et al.*, 2016). In the central nervous system (CNS) disease, although glutamate induced excitotoxicity has been thought to be affected by the NMDARs antagonist (Phencyclidine, ketamine, MK801 dizocilpine and memantine), due to the high affinity and long reside time of phencyclidine, ketamine, and MK801 on the receptor, their clinical trial have been stopped (Ellison, 1995). Besides, memantine shows a low affinity and is well accepted. Therefore, it is applied to the moderate to severe AD (Reisberg *et al.*, 2003; Pierson *et al.*, 2014; Song *et al.*, 2018). Furthermore, another glutamate release inhibitor (riluzole), is in phase II clinical trial for mild AD patients (Graham *et al.*, 2017).

Increasing evidences propose that neurotoxicity could be involved in the pathogenicity of AD (Parihar *et al.*, 2004). Neurotoxicity may be due to the chronic inflammatory response through the production of cytokines, reactive oxygen species (ROS), excitatory amino acids, proteases and nitric oxide. The inflammatory hypothesis has been highly reported as a key component of the pathogenesis of AD. Hence, NSAIDs has been tested in numerous clinical trials (Vlad *et al.*, 2008; Laino *et al.*, 2009).

The use of anti-oxidants for therapeutic as well as preventive treatments in AD has been questioned (Perkins *et al.*, 1999; Masaki *et al.*, 2000; Kim *et al.*, 2015). A study has proposed that anti-oxidant by scavenging free radicals lead to the suppression of hydrogen superoxide and thus suppressed the A β burden in AD (Lim *et al.*, 2001; Matsubara *et al.*, 2003).

Recently, experiments also verified the supposed role of peroxisome proliferator-activated receptor γ (PPAR γ) agonists to decrease the expression of Apolipoprotein $\epsilon 4$ (APO $\epsilon 4$) gene and showed neuroprotective effect during AD development in several mouse models (Bonet *et al.*, 2016; Moutinho *al.*, 2017; Koster *et al.*, 2017). Several PPAR γ agonists were tested for their role in AD, but owing to the large quantity health risks, including cardiovascular problems, weight gain, and fluid retention, the concept of neurodegeneration in AD attracts certain new therapeutic treatment for AD (Palop *et al.*, 2006).

4. Cognitive Enhancers

Since 1970s-1980s, it was found that acetylcholine (ACh) production, ACh levels, and its receptors were extremely decreased in AD brain (Kasa *et al.*, 1997). For many years and still today, there are no other clinical treatments available than acetylcholinesterase inhibitors (AChEIs) and memantine (non-competitive NMDA receptor antagonist). This drive other therapeutic strategies to be investigated such as the use of baicalein, a positive allosteric modulator of the GABA-A receptor, to improve cognitive dysfunctions (Zhang *et al.*, 2013).

In addition to AChEIs, several agonists and antagonists of the serotonergic, indirectly increase cholinergic neurotransmission are also being tested for AD. 5-hydroxytryptamine (5-HT) receptors (5-HT1A, 5-HT2A, 5-HT2C, 5-HT4, and 5HT-6) (Pauwels *et al.*, 2000) were found to be effective in animal models of AD (Butzlaff *et al.*, 2016). 5-HT1A partial agonist S15535 enhanced learning and memory in mouse models of AD (Millan *et al.*, 2004) and 5-HT2A receptor agonists improved cognitive dysfunctions (Zhang *et al.*, 2015). Last, a 5-HT6 receptor antagonist, SB271036, rescued memory impairment in a mouse model of AD by

inhibiting γ -secretase activity and reducing astrocyte and microglia activation (Yun *et al.*, 2015).

The application of pharmacological treatment discussed above have been demonstrated to present a direct effect on the progressive alteration of cognition (Neumann *et al.*, 1999; Birks *et al.*, 2006; Tampi *et al.*, 2007). Although these treatments display some beneficial aspects, numerous patients do not respond, and these compounds do not attenuate disease development (Giacobini *et al.*, 2000; Hansen *et al.*, 2007). In this case, establishment of other therapeutics for this complex disease will be a main aim.

Table 1 Different mechanism of therapeutic strategies in the treatment of Alzheimer’s disease

Hypothesis	Mechnism	Strategies
Aβ hypothesis	Inhibition of β -secretase	Acylguanides (Cole <i>et al.</i> , 2006) Aminobenzthiazines (May <i>et al.</i> , 2011) Aminoquinazolines (Madden <i>et al.</i> , 2010) OM99-2 (Ghosh <i>et al.</i> , 2000)
	Inhibition of γ -secretase	LY450139 (Henley <i>et al.</i> , 2009) DAPT (Morohashi <i>et al.</i> , 2006)
	Prevention of A β aggregation	Tramiprosate (Aisen <i>et al.</i> , 2007) Clioquinol (Crouch <i>et al.</i> , 2012) Colostrinin (Szaniszlo <i>et al.</i> , 2009)
	Increase A β clearance	Neprilysin (Shirovani <i>et al.</i> , 2001) IDE (Farris <i>et al.</i> , 2003) ACE (Hu <i>et al.</i> , 2001) Plasmin (Melchor <i>et al.</i> , 2003)
	Anti-A β immunotherapy	Gantenerumab (Roche) Solanezumab (Eli Lilly) Bapineuzumab (Pfizer) BAN2401(Logovinsky <i>et al.</i> , 2016; Piton <i>et al.</i> , 2018)
Tau hypothesis	Inhibition of tau phosphorylation	GSK-3 β (Gao <i>et al.</i> , 2012) Roscovitine (Zhang <i>et al.</i> , 2004)

	Anti-Tau immunotherapy	ACI-35 vaccine (Janssen Pharmaceuticals) AADvac1 vaccine, DC8E8 antibody (Axon Neurosciences) PHF1, MC1 antibodies (Eli Lilly) MAb86 (Roche) IPN001 and IPN002 antibodies (Bristol Meyers Squibb)
Neuroinflammation hypothesis	NSAIDs	CHF-5074 (Porrini <i>et al.</i> , 2015) Azeliragon (Burstein <i>et al.</i> , 2016) Oridonin (Wang <i>et al.</i> , 2014) Protocatechuic (Song <i>et al.</i> , 2014) Acetylpuerarin (Meng <i>et al.</i> , 2013) Indomethacin (Sung <i>et al.</i> , 2004) Mefenamic acid (Joo <i>et al.</i> , 2006) Ibuprofen (Dam <i>et al.</i> , 2010)
Oxidant hypothesis	Anti- Oxidant	Piracetam (Stockburger <i>et al.</i> , 2016) Gingko Biloba (Liu <i>et al.</i> , 2015) Blueberries (Tan <i>et al.</i> , 2017) Flavonoids (Ruan <i>et al.</i> , 2018) Polyphenols (Thenmozhi <i>et al.</i> , 2016)
AChE/BChE antagonism	Inhibition of AChE/BChE	Metrifonate (Mehta <i>et al.</i> , 2012). γ -carboline-phenothiazine (Makhaeva <i>et al.</i> , 2015) Phenylbenzofuran analogues (Kumar <i>et al.</i> , 2018)
NMDA Rs antagonism	Inhibition of NMDARs	Memantine (Atri <i>et al.</i> , 2008) Neramexane (Rammes, 2009) Ifenprodil (Costa <i>et al.</i> , 2012)
GABA-A Rs antagonism	Modulation of GABAARs	SGS742 (Bollock <i>et al.</i> , 2005) Baicalein (Zhang <i>et al.</i> , 2013)
PPAR-γ agonism	activation of PPAR- γ	Pioglitazone (Sato <i>et al.</i> , 2011) Peroxisome (Combs <i>et al.</i> , 2000)
Serotonergic hypothesis	Modulation of Serotonergic	SC-53116, ML-10302, Donecopride, RS-67333, Prucalopride (Geldenhuys <i>et al.</i> , 2011; Rochais <i>et al.</i> , 2015) MS-245, SB-271046 (Geldenhuys <i>et al.</i> , 2011)
PDE inhibition	Inhibition of Phosphodiesterase	BAY60-7550 (Zhu <i>et al.</i> , 2013) Rolipram, ibudilast (Mizuno <i>et al.</i> , 2004; Blokland <i>et al.</i> , 2006) BAY 73-6691, PF-04447943 (Prickaerts <i>et al.</i> , 2017).

B. Neuroinflammation

Over the last two decades, an increasing range of evidence has demonstrated that neuroinflammation involving the activation of glial cells played a pivotal role in the disease progression and pathology (Akiyama *et al.*, 2000; Heneka and O'Banion, 2007; Heneka *et al.*, 2015, 2018). There are considerable evidence that cytokines generated by activated glial cells during neuroinflammation, *i.e.*, tumor necrosis factor alpha (TNF- α), interleukin-1 β (IL-1 β), and interleukin 6 (IL-6) (Quan *et al.*, 1998; D'Mello *et al.*, 2013; Thomson *et al.*, 2014) have profound effects on synaptic transmission and plasticity (Pickering *et al.*, 2007; Nistico *et al.*, 2013) and may underlie cognitive and behavioral alterations (Raison *et al.*, 2006; Galic *et al.*, 2012; Santello and Volterra, 2012; Camara *et al.*, 2015).

a. Neuroinflammation in AD

Despite the considerable corpus of data pointing out the crucial role of neuroinflammation in AD, it is still unclear if neuroinflammation is causative or correlative of the pathology (Heneka *et al.*, 2015; Kinney *et al.*, 2018; Chaney *et al.*, 2018; Lin, 2018; Cao *et al.*, 2018; Kumar *et al.*, 2018). It has been suggested that A β deposition and NFTs activate microglia and increases level of pro-inflammatory cytokines (IL-1 β , IL-6, TNF- α ...) (Figure 6). These cytokines enhance APP processing via β -secretase and therefore promote A β production (Blasko *et al.*, 2000; Tuppo *et al.*, 2005). Rodent models overexpressing mutated hAPP protein have highlighted a direct connection between the level of A β and increased levels of TNF- α , IL-6, IL-12, IL-1 β , and IL-1 α (Patel *et al.*, 2005).

The inflammatory procedure in AD is promoted by the activation of microglia and astrocytes via pro-inflammatory molecules and associated signaling pathways, therefore resulting in synaptic impairment, neuronal loss, as well as the other inflammatory molecules produced (Perlmutter *et al.*, 1990; McGeer *et al.*, 1993; Aisen *et al.*, 1997). In spite of the fact that, the role of A β as a potential molecule to induce the inflammation is not clear, the aggregation applies an effect through activation of caspases and transcription factors including NF- κ B and AP-1, which prone to produce many inflammation mediators (IL-1 β , TNF- α , and IL-6) and further β amyloid (Ho *et al.*, 2005). Pro-inflammatory cytokines (TNF- α and IL-1 β and IL-6) act directly on the neurons and cause apoptosis.

Pro-inflammatory cytokines might be injurious to synapses by themselves and thus impair neuronal function such as long term potentiation (LTP) of synaptic transmission (Curran *et al.*, 2001; Griffin *et al.*, 2006; Hickman *et al.*, 2008; Tachida *et al.*, 2008; Ojala *et al.*, 2008; Dugan *et al.*, 2009; Park *et al.*, 2010; Chakrabarty *et al.*, 2010; Zhao *et al.*, 2011; Kitazawa *et al.*, 2011; Spooren *et al.*, 2011; Sutinen *et al.*, 2012;). In animals, *in vivo* (Murray *et al.*, 1998; Vereker *et al.*, 2000) and *in vitro* (Bellinger *et al.*, 1993; Tong *et al.*, 2012) electrophysiological studies have demonstrated that IL-1 β decreases LTP in the hippocampus. While IL-18 also suppresses LTP in the hippocampus, the IL-1 receptor antagonist (IL1RA) reverse the impairment of LTP by IL-18 (Curran *et al.*, 2001), thus illustrating that IL-18 affects LTP through an IL-1 dependent pathway. Cytokines can locally affect synaptic transmission between glia and neuron signaling. For example, a recent study has shown that TNF α enhances presynaptic activity via glutamate/NMDAR by measuring the frequency of miniature excitatory postsynaptic currents (mEPSCs) in mouse hippocampal slices (Habbas *et al.*, 2015).

Neurons also seem to lead to the accumulation of neuroinflammatory products in AD, although neurons were normally thought to be passive onlookers during neuroinflammation. Neurons generate several molecules such as IL-1, IL-6 and TNF- α (Tchelingerian *et al.*, 1996; Friedman *et al.*, 2001). It has been demonstrated that neurons are available to work as the origin of complement proteins (Shen *et al.*, 1997; Strohmeyer *et al.*, 2000), (pentraxins, C-reactive protein, amyloid P, prostanoids, NOS) (Yasojima *et al.*, 2000; Heneka *et al.*, 2001; Pavlov *et al.*, 2005; Simard *et al.*, 2006). Proinflammatory molecules produced by neurons may actually promote further neuroinflammatory actions and result in even more serious neuronal impairment as well as the pathological development of AD.

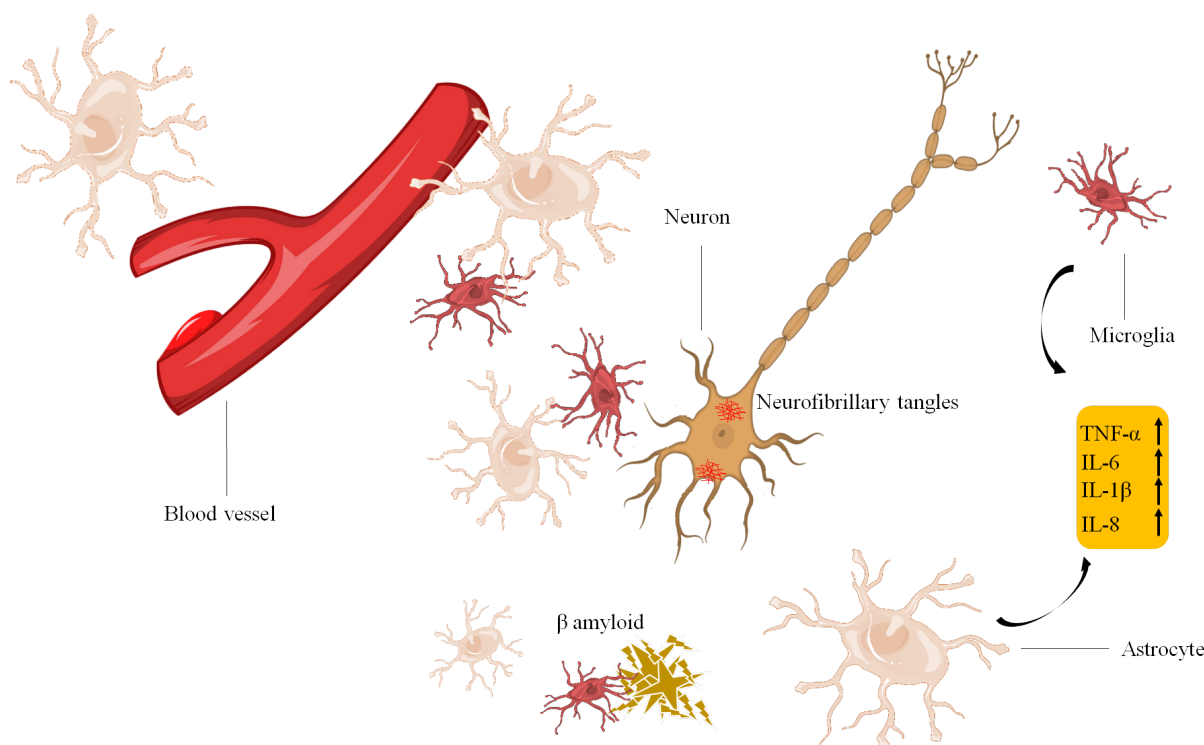


Figure 6. Neuroinflammation in AD

Representation of changes induced by abnormal accumulation of A β in the brain. A β oligomers and plaques set up from increased amyloidogenic processing of the APP and/or disrupted A β clearance circuits. Importantly, aggregation of intracellular A β leads to hyperphosphorylation of tau at postsynaptic sites, which disturbs tau function and changes recruitment of crucial proteins essential for synaptic potentiation. Furthermore, the increased A β load leads to abnormal activation and dysfunction of astrocytes and microglia, resulting in excessive generation of different inflammatory cytokines and chemokines, and eventually impairment in functions.

1. Microglia

Microglia are the resident brain macrophages and are usually considered as "chef d'orchestre" of the brain inflammatory response during infections and disease. Microglia accounts for 5%–12% of all glial cells in the rodent brain and for 0.5%–16% in human brain (Lawson *et al.*, 1990; Mittelbronn *et al.*, 2001; Nicola *et al.*, 2015).

In the course of neurodegenerative diseases, microglia become activated and phagocytic, proliferates and undergoes profound morphological modifications (Ransohoff *et al.*, 2009; Glass *et al.*, 2010). However, it is still debated if these modifications are beneficial

or detrimental to the organism, especially in the light of the time course of the disease (Sierra *et al.*, 2013).

Microglia take also part in forming appropriate neuronal connectivity during CNS development by pruning supernumerary and improper synapses to reinforce the appropriate ones based on neuronal activity and experience (Pierre *et al.*, 2017). Additionally, microglia modulate in the adult brain complicated cognitive process such as learning and memory (Parkhurst *et al.*, 2013; Udeochu *et al.*, 2016). Microglia are quiescent in resting status with small cell body and branching processes. Under the condition of disease, microglia turn into activated microglia (Stence *et al.*, 2001). Differing to the resting status, activated microglia are recognized by their amoeboid procedures, release of both pro-inflammatory and anti-inflammatory molecules, as well as phagocytic removal of apoptotic cells (Gehrmann *et al.*, 1995; Neumann *et al.*, 2008). Microglia continuously scan the brain parenchyma by sending and retracting its processes (Nimmerjahn *et al.*, 2005; Davalos *et al.*, 2005; Haynes *et al.*, 2006; Tremblay *et al.*, 2011) and play an important role in the plasticity of neuronal circuits.

During the course of AD, A β plaques are considered by microglial cells as a danger signal inducing a series of morphological and functional changes. These cells are termed "reactive" (Bornemann *et al.*, 2001). This innate immune reaction is considered to be beneficial to the organism if it settles down the danger signal. If not, microglia might topple in a chronically activated status leading to detrimental functions. Hence, reactive microglia become tightly involved in A β deposition via cell membrane receptors (SCARA1, CD36, CD14, α 6 β 1 integrin, CD47, and Toll-like receptors) in brains from AD patients and AD mouse models (McGeer *et al.*, 1987; Griffin *et al.*, 1989; Tooyama *et al.*, 1990; Geaghan *et al.*, 2009; Frenkel *et al.*, 2013; Gold *et al.*, 2015; Heela *et al.*, 2017). Furthermore, the expression of some proliferation and inflammation markers by reactive microglia (CSF1, IL34, TGF β) correlate with disease severity (Alonso *et al.*, 2016), also suggesting a pathological role of microglia proliferation and activation in disease progression.

2. Astroglia

Astrocytes are also CNS-resident glial cells and respond to pathological stimuli through reactive gliosis aimed at neuroprotection (Sofroniew *et al.*, 2010; Burda *et al.*, 2014). However, morphological remodeling of astrocytes may lead to cognitive deficits via reducing the number of synapses (Zorec *et al.*, 2015) and might contribute to synaptic weakness and death. Like microglia in AD, astrocytes are surrounding A β plaques (Medeiros *et al.*, 2013)

but interestingly, their activation precede A β plaque deposition in APP transgenic mice, that is at an early stage of the pathology (Heneka *et al.*, 2005).

In AD, reactive astrocytes go through several complex alterations that vary between brain regions and happen at early stage of the disease, even before A β deposition (Kummer *et al.*, 2014). Reactive astrocytes are characterized by enhanced expression of glial fibrillary acidic protein (GFAP) and also marks of functional abnormality (Olabarria *et al.*, 2011). In mice models of AD, the early response is marked by astrocytes function decline, which might have profound and lasting effects on synaptic function, because astrocytes are key players of synaptic transmission (Kang *et al.*, 1998; Olabarria *et al.*, 2010, 2011). Similar to microglia, in response to A β , astrocytes release pro-inflammatory cytokines, nitric oxide, and other cytotoxic molecules fueling therefore the neuroinflammatory process.

3. Cytokines

Microglia and astrocytes are the main source of pro-inflammatory cytokines in AD. Cytokines take part to almost every step of the neuroinflammatory process but also A β deposition. Activation of microglia is designed by cytokines. Hence, in old APP/PS1 mice (15 months of age) the pathological accumulation of A β seems directly related to increased levels of pro-inflammatory cytokines, including TNF α , IL-6, IL-1 α and IL-1 β (Patel *et al.*, 2005). Interestingly, *in vitro* incubation of microglia with soluble A β ₁₋₄₂ increase proinflammatory cytokines production (Lue *et al.*, 2001) such as IL-1 β (Akama *et al.*, 2000). High levels of the proinflammatory cytokine IL-1 β are also found in microglial cells nearby A β plaques in AD patients.

Intracellular caspases proteases family are central of apoptosis and inflammation. In the caspase's family, caspase 1 activation is required to subsequent cleavage of interleukin 1 β and interleukin 18 into their active forms (Schroder *et al.*, 2010; Veerdonk *et al.*, 2011). A β can initiate NLRP3 through lysosomal impairment in microglia (Halle *et al.*, 2008). Increased active caspase 1 levels are found in AD brains and APP/PS1 mice. In addition, APP/PS1 mice lacking of NLRP3 or caspase 1 are mainly prevented from spatial memory impairment, hippocampal synaptic plasticity loss and related behavioural dysfunction associated with AD (Heneka *et al.*, 2013). Lacking of NLRP3 or caspase 1 in APP/PS1 mice appeared to change microglial from a proinflammatory type to an anti-inflammatory type (Heneka *et al.*, 2013). Even more, activation of microglia with different proinflammatory factors resulted activation of apoptotic caspase 8 and caspase 3 as well as caspase 7. Caspase 3 activation regulates NF-

κ B activity through PKC δ and increases the levels of neurotoxic proinflammatory factors, such as IL-1 β , TNF α . Inhibition of these caspases prevented microglia activation and neurotoxicity (Fricker *et al.*, 2013). Pharmacological investigations to inhibit activated caspases have been described to reasonable play neuroprotective roles in AD mouse models (Rohn *et al.*, 2009; Biscaro *et al.*, 2012).

Under the head injury condition, IL-1 β could promote A β deposition by increasing the expression of β -APP (Goldgaber *et al.*, 1989; Forloni *et al.*, 1992) through the β APP promoter. (Buxbaum *et al.*, 1992; Mrak *et al.*, 1995). On the contrary, proinflammatory signalling pathways can be shown in APP/PS1 mouse model where transgenic expressed IL-1 β in APP/PS1 mice resulted in strong neuroinflammation but decreased A β plaque pathology (Shaftel *et al.*, 2007; Ghosh *et al.*, 2013).

IL-12 and IL-23, two pro-inflammatory cytokines, are produced by leucocytes and microglia in mouse models of AD (Berg *et al.*, 2012), and their inhibition via the common subunit (p40) subsequently attenuate AD-like pathology and reverse cognitive deficits (Berg *et al.*, 2012; Tan *et al.*, 2014). An increase of CSF levels of the proinflammatory cytokine TNF α together with a concomitant decrease of the anti-inflammatory cytokine TGF β has been reported in patients with mild cognitive impairment at risk to develop AD (Tarkowski *et al.*, 2003) highlighting the detrimental role of neuroinflammation in the pathogenesis of AD.

4. Prostaglandins E2

Prostaglandin E2 (PGE₂) plays pivotal functions in neuroinflammation. PGE₂ is produced from arachidonic acid by the microsomal prostaglandin-E2 synthase (mPGES) and by two rate-limiting enzymes, Cox-1 and Cox-2 (Smith *et al.*, 1991) which are expressed by hippocampal neurons (Yasojima *et al.*, 1999), astrocytes and microglia (Nieves *et al.*, 2012) (Figure 7). Whereas Cox-1 is constitutively expressed, Cox-2 and mPGES are strongly activated by and during neuroinflammation (Nieves *et al.*, 2012).

PGE₂ plays an important role in the pathophysiology of AD. It is present at high concentration in the course of the disease and by fostering the production of others inflammatory molecules (TNF- α , IL 1 β , ROS, nitrite) may lead to neuronal death (Gehrmann *et al.*, 1995; Montine *et al.*, 1999; Aloisi *et al.*, 2001; Combrinck *et al.*, 2006; WEI *et al.*, 2010; Shi *et al.*, 2012). Study also proposes that PGE₂ promotes A β generation in a mouse model (Hoshino *et al.*, 2009). PGE₂ can bind to four different subtypes of G-protein-coupled receptors (EP1-4) which regulate adenylyl cyclase (AC) activity and/or phosphoinositol

turnover and intracellular calcium mobilization (Breyer *et al.*, 2001). Depending on which subtype of receptor it is acting, PGE₂ may have opposite effects on neuroinflammation. Activation of EP1 receptor triggers an increase of the concentration of intracellular Ca²⁺, whereas activation of the EP2/EP4 receptors are linked to increased levels of intracellular cAMP via Gs (Honda *et al.*, 1993; Bastien *et al.*, 1994; Nishigaki *et al.*, 1995). Conversely, EP3 receptor couples negatively to cAMP generation via Gi (Breyer *et al.*, 1994, 2001).

Consistent with data of both favorable and toxic results of COX activity, more and more study shows both beneficial and toxic effects for EP receptors (Andreasson *et al.*, 2010; Johansson *et al.*, 2015).

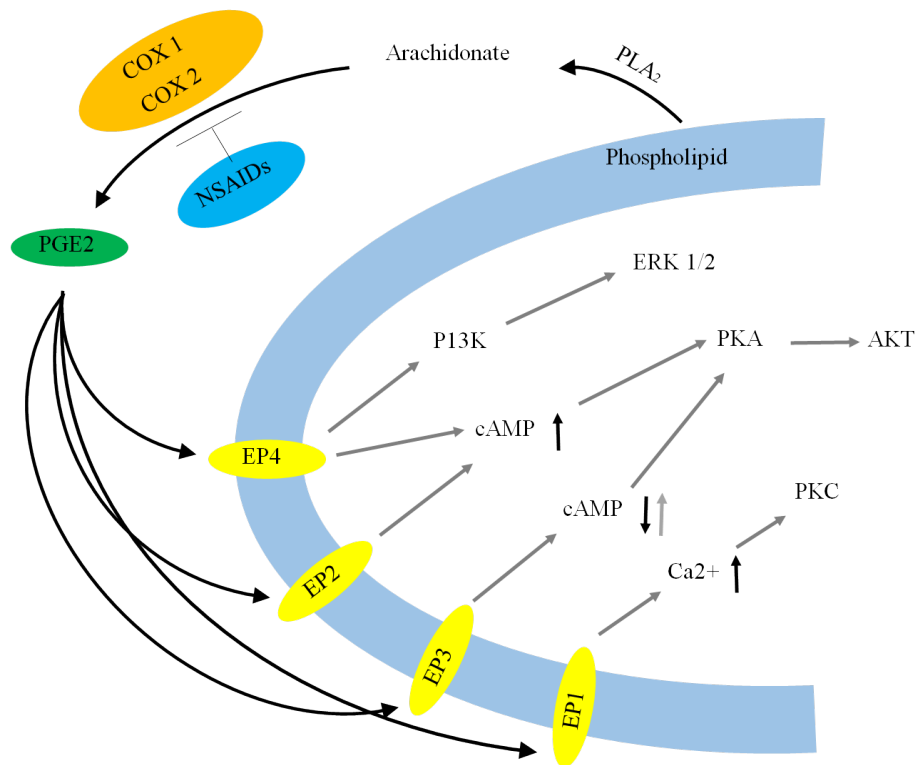


Figure 7. Prostaglandin synthesis

PGE₂ is mainly produced by two rate-limiting enzymes: Cox-1 and Cox-2. Cellular actions of PGE₂ are performed via G protein coupled receptors family named EP1-EP4. EP1 is coupled to calcium mobilization. EP2 and EP4 activation increased cAMP levels, while EP3 activation can lead to a decrease of cAMP levels. By inhibiting PGE₂ synthesis with nonsteroidal anti-inflammatory drugs (NSAIDs) or particular EP antagonists, neuroinflammation can be alleviated.

1) EP1 receptor

EP1 receptor is expressed in the hippocampus (Kawano *et al.* 2006). It is coupled to G α_q , and its activation leads to an increase in intracellular Ca²⁺ concentration. The role of EP1 receptor in neurodegenerative disease is still not clear but it may play a role in AD as its genetic deletion in APP/PS1 mice attenuated memory loss induced by ischemic insult and reduced the A β plaque load (Zhen *et al.*, 2012).

2) EP2 receptor

By contrast, the role of EP2 receptor has been well assessed in neurodegenerative models. EP2 receptor is positively coupled via G s to the generation of cAMP and is densely expressed in pyramidal neurons of hippocampus where it modulates synaptic transmission (Sang *et al.*, 2005). The genetic deletion of EP2 in APP/PS1 mice leads to reduced brain lipid oxidation and decreased A β levels, possible via decreased the activity of BACE-1 (Tamagno *et al.*, 2002; Liang *et al.*, 2005). EP2 receptor is a key mediator of the microglial response to A β . The blockade of microglial EP2 receptor inhibits the ability of microglia to engulf A β (Shie *et al.*, 2005) possibly through the blockade of the expression of CD36, a key player in A β phagocytosis by microglia (Li *et al.*, 2015). The specific genetic deletion of microglial EP2 receptors in APP/PS1 mice induces alterations in growth factor levels, working memory and synaptic density (Johansson *et al.*, 2015).

3) EP3 receptor

EP3 receptor is coupled to G proteins through a G i/o subunit (Hatae *et al.*, 2002) which negatively regulates AC activity. In APP/PS1 mice, PGE₂-EP3 signaling pathway is associated with the neuroinflammatory response to A β and its genetic deletion reduced proinflammatory gene expression, IL-1 β and TNF α production and oxidative stress (Shi *et al.*, 2012). More recently we have shown that PGE₂-EP3 pathway was responsible for the impairment of hippocampal presynaptic long term plasticity at the Mf/CA3 synapse in aged APP/PS1 mice and that impairment was fully reversed by the specific blockade of EP3 receptor (Maingret *et al.*, 2017).

4) EP4 receptor

EP4 receptor is positively coupled via G s to the generation of cAMP and expressed in neurons at low levels (Li *et al.*, 2008). EP4 receptor may provide neuroprotection in AD

models and hypoxic damage (Liang *et al.*, 2011). In agreement to neuroprotective action, the stimulation of EP4 receptors in cultured microglia decreases the response to LPS (Minami *et al.*, 2008; Shi *et al.*, 2010) and its selective genetic deletion in microglia extends neuroinflammatory status (Shi *et al.*, 2010). The stimulation of EP4 receptors in primary culture of microglia reduces levels of A β ₄₂ - induced inflammatory factors (TNF α , IL-12, iNOS, Cox-2) and conditional deletion of microglial EP4 receptors in APP/PS1 mice conversely increased inflammatory gene expression, oxidative protein modification and A β plaques load in brain at early stages of pathology (Woodling *et al.*, 2014). All together, these results highlight an anti-inflammatory and neuroprotective role of microglial EP4 signaling pathway in AD mice model.

b. Nonsteroidal anti-inflammatory drugs (NSAIDs)

Numerous epidemiological studies have shown that chronic intake of NSAIDs reduced the prevalence of AD (McGeer *et al.*, 2007). Then clinical trials revealed that NSAIDs, when given to asymptomatic patients, reduced AD incidence whereas they have deleterious effects on AD pathogenesis in its later stages (Szekely *et al.*, 2010; Breitner *et al.*, 2011; Wang *et al.*, 2015; Zhu *et al.*, 2018; Kinney *et al.*, 2018).

NSAIDs inhibit cyclooxygenase activity by blocking Cox-1 and Cox-2 (Kaufmann *et al.*, 1997; Vlad *et al.*, 2008). Although NSAIDs are likely to be the safest drug option for AD, meta-analysis of different cohorts using NSAIDs on short, intermediate and long term treatment yielded no conclusive results (Etminan *et al.*, 2003).

The chronic intake of NSAIDs by patients treated for inflammatory pathologies for more than 24 months clearly reduced the prevalence (40 to 70 %) of AD (Stewart *et al.*, 1997; Veld *et al.*, 2001; Lindsay *et al.*, 2002; McGeer *et al.*, 2007) whereas most of the clinical trials looking at the effects of NSAIDs on AD's patients failed to show positive results or even yielded deleterious side effects mostly on the gastrointestinal tractus (McGeer *et al.*, 2007). In an effort to reduce side effects, Cox-2 specific inhibitors (nimesulide: Aisen *et al.*, 2003; rofecoxib: Aisen *et al.*, 2003, Thal *et al.*, 2005; celecoxib: Soininen *et al.*, 2007; Lyketsos *et al.*, 2007) have been tested but also failed to demonstrate beneficial effects on AD's patients. A subset of NSAIDs known as "selective A β ₄₂ - lowering agents" (SALAs) which modify γ -secretase cleavage away from A β ₄₂ to A β ₄₀ and A β ₃₈ also failed to reduce AD risk (Szekely *et al.*, 2008).

From all these studies it seems that effects of NSAIDs on neuroinflammation may be beneficial in the very early stages of AD but may become detrimental for later stages and even worsen the clinical symptoms (Breitner *et al.*, 2011; Leoutsakos *et al.*, 2012).

C. Synaptic dysfunction

The human brain comprises about 86 billion neurons and supposedly ten times more glial cells (Herculano-Houzel, 2009). This unique network processes and transfers messages in the form of chemical and electrical signals (Figure 8). Transmission between neurons happens at special junctions named synapses. Synaptic development and connectivity is crucial to maintain neuronal network activity and normal brain functions. It is now widely accepted that processing but also storage of the information within the brain involves structural and functional synaptic plasticity. This plasticity is considered to be the foundation of learning and memory in the brain (for review see Malenka & Bear, 2004) and has awarded the Nobel Prize in Physiology or Medicine in 2000 to Eric Kandel. When it comes to pathology, it is doubtless that the improper loss of synapses and/or dysfunction in neuronal transmission can be the cause of various psychiatric and neurologic diseases, such as mental retardation (Pfeiffer *et al.*, 2009), schizophrenia (Stephan *et al.*, 2006), Parkinson's disease (Calabresi *et al.*, 2006), autism (Sudhof *et al.*, 2008), AD (Selkoe *et al.*, 2002), compulsive behavior (Welch *et al.*, 2007), and addiction (Kauer *et al.*, 2007).

a. Intrinsic and synaptic plasticities

1. Intrinsic plasticity

Passive and active membrane properties are related to ionic channels expressed at the plasma membrane. Resting membrane potential (RMP), membrane resistance (R_{in}) and the voltage threshold and/or the minimal current intensity (also termed rheobase) needed to generate action potentials (APs) are considered as passive membrane properties. Frequency of spike activity, *i.e.* discharge of APs, firing pattern *i.e.* frequency and/or spike frequency adaptation are considered as active membrane properties. Any modification of these non-synaptic intrinsic membrane properties will change neuronal activity and likely modify circuit properties (Santos *et al.*, 2010).

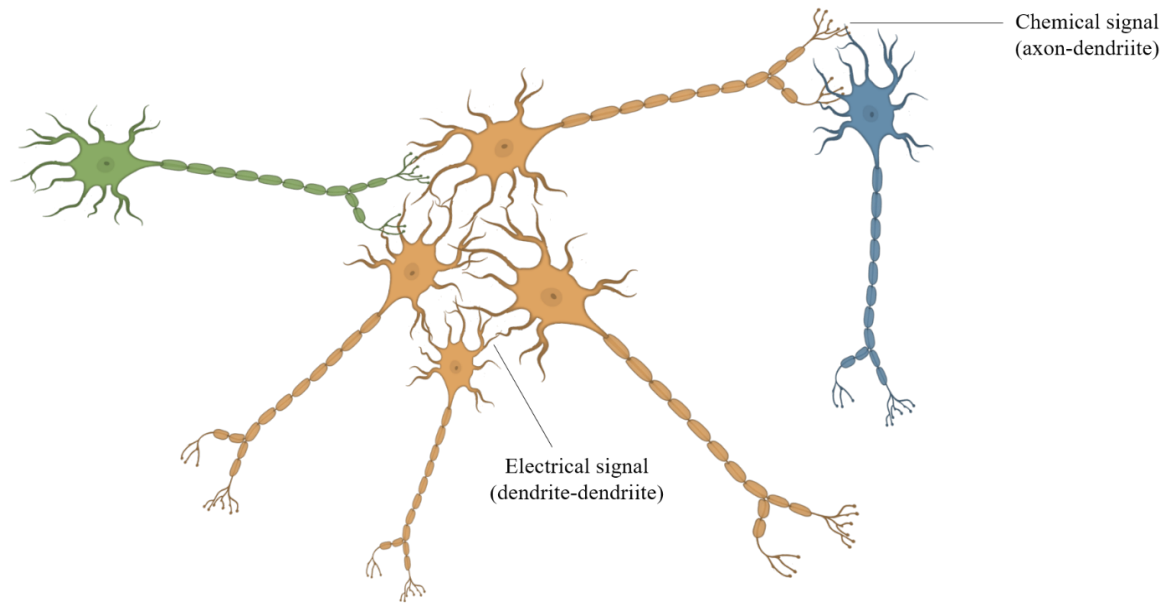


Figure 8. Synaptic transmission

Information from one neuron is transferred to another neuron through a minute gap termed synapse. At the synapse, electrical signals are changed into chemical signals so as to cross the gap. While on the other side, the signal turns back into electrical signals again. One neuron could connect to thousand other neurons.

As such, Ca^{2+} influx occurring during APs triggers Ca^{2+} release (CICR) from intracellular stores mediated by the inositol 1,4,5-trisphosphate (IP_3) receptor (IP_3R) via the activation of ryanodine receptor (RyR) channels (Berridge, 1998, 2002). Interestingly, in early stages of AD, increased RyR mediated Ca^{2+} signaling has been reported to affect small conductance calcium activated potassium (SK) channels through a decreased expression of FK506 binding protein that related to RyR2 activity (Gant *et al.*, 2014, 2015). This could result in a decline of neuronal excitability. Intracellular Ca^{2+} rise also activates large conductance potassium (BK) channels, which can modulate AP firing by accelerating the membrane repolarization (Stutzmann *et al.* 2006; Chakroborty *et al.*, 2009; Chakroborty *et al.*, 2012) and lead also to a decline of neuronal excitability.

Consequently, alteration of neuronal firing properties might induce functional deficits in the early stages of AD (Rowan *et al.*, 2005; Trommer *et al.*, 2005; Cuevas *et al.*, 2011; Palmer and Good, 2011).

2. Long term synaptic plasticity

Long term synaptic plasticity is an activity-dependent ability of a synapse to display a strengthened or weakened activity over time, on minutes to hours. The idea of synaptic plasticity and its involvement in learning was first proposed by Ramon y Cajal, who reported that the amount of neurons in the brain did not seem to change noticeably during lifetime (Cajal, 1894; Jones, 1994). The major forms of synaptic plasticity are long-term potentiation (LTP) and long-term depression (LTD), which may happen at either excitatory or inhibitory synapses, through presynaptic and/or postsynaptic mechanisms. Both mechanisms of plasticities are believed to be associated with information storage and consequently in learning and memory as well as other physiological procedures.

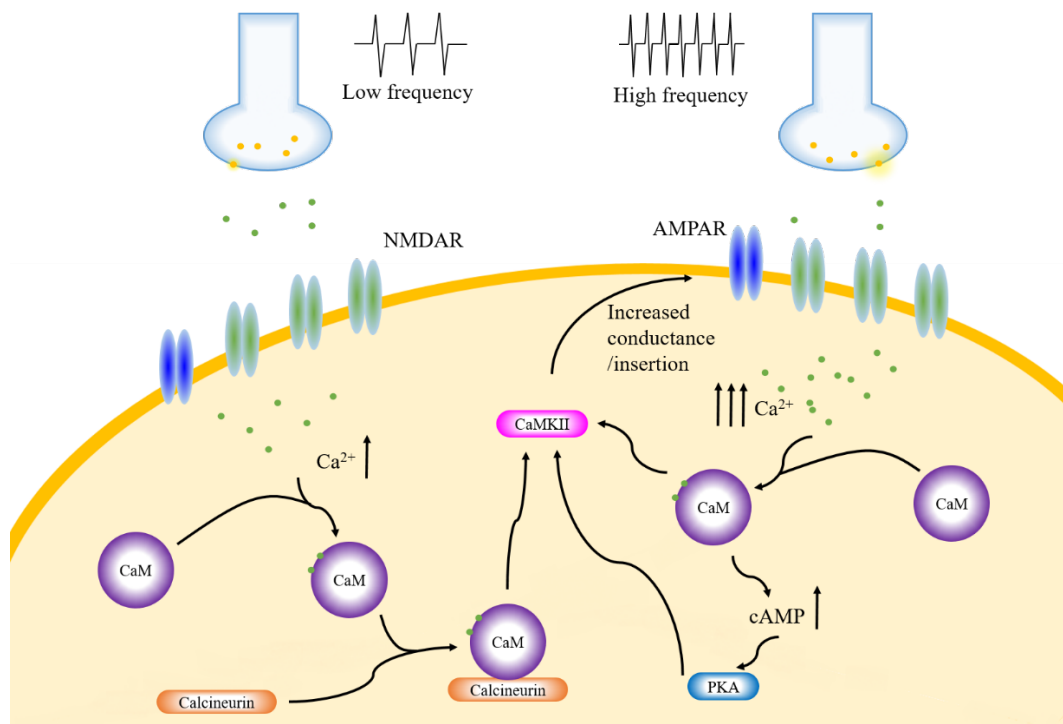


Figure 9. Signaling in LTD and LTP

Activation of NMDA receptor, inducing potentiation or depression of synaptic transmission. The levels of intracellular Ca^{2+} approached by the activation of NMDA receptors have an important role in characterization of long term potentiation (LTP) or long-term depression (LTD) (Lisman *et al.*, 1989; Lisman *et al.*, 1994). Based on the fact that, weak activation of the NMDA receptor (left) would induce low concentration increases of Ca^{2+} , leading to the activation of calcineurin (CaN) over CaMKII, leading to LTD. Conversely, strong activation of the NMDA receptor (right) would increase Ca^{2+} levels enough to activate CaMKII and PKC, leading to the generation of LTP. It was suggested that the activation of calcium sensitive adenylyl cyclases by strong activation of NMDA receptor would result in the activation of cAMP dependent protein kinase (PKA).

Classical LTP is dependent the activation of post-synaptic NMDA receptors and the insertion of new AMPA receptors, whereas classical LTD would be the result of the activation of NMDA receptors, AMPA receptors as well as kainate receptors and metabotropic glutamate receptors (mGluRs) (Collingridge *et al.*, 2009; Granger *et al.*, 2014) (Figure 9). Changes in both LTP and LTD have been considered to be involved in AD (Wang *et al.*, 2002; Shankar *et al.*, 2008; Li *et al.*, 2009).

1) Long-term potentiation (LTP)

LTP is a long lasting enhancement in the strength of synaptic transmission involving either presynaptic or postsynaptic mechanisms or both, was first reported in rabbit hippocampus (Bliss and Gardner-Medwin, 1973).

LTP has been involved in a number of physiological events such as the building of new dendritic spines, increments in perforated postsynaptic densities (collecting more presynaptic input), and triggers an enlargement of spine heads (Harreveld and Fifkova, 1975; Savatic *et al.*, 1999; Nagörl *et al.*, 2004; Bosch and Hayashi, 2012). Hence, LTP alterations in the hippocampus in AD mice models have been extensively studied. LTP comprises two phases: early and late. The early phase depends upon protein kinase activation leading to the phosphorylation of synaptic AMPA receptors, enhanced activity, insertion of new AMPA receptors into the postsynaptic density leading to the enhancement of synaptic transmission. In the late phase, raised calcium levels at the postsynaptic side and constant activation of kinases (PKC, PKMzeta, and CamKIIa) trigger the activation of transcription factors, such as CREB. *In vitro*, the exogenous application of A β triggers an impairment of LTP (Chen *et al.*, 2000; Li *et al.*, 2011; Jo *et al.*, 2011; Varga *et al.*, 2015) but genetic deletion or treatment with blocking antibody showed that endogenous A β is necessary for hippocampal LTP *in vivo* (Puzzo *et al.*, 2011). This study together with other reports (Puzzo *et al.*, 2008; Osta and Alberini, 2009), suggest that endogenous low levels of A β do have a physiological function in synaptic plasticity.

LTP is also impaired in tau mutant mice (Fa *et al.*, 2016; Gelman *et al.*, 2018). These alterations were observed in old mice, likely the result of high levels of accumulated tau (Chong *et al.*, 2011; Sydow *et al.*, 2011). Moreover, similar age-dependent results on LTP were also reported in mutant mice overexpressing normal human tau (Polydoro *et al.*, 2009). Whereas tau acts on LTP by itself or downstream of A β or even in concert with A β is still debated (Jones *et al.*, 2014).

2) Long-term depression (LTD)

LTD is a long lasting decline of synaptic strength. It may occur by several ways, which not surprisingly have opposite effects to those observed in LTP, including internalization of AMPA receptors (Dudek and Bear, 1992; Massey and Bashir, 2007; Collingridge *et al.*, 2010), and calcineurin activation (Mulkey *et al.*, 1993; Li *et al.*, 2010).

LTD is believed to be important for the clearing of old memories and in circumstances requiring behavioral adaptability (Collingridge *et al.*, 2010). This "forgetting" feature of LTD could be disrupted during AD causing memory problems (Jones *et al.*, 2014). As for LTP, the molecular mechanisms of LTD which could be targeted by AD are poorly known. Interestingly, A β induces AMPA and NMDA receptors internalization through the same calcineurin-mediated pathways involved in LTD (Wang *et al.*, 2004; Snyder *et al.*, 2005; Hsieh *et al.*, 2006; Koffie *et al.*, 2011).

Knockout mice for tau at the CA1 region of hippocampus show impairment of LTD (Kimura *et al.*, 2014) suggesting an important role of tau in synaptic transmission. Besides, mutation of tau was found to be connected with glutamate signaling as well as excitotoxicity *in vivo*, further proposing a role for tau in modulation of synaptic transmission (Hunsberger *et al.*, 2015).

3. Short term synaptic plasticity

Short term plasticity (STP) takes place on milliseconds to few seconds scale (Zucker and Regehr, 2002; Abbott and Regehr, 2004). At some synapses, a decrease of neurotransmitter release contributes to short term depression by decreasing the release probability or reducing the readily releasable pool of vesicles (Eccles *et al.*, 1941; Feng *et al.*, 1941) (Figure 10). At other synapses, increases of neurotransmitter release probability (Katz *et al.*, 1968) contribute to short term depression by saturating of local calcium buffer (Felmy *et al.*, 2003; Blatow *et al.*, 2003) or increasing of calcium concentration at the presynaptic terminal (Kamiya *et al.*, 1994; Atluri *et al.*, 1996; Sippy *et al.*, 2003).

STP plasticity such as post-tetanic potentiation (PTP) and paired pulse facilitation (PPF) play essential roles in long-term memory formation and hence, STP impairments cause long lasting memory formation deficits (Silva *et al.*, 1996). Consistent long-term memory activity relies on Ca²⁺ mediated STP to convert short term to long term memory and set up synapse specificity (Frey *et al.*, 1997; Redondo *et al.*, 2011).

STP is not a trivial synaptic mechanism but seems instead to be a property to keep the balance between excitation and inhibition. Depending on the type of STP triggered at a particular synapse, it will shape the transferred information which **suggests** that the same presynaptic site may carry different information to distinct postsynaptic cells (Markram *et al.*, 1998). In this case, short-term facilitation and depression carry information in both low and high frequencies range (Fuhrmann *et al.*, 2002). These studies highlight the crucial roles of STP in information processing and strongly suggest the physiological relevance of STP in different cognitive tasks as for example, decision making or working memory (Shyu *et al.*, 2009; Deng *et al.*, 2011).

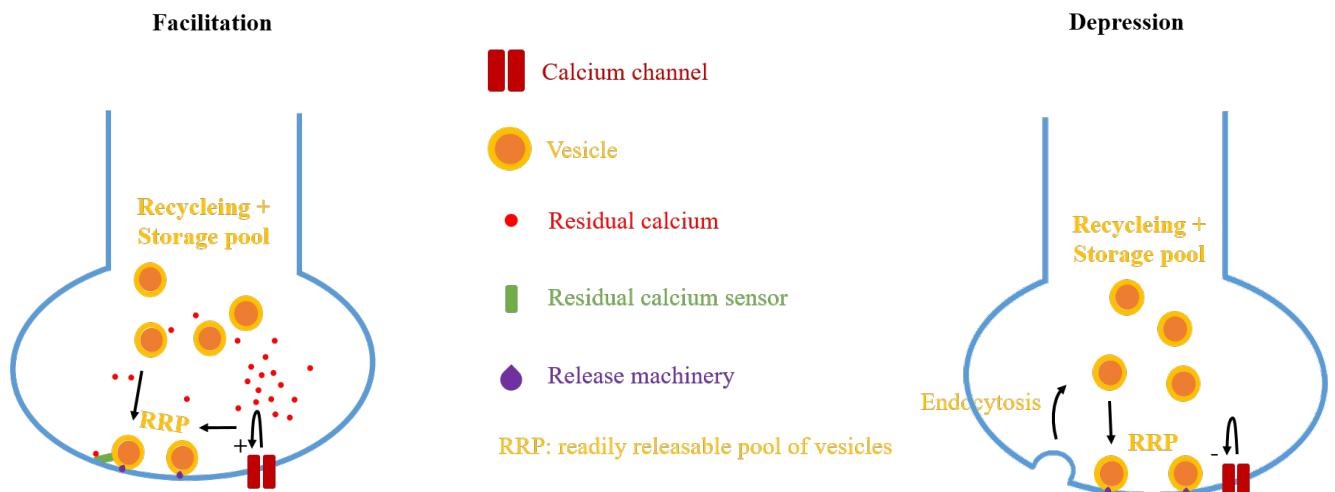


Figure 10. Short term synaptic plasticity

Presynaptic mechanisms of short term plasticity. Schematic graphs demonstrate possible mechanisms of facilitation and depression.

The characteristics of postsynaptic receptors could be also involved in short term plasticity. When exposed to neurotransmitter, the desensitization of postsynaptic receptors could lead to a decrease synaptic activity upon repeated activation (Trussell *et al.*, 1993; Jones *et al.*, 1996; Chen *et al.*, 2002; Sun *et al.*, 2002; Friedman *et al.*, 2003; Wadiche *et al.*, 2001; Foster *et al.*, 2002; Harrison *et al.*, 2003).

In early AD stage, a number of synaptic pathophysiological mechanisms induced by ryanodine receptors (RyR) of excessive Ca^{2+} have been reported, such as an enhanced frequency of spontaneous synaptic potentials, decreased PPF ratio as well as increase of

synaptic depression (Chakroborty *et al.*, 2009, 2012; Briggs *et al.*, 2013). Published studies have demonstrated that sizeable vesicle depletion happens at high release probability synapses, which has been illustrated in several studies of AD mice (Goussakov *et al.*, 2010; Scimemi *et al.*, 2012). For example, at excitatory CA3/CA1 synapses of mouse hippocampus, several functional Ca^{2+} channels may be located in the active zone at variable sites. This location results in a far and wide distribution of release probability through the vesicles at the active zone, and the depletion of the vesicles adjacent to Ca^{2+} channels can contribute to the Ca^{2+} dependent STP at these synapses.

4. Activity-dependent modulation of population spike (PS) plasticity

Population spike (PS) which can be recorded with extracellular electrodes is a composite response built by the extracellular summation of action currents during synchronous APs firing. With appropriate external stimulation, it is possible to trigger a potentiation of the PS which gives rise to an increase in the PS amplitude.

Noteworthy, at the LPP-DG synapse, the PS amplitude is comparatively larger than the amplitude of the excitatory postsynaptic potential (EPSP) (Bliss *et al.*, 1973; Andersen *et al.*, 1980; Yanagihashi *et al.*, 1992). In theory, an increase of the PS amplitude goes along with an increase of the field EPSP (fEPSP). Nonetheless, this close relation between PS and EPSP may vary while LTP is induced by tetanic stimulation. The potentiation of PS is relatively greater than the EPSP. This suggests that the potentiation mechanism underlying spikes is to some extent independent from the synaptic potentiation (Bliss *et al.*, 1973; Mozzachiodi *et al.*, 2010). Therefore, the increase in PS amplitude is not *sensus stricto* the exact equivalent of an increase in EPSP which is synaptic in essence. In other words, the mechanisms of an increase of PS amplitude may rely both on synaptic and non-synaptic mechanisms whereas an increase in the EPSP reflects synaptic mechanisms.

The "non-synaptic" part of PS potentiation has been observed in different brain regions with different LTP protocols (Abraham *et al.*, 1987; Noriega *et al.*, 1989). It shows an enhancement of the intrinsic excitability in the postsynaptic neurons via modulation of voltage Na^+ channels but also other channels (Ca^{2+} channels, K^+ channels) (Taube and Schwartzkroin, 1988; Wathey *et al.*, 1992; Bernard *et al.*, 1995; Xu *et al.*, 2005; Fink and O'Dell, 2009).

In some cases, alterations in intrinsic excitability without obvious changes in synaptic strength, *i.e.* LTP or LTD, have been reported. In the CA1 hippocampus, high frequency

stimulation potentiates EPSPs, but this potentiation is neither a prerequisite for, nor a correlation of a potentiation of PS amplitude (Taube and Schwartzkroin, 1988). Short trains (2-3 s) of Schaffer collateral fiber stimulation delivered at 5 Hz induce increase in the excitability of CA1 neurons in the absence of synaptic potentiation (Fink and O'Dell, 2009).

Furthermore, both EPSP and PS plasticities were found related to the activity of NMDARs (Jester *et al.*, 1995; Lu *et al.*, 2000). Remarkably, non-synaptic changes can promote synaptic modulations (Byrne *et al.*, 1987) such as depolarization of the resting membrane potential, could promote the induction of synaptic plasticity which is necessary for memory storage (Crow *et al.*, 1980; Mozzachiodi *et al.*, 2008). Generally, these results show that neuronal activity can be modified respectively in different sections within the same cell (Losonczy *et al.*, 2008) and reveal therefore that modulation of neuronal activity are more intricate than previously thought.

5. Miniature excitatory postsynaptic currents (mEPSCs)

Miniature excitatory postsynaptic current (mEPSC) is due to the spontaneous release of a single vesicle into the synapse in the absence of the occurrence of APs as they are recorded in the presence of TTX. Synaptic plasticity mechanisms modifying neuronal activity can be studied at the level of mEPSCs (Bekkers *et al.*, 1990; Turrigiano *et al.*, 1998; O'Brien *et al.*, 1998; Watt *et al.*, 2000) which the frequency and the amplitude are related to pre and postsynaptic loci of action respectively (Prange *et al.*, 1999; Ling *et al.*, 2006).

The increase in mEPSCs frequency depends on calcium influx but not on the activation of voltage-dependent Ca^{2+} channels (Gray *et al.*, 1996; Capogna, *et al.*, 1996; Sharma *et al.*, 2003). mEPSCs frequency changes are often associated to changes in the transmitter release probability (Scholz *et al.*, 1992; Debanne *et al.* 1996; Prange *et al.*, 1999; Sharma *et al.*, 2003), but mEPSCs frequency can change without having an effect on the transmitter release (Eliot *et al.*, 1994; Yoshihara *et al.*, 1999; Béïque *et al.*, 2003). Hence, the increase of docked vesicles number can lead to an increase of mEPSC frequency (Tyler *et al.*, 2001; Murthy *et al.*, 2001; Lauri *et al.*, 2003) but also the increase of the number of functional release sites, *i.e.* via the unmasking of "silent synapses" into functional ones (Isaac *et al.*, 1995; Liao *et al.*, 1995; Durand *et al.*, 1996; Liao *et al.*, 2001).

Changes in mEPSCs amplitude are considered to reflect postsynaptic mechanisms. Unless if the quantal size of a vesicle is modified, the modification of mEPSCs amplitude is believed to reflect a change in the receptor number (Turrigiano *et al.*, 1998; Watt *et al.*, 2000;

Ling *et al.*, 2006). But it is also possible that increases or decreases in the glutamate content of synaptic vesicles lead to the change in mEPSC amplitude. (Watt *et al.*, 2000; Liu *et al.*, 1999; McAllister *et al.*, 2000). Although there is no direct study supporting alterations in glutamate content in vesicles, there is direct study for alterations of sensitivity in postsynaptic receptors (Turrigiano *et al.*, 1998; Lissin *et al.*, 1998; O'Brien *et al.*, 1998).

Numerous data report that soluble A β increases neuronal activity (Bausch *et al.*, 2006; Busche *et al.*, 2012). A β_{1-40} triggers an increase in the frequency of mEPSCs together with firing activity (Cuevas *et al.*, 2011). Pharmacologically study to identify synaptic activity indicated that the activity of NMDA mediated mEPSCs was obviously increased, implying enhanced excitation and synaptic plasticity (Berberich *et al.*, 2007; Hoe *et al.*, 2009).

b. Synaptic dysfunction in AD

1. Synaptic activity and A β homeostasis

AD, at least in its early stage is considered to involve synaptic dysfunction before synaptic and neuronal loss (Selkoe *et al.*, 2002; Jacobsen *et al.*, 2006; Sheng *et al.*, 2012). In good agreement, spatial and contextual learning and memory deficits reported in a mouse model of AD appear prior to A β plaques and tau pathology (Billings *et al.*, 2005). Therefore, it is no surprise that synapses appear as a major therapeutical target to at least alleviate the burden of the disease and possibly maintain cognitive and functional abilities. Furthermore, the brain location of A β plaques and tau tangles, the two main molecular hallmarks of AD, allows to link morphologic alterations to cognitive impairments such as learning and memory with synaptic dysfunction.

It is well known that A β peptide, lead to morpho-functional alterations and numerous harmful effects on neurons (Strooper *et al.*, 2016). A β oligomers directly promote neuronal apoptosis via cell-surface receptors (TRAIL, TNF-related apoptosis-inducing ligand death death receptors 4 and 5) (Fossati *et al.*, 2012). Furthermore, the long-term buildup of toxic A β species triggers DNA damage, injury of cellular organelles and dysregulation of intracellular calcium level — all of them leading to cell death (Kayed *et al.*, 2013). Although A β under certain conditions can bring rapid impairment of synaptic plasticity (Cullen *et al.*, 1997; Lambert *et al.*, 1998; Shankar *et al.*, 2008) as well as changes of dendritic spine morphology and nerve terminals of dendritic spines (Rönicke *et al.*, 2011) within hours, it takes years for A β accumulation to have noticeable and deleterious effects.

There are numerous data supporting synaptic pathological effects of A β (Hsia *et al.*, 1999; Mucke *et al.*, 2000; Walsh *et al.*, 2002; Kamenetz *et al.*, 2003; Lacor *et al.*, 2007). Depending on its extracellular concentration, A β may have opposite effects: low concentrations (100-200 pM) facilitate excitatory activity and may be involved in memory formation (Walsh *et al.*, 2007; Puzzo *et al.*, 2008, 2011; Osta and Alberini, 2009) while higher levels (>40 nM) suppress it (Yankner *et al.*, 1990). As A β depresses synapses and impairs synaptic plasticity (Hsieh *et al.*, 2006; Shankar *et al.*, 2008), the activity-dependent A β accumulation (Cirrito *et al.*, 2005) could be considered as a feedback mechanism protecting against synaptic hyperactivation and excitotoxicity (Kamenetz *et al.*, 2003).

In early stages of AD, in addition to the increased expression of RyR, its function is regulated by PS1 and/or PS2 (Shilling *et al.* 2014; Prete *et al.* 2014). PS is known to interact with proteins of the neurotransmitter release machinery and its genetic deletion impairs synaptic plasticity (Barthet *et al.*, 2018). At postsynaptic level, intracellular Ca²⁺ rise is enlarged by RyR-mediated CICR in dendritic spines which is required for the initiation of long term synaptic plasticity (Rossi *et al.*, 2008; Goussakov *et al.*, 2010; Briggs *et al.*, 2016). In summary, further investigations on physiological functions of A β will be most wanted to decipher new targets at which possible AD therapeutics could be performed.

1) A β -induced presynaptic dysfunction

Vesicles which are released within the synapse are present at presynaptic terminals and their release is controlled by a constellation of proteins (Jin *et al.*, 2008). In AD, A β accumulates at presynaptic terminals (Ye *et al.*, 2017) and not surprisingly may impair presynaptic mechanisms. In addition, A β oligomers co-localize with voltage-gated calcium channels to stimulate presynaptic calcium influx increasing therefore the release probability of neurotransmitter, leading to an unusually high level of glutamate in the extracellular space (Moreira *et al.*, 2011). A β oligomers can also damage axonal transport (Decker *et al.*, 2010). The mechanisms by which A β disturbs axonal transport of calcium influx through NMDA receptors activation, resulting in cytosolic calcium upregulation as well as the activation of calcineurin. GSK-3 β dependent activation of calcineurin setup a new link between toxic A β and AD pathology (Pigino *et al.*, 2009; Decker *et al.*, 2010; Ramser, *et al.*, 2013; Gan & Silverman, 2015). A recent study has shown that the axonal binding of A β oligomers was leading to neuronal degeneration (Baleriola *et al.*, 2014).

Synaptic vesicles are the key players that pass on synaptic signals and A β could affect the key procedures of vesicle cycling, including trafficking, release, and recycling (Ovsepian *et al.*, 2018). Additionally, A β may damage vesicle recycling by lowering the recycling pool and increasing the resting pool (Park *et al.*, 2013).

2) A β -induced postsynaptic dysfunction

A β oligomers interact with the postsynaptic side to alter the structure, the composition, and the function of the synapse (Koffie *et al.*, 2009). The activity of A β oligomers at postsynaptic sites can be mediated via their interaction with membrane molecules, eventually leading to synaptotoxicity.

NMDA and AMPA receptors are the main ionotropic glutamate receptors expressed by postsynaptic excitatory synapses (Scheefhals & MacGillavry, 2018). The activation of NMDA receptor promotes calcium influx and triggers intracellular signaling pathways such as calcineurin-mediated Ca²⁺ signaling and Ca²⁺/calmodulin-dependent protein kinase II (CaMKII) (Scheefhals & MacGillavry, 2018). Consequently, evoking dysregulation of intracellular signaling cascades may induce the disruption of neuronal transport in primary neurons (Rui *et al.*, 2006; Decker *et al.*, 2010). Similarly, the removal of AMPA receptors from excitatory synapses can be induced by increased A β levels, which result in dendritic spines loss and synaptic depression (Chang *et al.*, 2006; Hsieh *et al.*, 2006; Molina *et al.*, 2011; Guntupalli *et al.*, 2016).

Metabotropic glutamate receptors 5 (mGluR5) play a key role in modulating transmission at glutamatergic synapses and NMDA receptor-dependent synaptic plasticity (Harney *et al.*, 2006) (Figure 11). In addition, mGluR5 seem essential in A β -induced synaptotoxicity and could be a possible therapeutical target for AD (Kumar *et al.*, 2015). A β induces the clustering and the overstabilization of mGluR5 at the postsynaptic side and thus may lead to early synaptic failure (Renner *et al.*, 2010).

2. Synaptic activity and tau homeostasis

Tau is present at synapses in both physiological and pathological conditions (Pooler *et al.*, 2014). Normal tau is detected at both pre and postsynaptic terminales in control humans brains but a hyperphosphorylated form of tau (pS396/pS404) is found in AD synaptosomes, a key location to mediate proteotoxicity which disrupts synapses in AD (Tai *et al.*, 2012) (Figure 11).

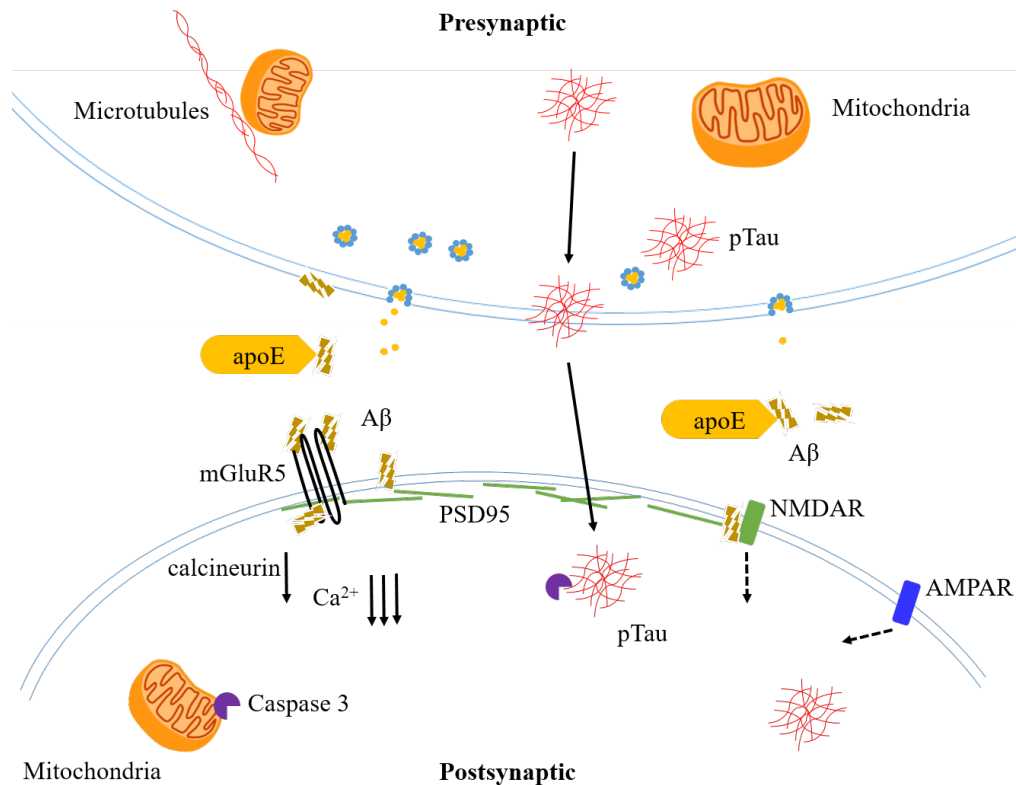


Figure 11. A β and Tau pathology in AD

A β may be transported to the synapse by apoE4 and binds to postsynaptic receptors, leads to an increase in calcium levels, activation of CaN, caspase-3 and downstream synaptic receptors internalization. Tau has also been implicated in synapse dysfunction induced by A β , and phosphorylated forms of tau (pTau) are transported "through" synaptic activity. However, which forms of pTau are transferred and how they are transported remains unknown.

Numerous publications have demonstrated that when administrated to extracellular cultured neuronal cells, tau oligomers promoted tau neuronal uptake and enhanced intracellular calcium levels (Gomez-Ramos A *et al.*, 2008). In mice, *in vivo* application of tau oligomers induces mitochondrial and synaptic dysfunction (Berger *et al.*, 2007; Reeves *et al.*, 2011).

Strikingly, synaptic activity induced in mouse cultured cortical neurons promoted tau phosphorylation, translocation of endogenous tau from the dendritic shaft to spines whereas the addition of A β oligomers induced mislocalization of tau into the spines at rest *i.e.* without synaptic activation, and blocked the activity-dependent enrichment of tau within spines (Frandemiche *et al.*, 2014).

The abnormal synaptic localization of tau and the disruption of the activity-dependent enrichment of tau within spines by A β oligomers could play a role in the progression of the

pathology (Hoover *et al.*, 2010; Roberson *et al.*, 2011; Tai *et al.*, 2012). Furthermore, synaptic activity and A β likely promote tau-phosphorylation on different sites (Frandsen *et al.*, 2014) leading to different forms of phosphorylated tau (toxic vs non-toxic) which may have different impact on synaptic transmission and plasticity.

1) Tau-induced presynaptic dysfunction

In the course of AD, hyperphosphorylation of tau leads to its dissociation from microtubules, followed by diminished cytoskeletal solidity and impaired axonal transport (Wagner *et al.*, 1996; Patrick *et al.*, 1999). Additionally, extracellular tau oligomers can be taken up by neurons, accumulated within axons and dysregulate axonal transport (Wu *et al.*, 2013; Swanson *et al.*, 2017). pTau binds to synaptic vesicles via its N-terminal domain and synaptogyrin-3 (a transmembrane vesicle protein) and interferes with presynaptic synaptic vesicles (SV) mobilization and release rate as well as down regulation of neurotransmission. (Zhou *et al.*, 2017; McInnes *et al.*, 2018). Accumulation of presynaptic pTau triggers a transient increase of spontaneous neurotransmitter release mediated by intracellular calcium release. This is followed by SV exocytosis block, leading to consistent synaptic transmission failure owing to the consequent toxicity activities (Moreno *et al.*, 2011, 2016).

In an inducible mouse model expressing pro-aggregant tau, a severe depletion of synaptic vesicles pool was observed together with an impaired LTD (Decker *et al.*, 2015) and the acute addition of recombinant human tau oligomers produced the impairment of LTP (Fà *et al.*, 2016).

2) Tau-induced postsynaptic dysfunction

The accumulation of tau into dendritic spines leads to the decreased expression of both NMDA and AMPA receptors, induced impaired excitatory synaptic transmission and memory deficits (Hoover *et al.*, 2010; Xia *et al.*, 2015; Kim *et al.*, 2016; Yin *et al.*, 2016; Zhao *et al.*, 2016). Lack of tau in tau^{-/-} mutant mice disrupts the postsynaptic Src kinase Fyn localization resulting in the reduction of NMDA receptor phosphorylation and *in fine* protection against A β -induced excitotoxicity (Ittner *et al.*, 2010; Roberson *et al.*, 2011). In contrast to the A β toxicity mediated by phosphorylated tau protein, at least in the early stage of the disease, site-specific phosphorylation of tau mediated by p38 MAPKinase inhibits A β toxicity (Ittner *et al.*, 2016).

c. Synaptic dysfunction at hippocampus

Following entorhinal cortex, hippocampus is one of the earliest affected brain region in AD (Arriagada *et al.*, 1992). AD pathological lesions and neuronal loss are observed in multiple regions including hippocampus as well as trans-entorhinal and entorhinal cortices (Hyman *et al.*, 1984, 1990; Geddes, 1985; Isla *et al.*, 1996; Kordower *et al.*, 2001). The hippocampus is part of the limbic system, and plays important roles in the consolidation of information from short-term memory to long-term memory, and in spatial memory that enables navigation in rodents. As such, it is generally acknowledged that memory deficits in AD involve synaptic dysfunction and synaptic plasticity impairments in the hippocampus (Selkoe, 2002; Morrison and Baxter, 2012).

The hippocampus (Figure 12) has a general shape of a seahorse and therefore was named after by the Venetian anatomist Julius Caesar Aranzi (1587). It comprises the *dentate gyrus* (DG) formed by a condensed layer of granule cells bodies making a v-shape and a ram's horn (*Cornu Ammonis*) comprising CA4 (in humans), CA3, CA2 and CA1. The CA fields present densely packed pyramidal neurons (Hyman, 1987; Cajal, 1901; Lorente, 1934). The main input to the hippocampus is the entorhinal cortex (EC) through perforant pathway (PP). This pathway generates mostly from layers II and III of EC. Cells from layer II project to DG and CA3, while layer III neurons project to CA1 and the subiculum. Axons from granule cells of DG which project to CA3 pyramidal neurons are named mossy fibers. CA3 pyramidal neurons mainly project to CA1 via Schaffer collaterals (Amaral and Witter, 1989). These two routes, EC → CA1 and EC → DG → CA3 → CA1, drive CA1 activity and CA1 pyramidal neurons projects back into deep layer of EC (Figure 12).

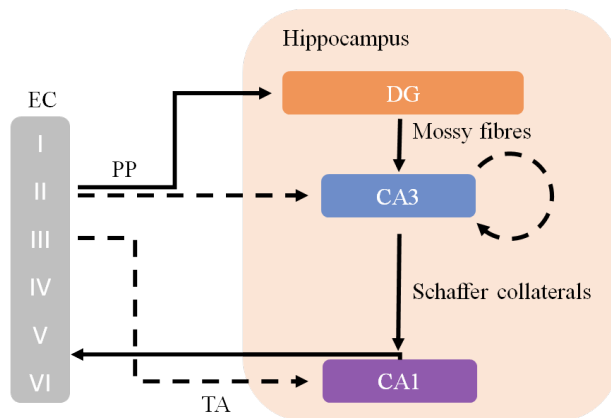
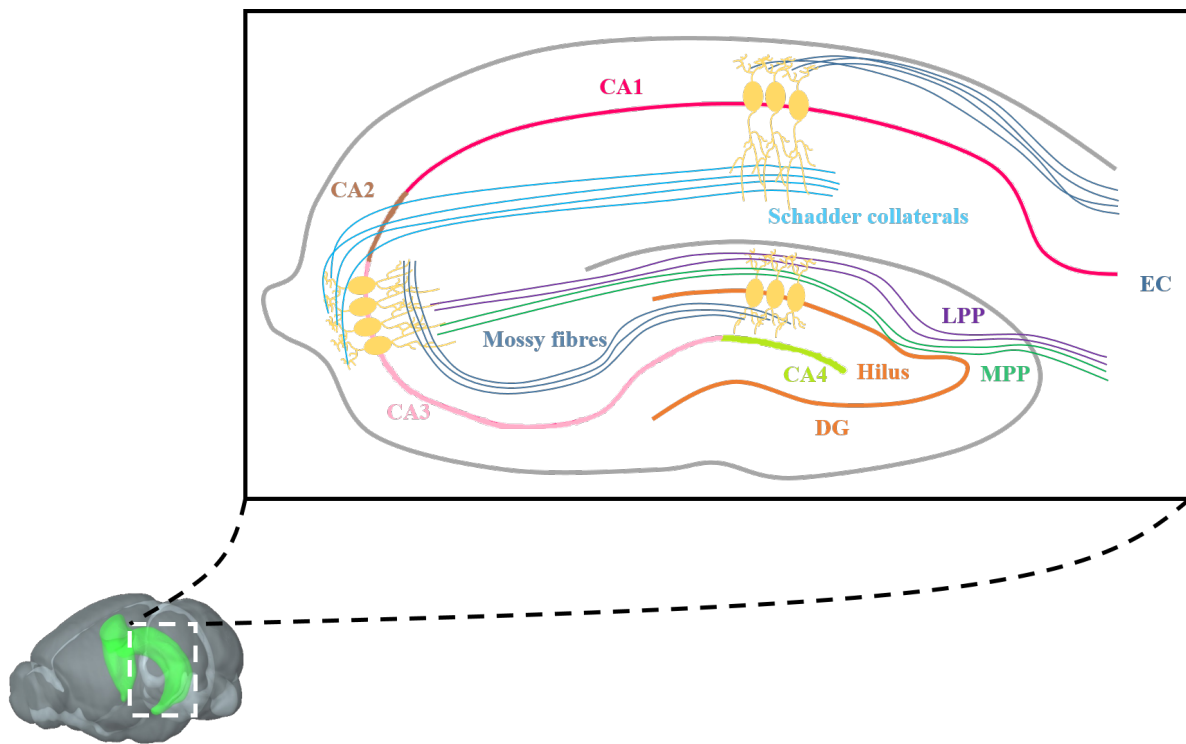


Figure 12. Synaptic circuits in hippocampus

The cartoon illustrates the hippocampal morphology and circuitry. The graph below shows the hippocampal connectivity. The classical excitatory pathway (EC–DG–CA3–CA1–EC) is illustrated by solid arrows. The layer II neurons in EC project to the DG via perforant pathway (PP), including the lateral perforant pathway (LPP) and medial perforant pathway (MPP). DG projects to CA3 pyramidal neurons through mossy fibres (Mf). CA3 pyramidal neurons pass on the information to CA1 pyramidal neurons via Schaffer collaterals. CA1 pyramidal neurons project back into deep layer of EC. CA3 pyramidal neurons receive also projections from EC layer II neurons through the PP. CA1 pyramidal neurons receive direct input from EC layer III via the temporoammonic pathway (TA). The DG cells also project to the mossy cells in the hilus and interneurons, which project to excitatory and inhibitory pathways, respectively, then back to the granule cells (adapted from Deng *et al.*, 2010)

1. Perforant path (PP)/Dentate Gyrus (DG) synapses

The major excitatory input to DG is the perforant path (PP) made of glutamatergic projections, which form functional connections during the 3rd week of development (Esposito *et al.*, 2005; Ge *et al.*, 2006; Zhao *et al.*, 2006; Toni *et al.*, 2007). In the 4th week, the strength of excitatory afferents enhances greatly whereas the membrane input resistance is still high, an intrinsic property of immature neurons (Mongiat *et al.*, 2009). According to this, immature granule cells (GCs) are much easier to fire AP than mature neurons (Ge *et al.*, 2007) and therefore intrinsic properties of GCs may highly affect the memory encoding procedure (Rolls and Kesner, 2006).

The perforant path consists of two subfields: the medial perforant path (MPP) and the lateral perforant path (LPP) (McNaughton, 1980). The MPP initiates from medial EC and projects to the middle third of the molecular cell layer, while the LPP initiates from the lateral EC and projects to the outmost third of the molecular cell layer. The excitatory input from both MPP and LPP onto GCs have different properties of short-term and long-term plasticity (McNaughton *et al.*, 1980; Bramham *et al.*, 1991; Colino *et al.*, 1993; Patten *et al.*, 2015). The MPP processes spatio-temporal informations involving NMDAR-dependent plasticity whereas the LPP processes novel object information through opiate receptor dependent plasticity (Hargreaves *et al.*, 2005; Hunsaker *et al.*, 2007, 2013) (Figure 13).

Both MPP and LPP take glutamate as main neurotransmitter (White *et al.*, 1977; Mathisen, 1981), but present different pharmacological and physiological features such as L-2-amino-4-phosphonobutyric acid (L-AP4) acting through the inhibition of voltage-dependent calcium channels (VDCCs) and/or potassium currents, leading to the suppression of glutamate release (Forsythe and Clements, 1990). L-AP4 contributes to a significant larger depressive effect on the excitatory postsynaptic potential (EPSP) evoked by LPP than by MPP stimulation (Koerner and Cotman, 1981), while baclofen has the opposite effect (Lanthom and Cotman, 1981). Carbachol acting through postsynaptic sites, more likely suppresses MPP EPSP (Kahle and Cotman, 1989), and antagonists of NMDA receptor (D- (-)-2-amino-5-phosphonovaleric acid, D-APV and 3-[(+)-2-carboxypiperazin-4-yl]-propyl-1-phosphonic acid, CPP) have been shown to decrease the late part of the MPP EPSP whereas with the LPP EPSP is unchanged (Dahl *et al.*, 1990). Short term synaptic plasticity events such as PPF and PTP also vary between LPP and MPP (McNaughton, 1980).

When it comes to AD, both pathological tau and amyloid collaboratively increases

cytotoxicity leading to PP degeneration. A β oligomer were demonstrated to be transported from cell bodies in the entorhinal cortex to the PP, and partly from the posterior parietal cortex (Morales *et al.*, 2012; Bero *et al.*, 2012).

Most interestingly, tau pathology seems confined initially to PP (Sierra *et al.*, 2000; Calignon *et al.*, 2012; Liu *et al.*, 2012; Polydoro *et al.*, 2014). These data strongly support the concept of a “spread” of the pathology following the perforant pathway. Furthermore, PP projection to DG is specifically vulnerable in terms of synaptic plasticity in AD murine models (Palop *et al.*, 2007).

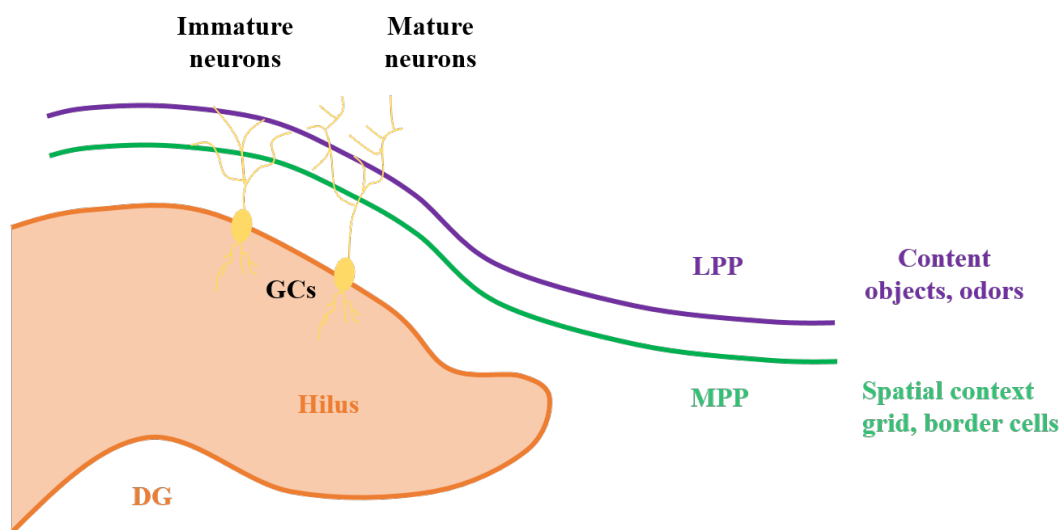


Figure13. Synaptic circuits of PP/DG in hippocampus

The schematic shows the arrangement of afferent inputs to the molecular layer of the DG. Inputs from lateral perforant path (LPP), medial perforant path (MPP) connect the outer, middle molecular layer, respectively. Hippocampal granule cells bodies (yellow) located at the dentate granule cell body layer (DG).

2. Mossy fibre (Mf) /CA3 synapses

Since the first classified of CA3 region in the hippocampus (Lorente, 1934), there has been more and more interest in the different CA3 neuron characteristics. Intrinsic functional connectivity in CA3 is a network of highly structured connection (Willshaw *et al.*, 1969; Marr *et al.*, 1971; Hopfield *et al.*, 1982; McNaughton *et al.*, 1987; Treves *et al.*, 1994; Bennett *et al.*, 1994; Lisman *et al.*, 1999; Rolls *et al.*, 2013). CA3 circuits display extensive excitatory

interconnections between CA3 pyramidal cells (PCs) through associational and commissural fibres (for review see Rebola *et al.*, 2017) but also connections to inhibitory neurons (Lorente, 1934). This region is as likely as involved in forming spatial representations and episodic memories of initial acquisition stages (Scoville and Milner, 1957; O'Keefe and Nadel, 1978; Kesner and Rolls, 2015).

CA3 region receives inputs from the entorhinal cortex either directly via the PP or from DG through mossy fibres (Mfs) (Amaral and Witter, 1989). Mf form giant boutons (5–8 μm in diameter) that have an average of 20 release sites *per* bouton. The Mf/CA3 pathway acts as a filter that transforms complex coded signals to specific hippocampal information, required for memory encoding. This procedure really depends on the firing pattern of DG granule cells (GCs) and leads to more positive stimulus performance in the CA3 region (Leutgeb *et al.*, 2007).

Pattern separation and pattern completion are often considered as associated with DG and CA3 region, respectively, with the DG granule cell and Mf setting the pathway to CA3 (Figure 14). However, CA3 pyramidal cells also send 'back' to DG (Scharfman *et al.*, 2007). The projection from CA3 to DG might have an effect on the circuits in the DG and CA3 (Penttonen *et al.*, 1997; Lisman *et al.*, 2005), and this pathway might contribute to the information process. A study in the computational model showed that this backprojection has an important role in the ability of the computational model to present pattern separation and completion (Myers *et al.*, 2011). In the hippocampus, only CA3 contains recurrent pathway: more than 95% of input to CA3 region is from CA3 recurrent connections. These associated fibers might promote the pattern completion (Guzowski *et al.*, 2004).

Importantly, Mf-CA3 synapses present a wide range of short term plasticity (frequency facilitation, paired-pulse facilitation, post-tetanic potentiation) but also presynaptic LTP and LTD that are depending on NMDAR activation (Nicoll and Schmitz, 2005; Rebola *et al.*, 2017). Furthermore, NMDARs contribute to LTP characterized by a selective enhancement of NMDA receptor-mediated transmission which requires coactivation of NMDA and mGlu5 receptors and a postsynaptic calcium rise at Mf-CA3 synapses (Kwon and Castillo, 2008).

Up to now, most of the studies in AD mouse models have been done at the archetypical CA3-CA1 synapse which is characterized by a canonical postsynaptic LTP. On the opposite, few studies have investigated Mf-CA3 synapses alterations in AD (Witton *et al.*, 2010; Marchetti and Marie, 2011; Wilke *et al.*, 2014; Silva *et al.*, 2016; Maingret *et al.*, 2017).

In APP/PS1 mice at 6 months of age, postsynaptic LTP of associative/commissural (A/C) inputs to CA3 pyramidal cells was totally impaired (Silva *et al.*, 2016) whereas the presynaptic LTP at the Mf-CA3 synapse was unaffected (Maingret *et al.*, 2017). Interestingly, APP/PS1 mice at 6 months of age displayed altered fear conditioning (Kilgore *et al.*, 2010).

A β -overproducing Tg2576 mouse display impairment of short term frequency facilitation and absence of NMDAR independent LTP at the Mf pathway (Witton *et al.*, 2010). On the contrary, tau oligomers lead to presynaptic and postsynaptic morphological changes (toxic function) at the Mf linked to the CA3 region in mutant human tau mice (Decker *et al.*, 2015).

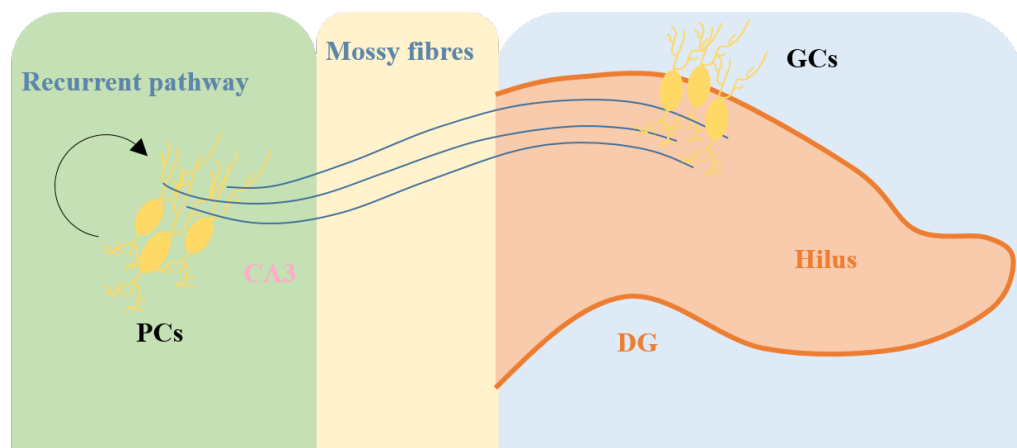


Figure 14. Synaptic circuit of Mf/CA3 in hippocampus

Mossy fibre (Mf) projection contains axons of the DG granule cells in the hippocampus.

3. Schaffer collaterals (SC)/CA1 synapses

CA3 are important for the fast combination different information and pattern completion, while CA1 may be helpful in identifying a new event or a new context (Lisman *et al.*, 2001; Kumaran *et al.*, 2007). In the hippocampus, CA1 receives projections from CA3 through Schaffer collaterals (SC) (Li *et al.*, 1994) (Figure 15). Different layers of entorhinal cortex (EC) transport vary kinds of information to the CA3 and CA1 regions (Sargolini *et al.*, 2006). This has lead to detailed physiological studies of these regions in the hippocampus.

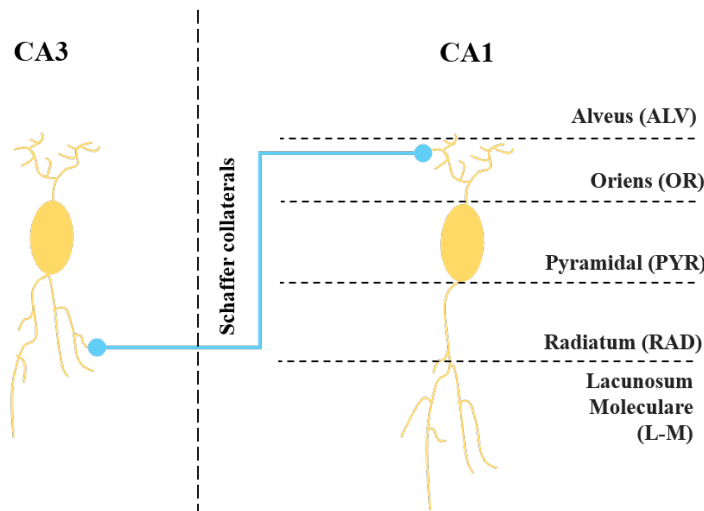


Figure 15. Synaptic circuit of CA3/CA1 in hippocampus

Sites of termination of axons of CA1 and CA3 pyramidal cells

Regional differences in firing patterns corresponding to different environmental factors have been found (Lee *et al.*, 2004; Leutgeb *et al.*, 2004, 2005; Vazdarjanova and Guzowski, 2004; Dragoi and Buzsaki, 2006; Lee and Knierim, 2007; Leutgeb and Leutgeb, 2007; Alvernhe *et al.*, 2008). When testing rat in a spatial environment, both CA1 and CA3 neurons are selectively firing when the animal moves to particular places in the environment. These ‘place cells’ are thought to set as parts of a cognitive map in the environment (O’Keefe *et al.*, 1978) or representations of places where significant events happen (Eichenbaum, 1999, 2004). Thus, investigations of age-related alterations in the firing patterns of place cells maybe informative of disruptions of memory procedure in aged hippocampus. Spatial information processed by place cells is changed in aged rats in several studies (Rosenzweig *et al.*, 2003). Particularly, many studies have indicated that CA3 and CA1 place cells of aged rats could not form new spatial or task information quickly (Tanila *et al.*, 1997; Oler *et al.*, 2000; Wilson *et al.*, 2003, 2004, 2005).

In the hippocampus, synaptic plasticity dysfunction of AD has been demonstrated by the application of A β peptides and strongly inhibits LTP induction at Schaffer collateral/CA1 synapses (Chen *et al.*, 2000). This result is also complementary to an early *in vivo* study showing impaired maintenance of CA1 - LTP in rats injected i.c.v. with A β ₁₋₄₀ or A β ₁₋₄₂ (Cullen *et al.*, 1997). Another study illustrates that basic synaptic transmission was disrupted at SC/CA1 synapse in htau mouse with an age dependent mode (Polydoro *et al.*, 2009). Only

aged htau mice displayed an alteration in PPR that is symptom of a decreased glutamate release probability. Aged htau mice display cognitive impairment in hippocampal related behavioral tasks, and changes in basic synaptic function. Additionally, HFS failed to induce LTP in aged htau mice. Together these results suggest that tau pathology may underlie age-dependent learning impairment via disruption of synaptic function.

II. Study objectives

Alzheimer's disease (AD) is a progressive neurodegenerative disease characterized by early cognitive deficits linked to synaptic dysfunction (Selkoe, 2002; Jacobsen et al., 2006; Sheng *et al.*, 2012). Numerous evidences show that neuroinflammation plays a key role in the pathogenesis of AD (Haruhiko *et al.*, 2000; Rojo *et al.*, 2008) and most importantly, neuroinflammatory molecules like prostanoids and cytokines are well known to modulate synaptic transmission and plasticity (Sang *et al.*, 2006; Yang *et al.*, 2008; Riazi *et al.*, 2015). Multiple epidemiological studies have revealed that chronic intake of nonsteroidal anti-inflammatory drugs (NSAIDs) reduced considerably the prevalence of AD (McGeer and McGeer, 2007) and clinical trials have shown that when given to asymptomatic patients, NSAIDs reduced AD incidence (Breitner *et al.*, 2011). Among the proinflammatory molecules generated during AD, prostaglandin E₂ (PGE₂) plays a pivotal role (Yang *et al.*, 2008). PGE₂ is produced from arachidonic acid by two rate limiting enzymes, Cox-1 and Cox-2 and binds with four main subtypes of receptors, that is EP1, EP2, EP3, and EP4 (Breyer *et al.*, 2001). In a previous study we demonstrated that the presynaptic long-term potentiation (LTP) at hippocampal Mf-CA3 synapse was impaired in APP/PS1 males due to the engagement of a PGE₂-EP3 signaling pathway (Maingret *et al.*, 2017). Mf-CA3 synapse is characterized by prominent presynaptic short term plasticity such as paired-pulse facilitation (PPF), frequency facilitation (FF), and presynaptic long-term plasticity (LTP). LTP has been described to be involved in the novel memory fast encoding (Kesner *et al.*, 2007), a procedure particularly affected in AD.

Among different major risk factors for developing the disease revealed by epidemiological and genetic studies (for review see Hickman *et al.*, 2016), surprisingly sex, which is one of the major risk, has received relatively little attention so far. However, there is a prominent sexual dimorphism in the human pathology, with a much higher prevalence (two to three times) of AD-related cognitive decline and greater AD neuropathology (Buckwalter *et al.*, 1993; Letenneur *et al.*, 1994; Henderson and Buckwalter, 1994; Brayne *et al.*, 1995;

Fratiglioni *et al.*, 1997; Andersen *et al.*, 1999; Ruitenbergh *et al.*, 2001; Corder *et al.*, 2004; Barnes *et al.*, 2005; Sinforiani *et al.*, 2010) in women compared to men. Remarkably, this sexual dimorphism is also found in mouse models of AD with females displaying precocious and stronger memory deficits (King *et al.*, 1999; Clinton *et al.*, 2007; Pistell *et al.*, 2008; Carroll *et al.*, 2010) as well as higher levels of A β accumulation in the brain (APP23: Sturchler-Pierrat and Staufenbiel, 2000; 3xTg-AD: Carroll *et al.*, 2010), than males. In APP/PS1 mice, A β deposition could be detected at 6 months, leading to a massive load of A β plaques in the hippocampus and cortex by 9 months (Jankowsky *et al.*, 2004) further increased at 12 months (Garcia-Alloza *et al.*, 2006). Higher levels of insoluble A β_{42} and A β plaque load are observed in females compared to age-matched males (Wang *et al.*, 2003; Taniuchi *et al.*, 2007; Gallagher *et al.*, 2013) but also earlier deficits in several hippocampal-dependent spatial memory tasks (Taniuchi *et al.*, 2007; Gallagher *et al.*, 2013; Richetin *et al.*, 2017). Amyloid- β peptides (A β) are one of the most important hallmark in AD pathogenesis (Robin Roychaudhuri *et al.*, 2009). Neuroinflammation in AD is reported to be associated with A β , likely leading to microglia and astrocytes activation (Frezza *et al.*, 2013). Strikingly *in vitro* and *in vivo* studies have reported that neuronal electrical activity was enhanced in the vicinity of A β plaques (Busche *et al.*, 2012; Busche & Konnerth, 2016). Female transgenic mice displayed both earlier memory deficits and more severe A β plaques deposition compared to age-matched males (Richetin *et al.*, 2017; Yang *et al.*, 2018). Therefore, we first investigated presynaptic LTP at Mf-CA3 synapses in APP/PS1 female mice at 4 and 6 months, an age for which behavioural impairments are already present. Unexpectedly, we did not observe any difference between WT and APP/PS1 female mice. Then we chose to study synaptic transmission and plasticity in a region which is highly enriched with A β plaques, the *stratum moleculare* in the vicinity of the *dentate gyrus*. This region receives axonal projections from entorhinal cortex via the perforant pathway (PP) which convey spatial informations (Deng *et al.*, 2010) and make synapses onto DG granule cells. PP projections carry to DG granule cells cortical inputs responsible for pattern separation and spatial information and memory encoding (Hyman *et al.*, 1986; Bennet *et al.*, 2016). The PP is divided into two parts: the lateral perforant pathway (LPP) and the medial perforant pathway (MPP). MPP and LPP display different properties in term of synaptic plasticity (Petersen *et al.*, 2013) allowing to distinguish between LPP and MPP. In this study, we investigated whether abundant A β deposition in female APP/PS1 mice shows alterations of behaviours, neuron intrinsic properties and basal transmissions, as well as LTP at LPP/DG area.

III. Results

Plasticity of synaptic transmission and intrinsic excitability in the *dentate gyrus* is altered in a mouse model of Alzheimer's disease

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Summary

Alzheimer's disease (AD) is a progressive and devastating neurodegenerative disease characterized by molecular and cellular alterations accompanied by cognitive disorders and likely related to deficits in synaptic transmission and plasticity in the hippocampus. Strikingly, AD displays a sexual dimorphism with a higher prevalence and stronger AD neuropathology and related cognitive decline in women. This dimorphism has been poorly investigated in transgenic mouse models of the disease. We report here that females APP/PS1, a double transgenic mouse model of AD, displayed much earlier A β plaques load than males in the *stratum moleculare* of the *dentate gyrus* in parallel with prominent neuroinflammatory activation of astrocytes and microglia. These cellular alterations were accompanied by strong deficits in spatial memory in APP/PS1 females when males were spared until 12 months of age. Interestingly, both non-synaptic and synaptic alterations at the PP-DG circuit were observed in APP/PS1 female mice at 6 months of age. All together our data bring new insights in gender-specific molecular, cellular and functional alterations in a mouse model of AD.

Keywords

sexual dimorphism, hippocampus, dentate gyrus, glial cells, neuroinflammation, Alzheimer's disease, synaptic plasticity, perforant pathway

Highlights

- * Neuroinflammation develops faster and stronger in APP/PS1 females than males
- * Hippocampal-dependent memory is impaired early on in APP/PS1 females but not in males
- * Basal synaptic transmission at the lateral perforant path - DG synapse is altered in APP/PS1 female mice
- * Firing activity of type 1 DG granule cells is increased in APP/PS1 mice
- * Firing properties of DG granule cells are differentially affected by synaptic potentiation in WT *vs* APP/PS1 mice

Introduction

Alzheimer's disease (AD) is a progressive and devastating neurodegenerative disease which is responsible for more than 70 % of dementia in the elderly population worldwide (in "2018 Alzheimer's disease fact and figures", 2018). AD is characterized by histopathological hallmarks, *i.e.* the extracellular deposit of A β plaques mostly in the cortex and the hippocampus and the intraneuronal accumulation of fibrillary tangles of hyperphosphorylated Tau (Querfurth and LaFerla, 2010). These molecular events are associated with profound structural changes in the brain such as the shrinkage of the cortex, the enlargement of ventricles and the severe atrophy of the hippocampus.

Despite tremendous efforts both targeted at basic and clinical studies over the last three decades, there is still neither a treatment or a cure for AD nor efficient treatments to alleviate the symptoms. Nevertheless, at least in its early stage, AD is thought to affect synaptic circuits through alterations of synaptic transmission and plasticity, especially in the entorhinal cortex-hippocampal circuitry (for review see Selkoe, 2002; Spire-Jones and Hyman, 2014).

Impairment of synaptic transmission and plasticity has been extensively studied over the last 20 years using transgenic mouse model of AD (for review see Marchetti and Marie, 2011). These studies have revealed that soluble A β and numerous inflammatory molecules produced by the activation of glial cells contributed to the pathophysiology of the disease and its progression (Akiyama *et al.*, 2000; Heneka and O'Banion, 2007; Heneka *et al.*, 2015).

Epidemiological and genetic studies point to major risk factors for developing the disease (age, susceptibility genes, diabetes of type 2, obesity) (for review see Hickman *et al.*, 2016) but surprisingly sex, which is one of the major risk, has received relatively little attention so far. In amyloidogenic mouse models of AD, female mice display precocious and stronger memory deficits (Carroll *et al.*, 2010; Clinton *et al.*, 2007; King *et al.*, 1999; Pistell *et al.*, 2008) as well as higher levels of A β accumulation in the brain (APP23: Sturchler-Pierrat and Staufenbiel, 2000; 3xTg-AD: Carroll *et al.*, 2010), than males. In APP/PS1 female mice, higher levels of insoluble A β ₄₂ and A β plaque load are observed compared to age-matched males (Wang *et al.*, 2003; Taniuchi *et al.*, 2007; Gallagher *et al.*, 2013). Paralleling these events, age-related deficits in several hippocampal-dependent spatial memory tasks have been reported in APP/PS1 male mice (Jankowsky *et al.*, 2004; Puoliväli *et al.*, 2002; Savonenko *et al.*, 2005) but occurring earlier in females (8-9 months), an age for which males are spared (Gallagher *et al.*, 2013; Taniuchi *et al.*, 2007; Richetin *et al.*, 2017).

This sexual dimorphism is of high interest as it is also found in the human pathology, with a much higher prevalence (two to three times) of AD-related cognitive decline and greater AD neuropathology (Buckwalter *et al.*, 1993; Letenneur *et al.*, 1994; Henderson and Buckwalter, 1994; Brayne *et al.*, 1995; Fratiglioni *et al.*, 1997; Andersen *et al.*, 1999; Ruitenberg *et al.*, 2001; Corder *et al.*, 2004; Barnes *et al.*, 2005; Sinforiani *et al.*, 2010) in women compared to men.

In the present work we have compared the hippocampal progression of A β plaque deposition in male and female APP/PS1 mice at 3, 6 and 9 months of age in relation to the neuroinflammatory status. We focussed on the *dentate gyrus* (DG), a key region of the excitatory trisynaptic hippocampal pathway which receives axonal projections from the entorhinal cortex and conveys non-spatial as well as spatial informations (Deng *et al.*, 2010). We then studied hippocampus-dependent spatial memory using two different one-trial behavioral paradigms, *i.e.*, contextual fear conditioning (CFC) and novel object place recognition task (NORT) in both gender at different age. We then studied if and how the intrinsic properties of DG granule cells and the properties of synaptic transmission and plasticity at the perforant pathway - DG circuit were altered in APP/PS1 female mice at 6 months of age.

The main findings of our study are plural: APP/PS1 females display earlier A β plaques load than males in the *stratum moleculare* of the DG that are paralleled by neuroinflammatory

activation of glial cells. These hippocampal molecular and cellular alterations are accompanied by strong memory impairments in female but not male mice even in old animals (12 months of age). We show that APP/PS1 female mice at 6 months of age displayed alterations in intrinsic excitability in DG cells, as well as synaptic alterations at the PP-DG circuit.

Material and Methods

Ethical approval

Animal anesthesia and euthanasia were carried out in accordance with the Animal Protection of ethical standards and the French legislation concerning animal experimentation and were approved by the University of Bordeaux / CNRS Animal care and Use Committee (authorization #2573). All efforts to improve the animal welfare and to minimize animals suffering were made.

Animals

The animals used in this study were males and females APP^{swe695}/PS1 Δ E9 termed APP/PS1 obtained from Jackson Laboratory (Bar Harbor, ME, USA) and their wild-type (WT) littermates (C57BL/6J). Briefly, the APP/PS1 mice express a chimeric mouse/human amyloid precursor protein APP^{swe} (mouse APP695 harboring a human A β domain and mutations K595N and M596L linked to a Swedish familial AD) and a human presenilin 1 mutated in familial AD (PS1 Δ E9; deletion of exon 9). These bigenic mice were created by co-injection of both transgenes allowing for a co-segregation of the transgenes as a single locus (Jankowsky et al., 2004). Mice were generated in our animal facility from double-transgenic APP/PS1 males mated with C57BL/6J females. Transgenic mice (APP/PS1) and age-matched non-transgenic littermates (WT) were allowed free access to food and water and maintained in a 12 hours' dark-light cycle. Mice were genotyped and systematically regenotyped after each experiments (Transcriptomics Platform, Neurocentre Magendie, Bordeaux, France). For all experiments, females have been used without consideration to their estrous cycle.

Acute hippocampal slices

In accordance with Animal Protection Association of ethical standards and the French legislation, APP/PS1 and WT mice at 6 months were deeply anaesthetized with a mix of

ketamine (80 mg/kg; i.p) and xylazine (16 mg/kg; i.p) and then intracardially perfused for 2-3 minutes with a protective solution at 4°C containing the following (in mM): 2.5 KCl, 1.25 NaH₂PO₄, 0.5 CaCl₂, 7 MgCl₂, 20 glucose, 26 NaHCO₃, 76 sucrose, 3 pyruvic acid, 5 Na ascorbate, equilibrated with 95% O₂ and 5% CO₂ (300-310 mOsm). When the solution coming out of the heart was free of blood, the mouse was sacrificed by decapitation and the brain was quickly removed from the skull and stored in the same solution. The isolated brain was glued onto the stage of a vibratome (VT 1200S, Leica Microsystems, Nussloch, Germany) and parasagittal hippocampal slices (320 μm) were cut in the protective solution for which sodium chloride was replaced by sucrose, and then incubated at 33°C for 10-20 min in a resting solution containing the following (in mM): 125 NaCl, 2.5 KCl, 1.25 NaH₂PO₄, 2 CaCl₂, 1 MgCl₂, 20 glucose, 26 NaHCO₃, 5 Na ascorbate equilibrated with 95% O₂ and 5% CO₂ (300-310 mOsm). Slices were thereafter maintained at room temperature in the same solution until required. Unless stated otherwise, all experiments have been done on 6 months old female mice.

Electrophysiological studies on acute hippocampal slices

For recording, slices were transferred into a recording chamber where they were submerged and continuously perfused with an oxygenated (95% O₂ and 5% CO₂) extracellular solution at 32-34°C. Whole-cell patch clamp recordings (3-4 MΩ electrodes, -70 mV holding potential) were made from *dentate gyrus* (DG) granule cells visualized by infrared video-microscopy. For field recordings, electrodes of about 2 MΩ were used.

Patch clamp electrodes were pulled out from borosilicate glass (GF 150 F-10) and filled with an internal solution containing the following (in mM): 140 CsCH₃SO₃, 2 MgCl₂, 4 NaCl, 5 phospho-creatine, 2 Na₂ATP, 0.2 EGTA, 10 HEPES, and 0.33 GTP adjusted with CsOH (300 mOsm, pH 7.3). In some experiments a K⁺ based internal solution containing (in mM) was used: 115 KC₆H₁₁O₇, 10 KCl₂, 0.2 EGTA, 0.02, CaCl₂ 10 HEPES, 15 phospho-creatine, 4 MgATP and 0.3 NaGTP adjusted with KOH (300 mOsm, pH 7.3). Bicuculline (10 μM) was added to the bath to inhibit γ-Aminobutyric acid-A (GABA-A) receptors. Voltage-clamp recordings were performed on DG granule cells identified with a differential interference contrast microscope (Eclipse FN-1, Nikon, Champigny sur Marne, France) equipped with an infrared camera (VX 44, Till Photonics, Gräfelfing, Germany) using an Axopatch-200B amplifier (Axon Instruments, Sunnyvale, CA, USA). Signals were filtered at 2 kHz and digitized at 5 kHz via a DigiData 1322A interface (Axon instruments). Series resistance (10-

20 M Ω) was monitored during the recording using a -10 mV hyperpolarizing voltage step of 50 ms length occurring at the beginning of each recording.

To monitor the access resistance during the recording time, a hyperpolarizing voltage step (-5 mV, 10 ms) was applied at the beginning of each sweep and DG granule cells were rejected if more than a 20% change occurred during the experiment. DG granule cells with a holding current exceeding 250 pA at a holding potential of -70 mV were also rejected. Data were collected and analysed using pClamp software 9.2 (Axon Instruments). For extracellular recordings, because of the possibility of crosstalk between mossy cell fibers (MCFs) and the more proximal medial perforant path (MPP) we focused our study on lateral perforant path (LPP) transmission. To stimulate selectively the LPP, a stimulating glass electrode (1 - 2 μ m tip diameter) filled with extracellular recording solution was placed in the outer molecular layer of the DG (>100 μ m away from the granule cell layer) as close to the hippocampal fissure as possible. In addition to the location of the stimulating electrode, we characterized the LPP on its ability to exhibit paired-pulse facilitation (Fig. 4C) while the MPP does not (Fig. 4C). Signals were recorded using an Axopatch-200B amplifier (Axon Instruments, Sunnyvale, CA, USA), filtered at 20 kHz and digitally sampled at 50 kHz. All experiments were performed in the presence of bicuculline (10 μ M) to block GABA-A receptors. Input-output curves were collected by varying stimulation strength from 0 to 200 μ A in 50 μ A intervals. For subsequent experiments on basal transmission and synaptic plasticities, the stimulus strength was set to a level that produced about 30% of the maximal responses. The LTP-induction protocol consisted of 3 bursts of 100 stimuli at 100 Hz with a 10 s interburst interval (HFS).

E-S curve, which relates EPSP slopes to the associated action potential probability, was generated by measuring slopes during the first 2 ms (reflecting mainly the fast component of AMPA-receptor synaptic transmission) and sorted in 0.5mV/ms bins to the associated action potential probability. The AP firing probability was calculated for each bin and the EC₅₀ value (the value of the EPSP-slope that elicits an AP with 50 % of probability) was calculated in each cell.

EPSP amplitude/slope ratio histograms before and after HFS were generated as follows: at the start of the experiment, for each cell the amplitude of sub-threshold EPSPs (*i.e.* not triggering APs) was measured in current-clamp mode (the membrane potential was set to around -70 mV), and plotted vs the EPSP slope. Then a linear regression was fitted yielding a slope value.

The cell was rejected if the goodness of fit, $S_{y,x}$, which quantifies the scatter of the data points around the best fit line was < 1.5 . After this, the cell was switched to voltage-clamp mode and held at -70 mV. A baseline was recorded for 5 min and then the HFS protocol (3 bursts of 100 stimuli at 100 Hz with a 10 s interburst interval) was applied followed by a period of 20 min. Then the cell was returned back to current-clamp mode (set around -70 mV) and a new EPSP amplitude/slope ratio histogram was generated.

Immunohistological staining and analysis

WT and APP/PS1 male and female mice at 3, 6 and 9 months of age were anesthetized with intraperitoneal administration of pentobarbital (50 mg/kg body weight) and were fixed by transcardial perfusion with buffered saline and 50 ml of 4% paraformaldehyde (PFA). Brains were removed, postfixed in 4% PFA overnight and cut into 40 μ m-thick frontal sections on a vibratome. Sections were collected into PBS 0.1M and stored at $+4^{\circ}\text{C}$ until the staining protocol. After a thorough wash in TBST (Tris Buffer Sodium: 0.0384 M Trisma Base - 0.1263 M NaCl pH: 7.4 + 0.1 % Tween 20), free-floating sections were incubated with TBST - 10% Normal Goat Serum - 0.3% Triton X-100, for 1 hour at room temperature. Then sections were incubated with primary antibody in TBST - 3.5% Normal Donkey Serum overnight at 4°C (chicken anti - glial fibrillary acidic protein (GFAP): 1/1000 USBiologicals; rabbit anti - ionized calcium-binding adapter molecule Iba-1: 1/5000 Wako. A β plaques were stained either using Methoxy-X04 (a derivative of Congo red, 50 μ M, Tocris) or mouse anti-A β_{1-16} : 1/150 (Millipore).

After washes in TBST, sections were incubated with secondary antibodies for 1 hour (Goat anti-chicken Alexa 647, anti-mouse Alexa 555 and anti-rabbit Alexa 488: 1/500, Invitrogen). Slices were finally washed and mounted in mounting medium (Fluoromount + DAPI, SouthernBiotech). Slices were imaged using the up-right widefield microscope Leica DM5000 (Leica Microsystems, Nanterre, France) using objectives HC PL Fluotar 20X NA 0.5. Fluorescence excitation was performed by a LED SOLA Light (Lumencor, Beaverton, USA). Images were obtained by the resolute cameras CoolSnap HQ2 (Photometrics, Tucson, USA) and a cooled QICAM (QImaging, Surrey, Canada). A galvanometric stage (Leica Microsystems) allowed the z stack reconstructions. The mosaics were made using a motorized stage Scan (Märzhäuser, Wetzlar, Germany). This system was controlled by MetaMorph software (Molecular Devices, Sunnyvale, USA). Analyses were performed with Image J software (National Institutes of Health, New York, USA) on the maximum intensity

fluorescence projections of the 3D mosaics.

Behavioral studies: Contextual fear conditioning (CFC)

Mice were housed individually. Prior to the test, mice were handled 2 minutes per day during 5 days. At day 0, single-trial contextual fear conditioning was performed by placing mice into a conditioning chamber (context A) where they were allowed to explore for 150 seconds after which they received a mild electric footshock (2s, 0.7 mA) and were removed 28 seconds after the shock. The conditioning chamber was housed in a sound attenuated box with the four interior walls wearing a checkerboard pattern. The conditioning cage (17 cm wide, 17 cm deep and 25 cm high) was made of clear plexiglas and the floor consisted of a shock grid. Between each animal the cage was cleaned with 70% ethanol solution. At day 1, mice were reexposed to the conditioning context A then to a new context (context B) during 180 seconds for each. For the neutral context B, the chamber was housed in an identical box but cues made of round and stars pattern on a white background were placed around a rounded instead of a squared plexiglas cage. The floor grid was covered by plexiglas and bedding. The light was dimmed compared to context A and the cage was cleaned with 1% acetic acid between animals. Contextual fear memory retention was studied by measuring the freezing response in context A and context B. Automatic freezing was detected with the help of a software (Any-Maze, Ugo Basile, Gemonio, Italy).

Behavioral studies: Object Location Task (OLT)

Prior to the test, mice were handled 2 minutes per day during 5 days and habituated to the openfield (45 cm wide, 45 cm deep and 40 cm high) the day before the experiment during 20 minutes, in the absence of objects. The total covered distance was measured by video-tracking (Noldus-Ethovision, Nantes, France). The day of testing, mice were first placed for 10 minutes (habituation phase) in the arena where two identical plastic bricks were located 15 cm away from the walls in adjacent quadrants (A and C or B and D). Brick exploration was defined as exploration with the nose directing toward it within 2 cm. Absence of preference with one of the two bricks was checked during the habituation phase. After a delay of 30 minutes, mice were reexposed to the arena in which one of the two bricks was moved (for exemple from quadrant B to quadrant A or from quadrant D to quadrant C) in order to create a new spatial combination and mice were allowed to explore for again 10 minutes. The time spent exploring the bricks in the novel and familiar locations was measured during the first 5 minutes as mice have tendency to loose novelty detection behavior after few minutes (Cès *et*

al., 2018). The discrimination ratio was determined by the percentage of the time spent exploring the displaced brick over the time spent exploring both bricks.

Enzyme immunoassay determination of PGE₂

The concentration of PGE₂ was quantified both in WT and APP/PS1 at 3, 6 and 9 months of age. After anesthesia induction by intraperitoneal administration of pentobarbital (50 mg/kg body weight), the hippocampus was rapidly dissected and immediately frozen into liquid nitrogen. The concentration of PGE₂ present in the hippocampus was quantified with a specific PGE₂ competitive EIA kit (ACETM EIA kit, Cayman Chemical, Ann Arbor, MI, USA) following the procedures detailed in the instructions. The detection limit of this EIA kit was 15 pg/mL (80% B/Bo). Absorbance was measured at 415 nm by a microplate reader model 680 (Bio-Rad, Marnes-la-Coquette, France).

Statistical analysis

In order to minimize biases as much as possible, experimenters were kept blind to the genotype of the mice until analysis was completed. Values are presented as mean ± SEM of *n* experiments. Statistical analyses were performed with Prism 7.0 (GraphPad Software, La Jolla, CA, USA). Unless stated otherwise, a Student's *t*-test for 2 groups and a one-way ANOVA with Bonferonni's test for more than two groups were used for statistical comparison. Data distributions were analysed using the Kolmogorov-Smirnov test; for miniature events the distribution curve was calculated for each cell and all cells averaged per condition to create a single curve. The *p*-values given are two-tailed and were considered significant if *p*<0.05.

Drugs

Bicuculline, D-2-amino-5-phosphonopentanoate (D-AP5), LCCG-1, CGP55845 and Methoxy-X04 were obtained from Tocris. TTX, PGE₂, D-serine and PF-04418948 were obtained from Sigma-Aldrich. NBQX was obtained from HelloBio. ONO compounds were a generous gift from ONO Pharmaceuticals Co Ltd (Japan).

Results

A β plaques and neuroinflammation develops faster in APP/PS1 females than males

Males and females WT and APP/PS1 mice at 3, 6 and 9 months of age were compared in terms of number of A β plaques and glial activation (Fig. 1). While there are only very few and small A β plaques in APP/PS1 male at 9 months of age, there is a heavy load of A β plaques in APP/PS1 female at the same age (Fig. 1A). Strikingly, A β plaques are not uniformly distributed throughout the hippocampus but are clearly concentrated in the *stratum moleculare* (delineated by the white dotted-line) of the DG. At a higher magnification (lower panel), A β plaques appear either associated with a dense core commonly referred as "compact" dense-core plaques or diffuse without a dense core known as "cotton wool plaque" (Le *et al.*, 2001). Compact plaques are formed by a dense amyloid core (anti- A β_{1-16} , white signal) surrounded by a spider web of microglia (Iba-1, green signal) walled off by activated astrocytes (GFAP, red signal). Cotton wool plaques are larger structures devoid of a dense amyloid core but nevertheless surrounded by activated microglia and astrocytes. There is a progressive increase in the number of A β plaques in APP/PS1 males and females (Fig. 1B) starting at 3 months of age with significantly more A β plaques in females (3 months: male: 0.04 ± 0.03 , n=44; female: 1.89 ± 0.66 , n=29, ** $p < 0.01$; 6 months: male: 4.87 ± 0.79 , n=47; female: 8.48 ± 0.77 , n=56, ** $p < 0.01$; 9 months: male: 10.14 ± 1.42 , n=37; female: 21.53 ± 2.91 , n=29, *** $p < 0.001$).

To further evaluate the neuroinflammatory status at different age, gender and genotype, double immunostaining of microglia (Iba-1) and astrocytes (GFAP) were performed. As shown in Fig. 1C, in WT females at 6 months of age, astrocytes displayed elongated bodies with long and thin processes whereas in APP/PS1 females at the same age, astrocytes displayed rounded cell bodies with shortened and enlarged processes, a morphological hallmark of activated astrocytes (Wilhelmsson *et al.*, 2006; for review see Schiweck *et al.*, 2018). Microglia also displayed a typical phenotype of reactive cells (Nimmerjahn *et al.*, 2005) shifting from a quiescent morphology to an activated status with enlargement of cell bodies and shorter and thicker processes (Fig. 1D).

These data provide clear evidence that deposits of A β plaques and glial activation occur faster and stronger in APP/PS1 females vs males and are in good agreement with previous studies reporting higher level of A β plaques load (Taniuchi *et al.*, 2007; Gallagher *et al.*, 2013;

Sierksma *et al.*, 2013) and abundant hypertrophic astrocytes in the DG (Richetin *et al.*, 2017) in APP/PS1 females compared to age-matched males.

Hippocampal-dependent memory is impaired in APP/PS1 females but not in males

Hippocampal-dependent memory was tested in APP/PS1 males and females of 3 to 12 months of age and their age-matched WT littermates using two different one-trial behavioral paradigms: contextual fear conditioning (CFC) and object location task (OLT).

Contextual fear conditioning is a hippocampal-dependent task which results in a robust long lasting memory for a rodent (Phillips and Ledoux, 1992). Our protocol (Fig. 2A) was designed to yield approximately 40-50% freezing in the retrieval test (Fig. 2B). In context A, APP/PS1 males at 4 months of age showed levels of freezing nearly identical to age-matched WT littermates indicating no memory deficits (WT_{context A}: 36.78±10.6 %, n=8; APP/PS1_{context A}: 37.76±8.34%, n=8, *p*=0.942) (Fig. 2B). In contrast, a pronounced memory deficit was observed in APP/PS1 females at the same age (WT_{context A}: 37.43±7.02 %, n=14; APP/PS1_{context A}: 15.2±4.79%, n=9, * *p*<0.05) (Fig. 2B). When placed in a neutral context B, WT and APP/PS1 males displayed very similar low levels of freezing (WT_{context B}: 11.56±3.18 %, n=8; APP/PS1_{context B}: 13.93±3.51%, n=8, *p*=0.624) and WT females significantly froze longer than APP/PS1 (female WT_{context B}: 11.57±3.06 %, n=14; female APP/PS1_{context B}: 3.19±0.97%, n=9, * *p*<0.05).

At 6 months of age, APP/PS1 males still displayed no memory deficit (WT_{context A}: 46.59±4.94%, n=12; APP/PS1_{context A}: 37.99±6.36%, n=10, *p*=0.291) whereas APP/PS1 females showed memory deficit as expected (WT_{context A}: 50.23±5.09%, n=15; APP/PS1_{context A}: 30.77±6.78%, n=10, **p*<0.05) (Fig. 2C). The lack of behavioral deficit in this task was even observed in 12 months old male mice (WT_{context A}: 49.48±7.3 %, n=7; APP/PS1_{context A}: 50.47±7.47%, n=11, *p*=0.929) (Fig. 2D).

Importantly, mice for both genotype and gender at any age displayed low levels of freezing (data not shown) during the 150 s period before the footshock at the time of training (females 4 months: WT:0.97±0.24%, n=14; APP/PS1:0.97±0.48%, n=9, *p*=0.997; males 4 months: WT:7.22±3.59%, n=9; APP/PS1:5.25±1.32, n=8, *p*=0.678; females 6 months: WT:5.62±1.44%, n=15; APP/PS1:3.01±0.84%, n=10, *p*=0.186; males 6 months: WT:1.70±0.46 %, n=12; APP/PS1:0.48±0.21%, n=10, **p*<0.05; males 12 months: WT:4.16±1.92%, n=7; APP/PS1:5.73±1.14%, n=11, *p*=0.462). Such amounts of exploratory

activity were good evidence for a lack of significant anxiety-related behavior in our experimental conditions.

Next, APP/PS1 males and females of 3, 4 and 6 months of age and their age-matched WT littermates were subjected to OLT (Fig. 3A). Following the 30 minutes' retention period, WT female mice spent more time exploring the novel location of the object than the familiar one at all age, so that the discrimination ratio was significantly different from 50% (3 months: $65.41 \pm 3.99\%$, $n=14$, $**p < 0.01$; 4 months: $66.34 \pm 3.76\%$, $n=16$, $***p < 0.001$; 6 months: $66.25 \pm 2.9\%$, $n=15$, $**p < 0.01$; Fig 3B).

In contrast and as early as 3 months of age, APP/PS1 female did not explore the novel location of the object significantly more than the fixed one (3 months: 44.17 ± 4.66 , $n=15$, $p=0.232$; 4 months: $51.6 \pm 4.91\%$, $n=10$, $p=0.752$; 6 months: $47.76 \pm 2.32\%$, $n=13$, $p=0.354$; Fig 3B) indicating therefore that they were impaired in this task at a fairly young age. Our data provide clear evidence that whatever the age, APP/PS1 mice never explored more than WT mice the novel location of the object.

All together, these data clearly show that memory deficits displayed by APP/PS1 mice and tested by CFC and NORT appear much earlier in females than males. These memory deficits parallel the prominent increase in neuroinflammatory markers, *i.e.* A β plaques load, astrocytic and microglial activation ahead of few months in females compared to age-matched males.

Basal synaptic transmission at the lateral perforant path - DG synapse is altered in APP/PS1 female mice

Because the A β plaques appeared earlier and were more abundant in the *stratum moleculare* of the DG in female mice, we set out to characterize the basic properties of the lateral perforant path (LPP) input which make synaptic contact to granule cells of the DG in this layer. The DG receives information from layer 2 of the entorhinal cortex via a bundle of axons, the perforant path which is subdivided into the LPP that is assumed to convey novel contextual information whereas spatial information appears to be conveyed by fibres of the medial perforant path (MPP) (Hunsaker *et al.*, 2007).

The stimulating electrode was placed in the outer one-third molecular layer of the DG suprapyramidal blade and the recording electrode was located either in the DG granule cell layer for patch-clamp recordings as shown in Fig. 4A, or near the crest of the suprapyramidal blade for field recordings. In this way, we achieved reliable and selective activation of LPP

projections to the DG. To activate selectively MPP, the electrode was carefully positioned in the middle molecular layer, that is at a distance $< 100 \mu\text{m}$ from the DG granule cell layer. Increasing stimulus intensity (0 - 200 pA; 0.1 ms at 0.1 Hz) of the LPP triggered population spike the amplitude of which was plotted against stimulus intensity (Fig. 4B). Input-output relationships for stimulus intensity vs population spike amplitude were significantly different between genotypes, APP/PS1 female mice at 6 months of age always displaying a smaller population spike amplitude compared to the aged-matched WT female (Fig. 4B). LPP was also discriminated from MPP using a paired-pulse protocol (McNaughton, 1980; Wang and Lambert, 2003; Petersen *et al.*, 2013) which results in the facilitation of the second pulse when the LPP is stimulated but either no facilitation or even a depression of the second pulse when the MPP is stimulated (Fig. 4C). Stimuli were delivered at a low intensity, that is at 25-30% maximum pop spike amplitude to minimize as much as possible current spreading to the adjacent pathway but also to avoid paired-pulse facilitation of the MPP which is reported for higher stimulus intensity (Petersen *et al.*, 2013). The two pulses were separated by 50 to 100 ms inter-pulse interval. Both genotypes exhibited a facilitation for the LPP but not for the MPP (Fig. 4C).

The difference in the input-output relationships between the two genotypes suggest a decreased excitability of DG cells in APP/PS1 female mice compared to WT. We next examined the relative amplitude of synaptic currents mediated by AMPA and NMDA receptors in DG granule cells of APP/PS1 and WT female mice at 6 months of age. AMPA currents were recorded using the whole-cell configuration of the patch clamp technique in voltage-clamp mode at a holding potential of -70 mV, a potential at which NMDA currents were virtually absent due to the Mg^{2+} voltage-dependent block (Kupper *et al.*, 1998). NMDA currents were isolated at a holding potential of +40 mV in the presence of 50 μM NBQX to block AMPA and kainate currents. We found no difference in AMPA/NMDA ratio between WT (2.82 ± 0.45 , $n=10$) and APP/PS1 mice (2.35 ± 0.39 , $n=9$; $p=0.452$, Fig. 4D). Furthermore, the AMPA rectification index, a proxy to investigate for possible change of AMPA receptor function did not significantly differ between both genotypes (WT: 0.51 ± 0.06 , $n=10$; APP/PS1: 0.49 ± 0.08 , $n=9$; $p=0.794$, Fig. 4E) suggesting no major alteration in AMPA receptor function in APP/PS1 female mice.

We then recorded spontaneous AMPA and NMDA miniature excitatory postsynaptic currents (mEPSCs) from DG cells to investigate if they were altered in APP/PS1 compared to WT mice. AMPA mEPSCs (mAMPA) were recorded at a holding membrane potential of -70 mV

in the presence of 0.5 μM TTX to block action potentials and 10 μM bicuculline to block mIPSCs due to GABA-A receptors. For NMDA mEPSCs (mNMDA), recordings were made in nominal Mg^{2+} free solution with 50 μM D-serine (co-agonist of the NMDA receptor, Mothet *et al.*, 2000) and in the presence of 10 μM NBQX to block AMPA receptors. Synaptic activity was recorded for 15 minutes and all events occurring during this period were counted for both amplitude and frequency analysis.

The average mean frequency of mAMPA currents was similar between both genotype (WT: 14.00 ± 2.50 events/min, $n=16$; APP/PS1: 18.50 ± 2.91 events/min, $n=15$, $p=0.249$). In contrast, the frequency of NMDA currents was significantly larger in APP/PS1 mice (17.12 ± 1.29 events/min, $n=14$) when compared to WT (4.60 ± 0.43 events/min, $n=13$; *** $p<0.001$) (Fig. 5A).

The average mean amplitude of mAMPA (WT: 8.19 ± 0.27 pA, $n=15$; APP/PS1: 9.22 ± 0.51 pA, $n=15$, $p=0.088$) and of mNMDA (WT: 15.50 ± 0.94 pA, $n=15$; APP/PS1: 17.55 ± 1.09 pA, $n=14$, $p=0.166$) currents were similar between both genotype (Fig. 5A).

No significant differences were observed for the mean 10-90% rise time and the time constant (Tau) of the decay between genotypes for both mAMPA and mNMDA currents. The mean rise time of mAMPA and mNMDA currents was 0.65 ± 0.07 msec ($n=15$) and 0.77 ± 0.08 msec ($n=15$) respectively for WT mice and 0.93 ± 0.13 msec ($n=14$) and 0.69 ± 0.05 msec ($n=14$) respectively for APP/PS1 mice (Fig. 5B). The decay of the currents was monoexponential with a Tau of 6.22 ± 0.35 msec ($n=15$) for WT mAMPA and 4.92 ± 0.21 msec ($n=14$) for APP/PS1 mAMPA (Fig. 5B). For mNMDA, Tau was 15.27 ± 2.01 msec ($n=15$) for WT and 19.06 ± 1.68 msec ($n=14$) for APP/PS1 (Fig. 5B).

The cumulative probability analysis of mAMPA amplitudes showed a significant difference between WT and APP/PS1 mice (KS test, *** $p>0.0001$; 2556 events of 16 cells for WT and 3884 events of 15 cells for APP/PS1 mice) (Fig. 5C) whereas the amplitude distribution histogram was not statistically different between genotypes (paired t test, $p=0.126$) (Fig. 5C). The cumulative probability analysis of mNMDA amplitudes also showed a significant difference between WT and APP/PS1 mice (KS test, *** $p>0.0001$; 861 events of 15 cells for WT and 3461 events of 14 cells for APP/PS1 mice) but also the amplitude distribution histogram (paired t test, * $p<0.05$) (Fig. 5C).

In summary these results show a decreased basal transmission and an increased frequency of mNMDA currents for APP/PS1 vs WT mice.

The firing activity of type 1 DG granule cells is increased in APP/PS1 mice

Adult DG granule cells have been reported to be composed of a continuum of newly born and mature neurons reflecting their dynamic integration in the DG throughout the entire life of the organism (Aimone *et al.*, 2011; Nakashiba *et al.*, 2012). These two populations of DG neurons display different intrinsic properties, such as input resistance (R_{in}) and firing patterns, which have been used as specific hallmarks of the degree of maturation and integration in the circuitry. This prompted us to ask whether intrinsic properties of DG cells were differentially affected by A β pathology in APP/PS1 mice model. Whole-cell patch clamp recordings were carried out in DG cells from 6 months-old WT and APP/PS1 mice. Figure 6A shows representative electrotonus and action potentials (APs) in a DG granule cell from a WT mouse in response to short (200 μ s) depolarizing current step injections (10 μ A increments) in current-clamp mode at a membrane potential close to -70 mV. We did not observe significant differences between WT and APP/PS1 mice for input resistance (R_{in}), total amplitude of AP, amplitude of the overshoot, time to peak of the AP, time to reach the half-maximal amplitude (t_{50}), threshold potential (Table 1). The resting membrane potential of DG granule cells was on average slightly more hyperpolarized in APP/PS1 mice compared to WT mice (-72.7 ± 0.9 , $n=23$; -75.4 ± 0.7 , $n=15$ respectively, * $p<0.05$, Table 1).

The E-S curve (relates EPSP-slopes to the associated AP firing probability) was generated by applying increasing stimuli to span the all range, *i.e.* from a sub-threshold EPSP evoking no action potential to an EPSP evoking an action potential with 100% probability. There were no significant differences between genotypes in the E-S curves with a EC_{50} value of 5.96 ± 0.38 ($n=15$) for WT and 5.85 ± 0.39 ($n=15$) for APP/PS1; $p=0.849$ (Fig. 6A).

In order to discriminate the putative two types of DG cells, we then applied long current steps (250 ms) of 10 pA increments from -20 pA up to 200 pA to evoke trains of APs. As shown in Fig. 6B, DG granule cells could be divided into two distinct categories, termed type 1 and type 2 according to Nenov *et al* (2015). Type 1 DG cells exhibited spikes starting from a moderate intensity, increasing in frequency following steps increments (Fig. 6B). At current steps of high intensity (>120 pA), type 1 DG cells lacked sustained firing, APs became progressively smaller in amplitude and ceased before the end of the current step (Fig. 6B). In contrast, type 2 DG cells fired throughout all the current step without failure and exhibited

firing frequencies linearly proportional to the intensity of the current step, *i.e.* the maximum number of APs was always observed for the highest current intensity (Fig. 6B; Fig. 6C). Whatever the intensity of the current steps, WT DG cells significantly fired fewer spikes than APP/PS1 DG cells for both type 1 and type 2 (Fig. 6C; *** $p < 0.0001$, two-way ANOVA). WT type 1 DG cells had a high R_{in} ($368 \pm 20 \text{ M}\Omega$, $n=14$) whereas WT type 2 DG cells had a significant lower R_{in} ($279 \pm 19 \text{ M}\Omega$, $n=16$, ** $p=0.0033$) (Table 2). In APP/PS1 mice, type 1 DG cells had a R_{in} of $368 \pm 16 \text{ M}\Omega$ ($n=17$) and type 2 DG cells a R_{in} of $294 \pm 18 \text{ M}\Omega$ ($n=14$, ** $p=0.0048$) (Table 2). The proportion of type 1 vs type 2 DG granule cells was 46.7 % vs 53.3 % in WT mice ($n = 30$) and not significantly modified in APP/PS1 mice (54.8 % vs 45.2 %, $n = 31$, $p=0.366$, Chi-square = 0.815, Chi-squared test). Type 1 and type 2 R_{in} were not significantly different with respect to the genotype ($p=0.993$ and $p=0.562$ respectively) (Table 2).

To gain further information on ionic mechanisms that may underlie different spiking patterns between type 1 and type 2 DG cells according to genotype, we studied the first three interspike intervals (Fig. 6D). The first spike interval was $15.9 \pm 1.6 \text{ ms}$ ($n=13$) in WT type 1 DG cells and was significantly smaller in APP/PS1 type 1 DG cells (10.4 ± 0.8 , $n=17$, ** $p < 0.01$) whereas it was not different significantly between WT and APP/PS1 mice for type 2 DG cells (12.5 ± 1.2 , $n=17$ and 9.6 ± 0.7 , $n=14$ respectively, $p=0.069$). Both the second and the third spike intervals confirmed the same pattern with an increased spike frequency for APP/PS1 vs WT type 1 cells (second spike interval: WT: $20.5 \pm 2.1 \text{ ms}$, $n=13$ and APP/PS1: 13.3 ± 1 , $n=17$, ** $p < 0.01$; third spike interval: WT: $23.9 \pm 2.2 \text{ ms}$, $n=13$ and APP/PS1: $17. \pm 1.5$, $n=16$, * $p < 0.05$) whereas it was not different between WT and APP/PS1 type 2 cells (second spike interval: WT: $16.4 \pm 1.5 \text{ ms}$, $n=17$ and APP/PS1: $13.5 \pm 1.2 \text{ ms}$, $n=14$, $p=0.154$; third spike interval: WT: $18.9 \pm 1.4 \text{ ms}$, $n=17$ and APP/PS1: 18.2 ± 1.9 , $n=14$, $p=0.773$) (Fig. 6D). We also examined the last three interspike intervals in type 2 DG cells, as they were spiking throughout all the current step without failure, to search for possible differences, with respect to the genotype, in spike frequency adaptation, *i.e.* a reduction of the firing rate to a stimulus of constant intensity. Whatever the interval, *i.e.* the third, the second or the first, they were no differences between both genotype (third spike interval: WT: $31.6 \pm 1.7 \text{ ms}$, $n=15$ and APP/PS1: $28.9 \pm 1.1 \text{ ms}$, $n=14$, $p=0.206$; second spike interval: WT: $30.3 \pm 1.5 \text{ ms}$, $n=17$ and APP/PS1: $31.6 \pm 1.4 \text{ ms}$, $n=14$, $p=0.551$; first spike interval: WT: $34.5 \pm 1.4 \text{ ms}$, $n=17$ and APP/PS1: $33.3 \pm 1.8 \text{ ms}$, $n=14$, $p=0.623$) (Fig. 6E).

In summary these results show a selective increase in firing activity for type 1 DG granule cells (supposedly less mature; Mongiat *et al.*, 2009, Spampanato *et al.*, 2012, van Praag *et al.*, 2002, Nenov *et al.* 2015) in APP/PS1 mice and no change of intrinsic excitability in type 2 DG cells.

The firing properties of DG granule cells are differentially affected by synaptic potentiation in WT vs APP/PS1 mice

It has been recently reported that the intrinsic excitability of DG granule cells was highly plastic and modulated by synaptic potentiation (Lopez-Rojas *et al.*, 2015). Interestingly, the intrinsic firing properties of DG granule cells were affected in a single transgenic mouse model of AD (Nenov *et al.*, 2015).

At the LPP-DG synapse, long-term potentiation (LTP) of excitatory transmission is induced by high-frequency stimulation (HFS). As shown in Fig. 7A, HFS (3 bursts of 100 stimuli at 100 Hz with a 10-second interburst interval) triggered a robust and long-lasting potentiation (LTP) of the population spike amplitude (PSA) in WT female mice ($187 \pm 14\%$, $n=17$) which was completely blocked by 50 μM AP-V ($n=9$). This LTP was reduced by half in APP/PS1 female mice ($146 \pm 8\%$, $n=16$, * $p < 0.05$) (Fig 7. A, D). The levels reached for LTP in age-matched WT male mice ($150 \pm 9\%$, $n=15$, * $p < 0.05$) were similar to those observed in APP/PS1 female mice (Fig 7. A, D).

We also examined if the probability of discharge of the postsynaptic DG granule cells in response to excitatory synaptic stimulation was differentially affected in WT vs APP/PS1 mice. HFS induced a large shift to the left of the EPSP-to-Spike (E-S) potentiation curves (Fig. 7B) in both genotypes although with a larger amplitude for APP/PS1 mice (EC_{50} WT: 5.96 ± 0.38 , $n=15$, EC_{50} WT_{after HFS}: 4.51 ± 0.41 , $n=11$, * $p < 0.05$; EC_{50} APP/PS1: 5.85 ± 0.39 , $n=15$, EC_{50} APP/PS1_{after HFS}: 3.92 ± 0.27 , $n=10$, ** $p < 0.01$). These results show that HFS induces long lasting changes in intrinsic plasticity of DG granule cells. The shift to the left indicates an increase of excitability in DG cells induced by LTP, *i.e.* to a smaller EPSP slope corresponds a higher AP firing probability (Fig. 7B).

Then we looked to the relation between the EPSP amplitude and the EPSP slope for subthreshold EPSPs (*i.e.* not triggering APs) before and after HFS. We found a significant increase in the EPSP amplitude/slope ratio for APP/PS1 mice (APP/PS1: 4.55 ± 0.18 , $n=15$;

APP/PS1_{after HFS}: 6.08±0.31, n=11, *** $p<0.001$) but not for WT mice (WT: 4.90±0.30, n=15; WT_{after HFS}: 5.70±0.33, n=12, $p=0.08$) (Fig. 7C).

PGE₂-signaling pathway is not involved in the impairment of LTP at the LPP-DG synapse

In a previous work, we reported that PGE₂ acting on EP3 receptors impaired Mf-CA3 LTP in WT male mice and that the involvement of an inflammatory PGE₂-EP3 signaling pathway was responsible for the impairment of the LTP in aged APP/PS1 male mice (Maingret *et al.*, 2017). In the present work, the LTP of PSA is significantly impaired by half in APP/PS1 female (WT: 187±14%, n=17; APP/PS1: 146±8%, n=16, * $p<0.05$) (Fig 7A,7D). This prompted us to investigate the effects of exogenous PGE₂ and/or specific agonists and antagonists of different EP subtypes receptors on the LTP of PSA both in WT and in APP/PS1 female mice.

When adding 10 μM PGE₂, we did not observe any significant impairment of LTP in WT female mice (WT: 187±14%, n=17; WT+PGE₂: 161±15%, n=17, $p=0.2$). Neither a specific EP2 receptor agonist (ONO-AE1-259-01, 1 μM) nor a EP2 receptor antagonist (PF-04418948, 10 μM) or a EP3 receptor antagonist (ONO-AE3-240, 1 μM) significantly altered LTP (WT+ONO-AE1-259-01: 160±12%, n=17; WT+PGE₂+PF-04418948: 169±16%, n=16; WT+PGE₂+ONO-AE3-240: 154±8%, n=16) (Fig. 7D). These results ruled out the involvement of either PGE₂-EP2 or PGE₂-EP3 signaling pathway acting of LTP at the LPP-DG synapse in WT female mice.

Then we searched if the impairment of LTP observed in APP/PS1 was possibly related to PGE₂. Neither PF-04418948 (10 μM) nor ONO-AE3-240 (1 μM) rescued LTP (APP/PS1+PF-04418948: 140±8%, n=17, $p=0.619$; APP/PS1+ ONO-AE3-240: 147±11%, n=17, $p=0.191$) (Fig. 7D) which ruled out any significant involvement of inflammatory PGE₂-EP2 or PGE₂-EP3 signaling pathway in APP/PS1 female mice acting on LTP. These somewhat unexpected results with respect to the precocious neuroinflammatory status of APP/PS1 female mice led us to quantified PGE₂ within the hippocampus at different ages for both genotypes. No significant difference in PGE₂ levels was observed between WT and APP/PS1 female mice at any age (3 months: WT: 17.9±2.06 pg/mg, n=13; APP/PS1: 13.07±1.31 pg/mg, n=12; $p=0.073$; 6 months: WT: 18.98±4.31 pg/mg, n=10; APP/PS1: 19.31±4.02 pg/mg, n=10; $p=0.705$; 9 months: WT: 12.44±1.41 pg/mg, n=12; APP/PS1: 12.57±1.36 pg/mg, n=11; $p=0.951$) (Fig. 7E).

Discussion

Our findings show prominent sex-differences in the development of A β plaques load and glial activation in a transgenic mouse model of AD. The molecular and cellular alterations observed in the DG of APP/PS1 female mice paralleled functional deficits revealed by hippocampal-dependent memory tasks. Most interestingly, these alterations are concomitant with modifications of the plasticity of synaptic transmission and intrinsic excitability in the perforant path to DG granule cells circuitry, a key region of the integration of mnemonic information originated from the entorhinal cortex which display a high density of A β plaques.

Sex-differences in A β load and glial activation in the DG region

Our results clearly show that A β load starts much earlier in APP/PS1 females as compared to age-matched APP/PS1 males. Whatever the age, A β plaque burden was always significantly higher in females *vs* males.

According to Jankowsky et al (2004), APP/PS1 mice display a high ratio A β_{42} / A β_{40} which accelerates A β aggregation into A β plaques starting around 6 months of age. APP/PS1 females at 8-9 months of age display roughly twice as many A β plaques in the hippocampus and two to three times higher levels of A β_{42} (Gallagher *et al.*, 2013; Taniuchi *et al.*, 2007) than age-matched males. This sex-difference persists during aging and is still present in APP/PS1 up to 17 months of age (Wang *et al.*, 2003).

The physiological basis underlying this sexual dimorphism is still elusive but few potential mechanisms have been proposed to support it such as the increased expression of BACE-1 in APP/PS1 females leading to an enhanced production of A β (Gallagher *et al.*, 2013), a modified proliferation of hippocampal progenitors (Taniuchi *et al.*, 2007) yielding less DG adult-born neurons with a reduced life expectancy in APP/PS1 females (Richetin *et al.*, 2017) but also an increased activation of glial cells (microglia, Taniuchi *et al.*, 2007; astrocytes, Richetin *et al.*, 2017). Obviously, the putative influence of sex hormones has also been investigated. *In vitro* estrogen has been shown to be neuroprotective against A β through its ability to decrease lipid peroxidation (Behl *et al.*, 1995; Gridley *et al.*, 1997). Estrogen supplementation has been reported to delay the onset in AD and to reduce its prevalence in women (Henderson *et al.*, 2000) but further trials suggested that estrogen supplementation might have no effect on the course of the disease in elderly women (Fillit, 2002; Cholerton *et al.*, 2002).

There is considerable evidence that neuroinflammation is a major component of AD (Akiyama *et al.*, 2000; Heneka and O'Banion, 2007; Heneka *et al.*, 2015) contributing to its progression and its pathogenicity. Furthermore, and relevant to sex-differences, it has been suggested that the differential vulnerability to AD between man and woman could be mediated by a sex-specific systemic inflammatory response in early AD (Varma *et al.*, 2016). The cellular players of neuroinflammation *i.e.* microglia, the resident immune-competent brain cells, and astrocytes release a plethora of inflammatory molecules such as pro-inflammatory cytokines, prostanoids, nitric oxide and other cytotoxic molecules when activated. The agenda of glial activation in the course of AD is still a matter of debate, but it is doubtless that A β is a potent neuroinflammatory molecule which activates glial cells and thereby exacerbates the neuroinflammatory response (Heneka *et al.*, 2015). In the present work, hallmarks of activated glial cells were evidenced in APP/PS1 females and appeared much earlier than in age-matched APP/PS1 males. In APP/PS1 females, abundant hypertrophic astrocytes (see Schiweck *et al.*, 2018) and amoeboid microglia were densely expressed in the *stratum moleculare* of the DG, a region affected early by the spatiotemporal progression of the disease (Hurtado *et al.*, 2010). Activated glial cells were distributed in the *stratum moleculare* and densely packed around A β aggregates to form A β plaques.

Sex-differences in hippocampal-dependent memory tasks

Our results show that APP/PS1 females exhibit much earlier spatial memory impairments than age-matched males. In order to relate hippocampal A β load and neuroinflammatory glial activation, we chose to compare mnemonic performances of APP/PS1 females and males in two behavioral tasks (contextual fear conditioning and object location task) testing hippocampal-dependent memory. APP/PS1 females were impaired as early as 4 months of age, *i.e.* an age for which there are only very few A β plaques, whereas age-matched males were spared. Remarkably, APP/PS1 males displayed unaltered CFC behavior until at least 12 months of age, despite a noticeable hippocampal A β plaques load (Maingret *et al.*, 2017). As noticed by Richetin *et al.* (2017) and strengthened by our study, it suggests that hippocampal-dependent memory deficits were not directly linked to A β plaques but more likely to the presence of A β peptides. Interestingly, it has also been suggested that impaired synaptic integration of disabled adult-born neurons related to the neuroinflammatory activation of astrocytes in APP/PS1 females could be involved in those hippocampal-dependent memory deficits (Richetin *et al.*, 2017).

Electrophysiological properties of type 1 and type 2 DG granule cells in WT and APP/PS1 mice

Our results show, supported by previous studies, that granule cells in the DG can be segregated into two distinct populations *i.e.* type 1 and type 2, with different intrinsic properties, based on R_{in} and firing patterns (Schmidt-Hieber *et al.*, 2004; Mongiat *et al.*, 2009; Nenov *et al.*, 2015). Type 1 DG cells are considered to be young neurons whereas type 2 cells correspond to more mature cells (van Praag *et al.*, 2002; Mongiat *et al.*, 2009; Spampanato *et al.*, 2012). Remarkably, the proportion of type 1 and type 2 was not affected by the genotype in contrast to what was reported by Nenov *et al.* (2015). Type 1 DG cells had a high R_{in} and did not fire APs throughout the entire duration of the current step whereas type 2 DG cells has a low R_{in} but fired APs without failure during the 250 ms current step. Notably, we observed no differences in R_{in} due to genotype whereas Nenov *et al.* (2015) reported a significant difference of R_{in} for type 2 DG cells between WT and single transgenic Tg2576 mice.

We have no firm explanation for these discrepancies but they might be related to differences between Nenov's data and ours. First, in our study, we were using only female mice whereas male and female were used indifferently in Nenov's study. Second the transgenic mouse model was different (single transgenic Tg2576 for Nenov *et al.*, 2015; double transgenic APP/PS1 in our work) and last the age was also different (9 months old mice for Nenov *et al.*, 2015; 6 months old mice in our work). Interestingly, Nenov *et al.* (2015) suggested that differences observed in WT *vs* Tg2576 DG cells could be due to a decrease in proliferation, survival, maturation or integration of newborn DG neurons within DG. If true, the younger age of our mice (6 months *vs* 9 months in Nenov's study) could blunt differences due to these mechanisms. Worthy of note, the estrous cycle is known to alter hippocampal spinogenesis and spine densities (Gould *et al.*, 1990; Hara *et al.*, 2015) and adult-born DG cells in APP/PS1 female mice at older age than in the present study (*i.e.* 7-9 months of age) display severely impaired dendritic spine density contributing to reduced survival rate (Richetin *et al.*, 2015).

Differences in the plasticity of intrinsic excitability in DG granule cells between WT and APP/PS1 female mice

Whatever the intensity of the current steps, APP/PS1 DG cells fired significantly more spikes than WT DG cells for both type 1 and type 2 hence revealing an increased excitability of DG

cells in APP/PS1 female mice. An increased excitability, although not statistically significant, was also reported for Tg2576 type 2 DG cells (Nenov *et al.*, 2015). To characterize further the firing pattern, we examined both the instantaneous firing frequency by measuring the interspike interval (ISI) during the first three intervals and the spike frequency adaptation by measuring ISI for the last three intervals for type 1 and type 2 DG cells. Type 1 APP/PS1 DG cells showed a significant reduction in ISI during the first three intervals compared to WT but not for type 2. No differences in spike frequency adaptation was observed for type 2 DG cells between genotypes.

The distinct firing patterns of type 1 and type 2 DG cells imply a diversity of underlying voltage-dependent ion channels and their different contribution may be related to the observed changes in firing frequency in APP/PS1 DG cells. Hence, a decreased expression of somatic Na⁺ channels produced an increase of the instantaneous spiking frequency in PS/APP double transgenic mice (Brown *et al.*, 2011). Related synaptic mechanisms may also impact neuronal excitability such as a relative decrease in GABAergic tonus reported in cortical neurons from APP23/PS45 double transgenic mice (Busche *et al.*, 2008) but unlikely to be involved in our experimental conditions always in the presence of bicuculline.

Synaptic plasticity and intrinsic excitability in DG granule cells of WT vs APP/PS1 females

The difference in the input-output relationships between WT and APP/PS1 at the LPP-DG synapse lead us to investigate if the synaptic content in AMPA and NMDA receptors were affected by the genotype. Both the AMPA/NMDA ratio and the AMPA rectification index were similar between genotypes suggesting neither major change in NMDA receptor activity nor in AMPA receptor function. Strikingly, the frequency of NMDA minis but not AMPA minis was increased in APP/PS1 DG granule cells. The most reasonable explanation for this increased frequency of NMDA minis would be an increased number of silent synapses expressing NMDA receptors in APP/PS1 DG granule cells but devoid of functional AMPA receptors. Remarkably, an increase in hippocampal silent synapses in transgenic mouse models of AD, by removal and/or internalization of AMPA receptors, has been previously reported (Hsieh *et al.*, 2006; Price *et al.*, 2014, reviewed in Chater and Goda, 2014), although in our conditions we found no decrease in AMPA receptor mediated synaptic transmission.

Our data show a strong impairment of HFS-induced potentiation of the population spike amplitude in APP/PS1 female mice, when compared to aged-matched WT female mice. Thus it is important to unravel the intermingled relationships between synaptic plasticity and

plasticity of intrinsic excitability of DG granule cells to decipher how they might impact each other. The LTP protocol induced a prominent modulation of the E-S curve linking EPSPs slope to the probability of firing an action potential. HFS elicited significant shifts to the left for both genotype with a larger shift for APP/PS1 vs WT female mice.

Taken together, these results indicated that synaptic potentialisation elicited changes in intrinsic excitability of DG granule cells with a larger increase for APP/PS1 vs WT. Strikingly, the increase in CA1 neuronal activity induced by A β oligomers impaired by half the potentialisation of population spike amplitude (Lei *et al.*, 2016).

Interestingly, we did not observe any significant changes in the threshold for action potentials and only a fairly minor change (less than 3 mV) in the resting membrane potentials that could account for excitability changes. However, we observed a significant difference in the EPSP amplitude/slope ratio before and after HFS for APP/PS1 but not WT DG cells. As EPSP slope was measured during the first two milliseconds which reflects mainly the AMPA component of synaptic transmission, these data suggested that changes in dendritic processing of synaptic input in APP/PS1 DG cells could account for excitability changes. Interestingly, changes in intrinsic excitability of DG cells have been recently attributed to the modulation of dendritic A-type potassium channels (Lopez-Rojas *et al.*, 2016) and dendritic hyperexcitability induced by Kv4.2 deficiencies in the hippocampal CA1 region was reported in a single transgenic (hAPP) mouse model of AD (Hall *et al.*, 2015).

Prostanoïds and LTP impairment in APP/PS1 females

In a previous work, we reported that PGE₂-EP3 signaling pathway was impairing hippocampal presynaptic LTP at the mossy fiber - CA3 synapse in aged APP/PS1 males (Maingret *et al.*, 2017). In the present work, neither PGE₂-EP2/EP4 nor PGE₂-EP3 pathways were involved in the impairment of PSA-LTP at LPP-DG synapses. Furthermore, in contrast with our previous study (Maingret *et al.*, 2017), hippocampal levels of PGE₂ were not increased with time in APP/PS1 vs WT females. Interestingly, deficiency of estrogen augments PGE₂ levels in ovariectomized female rats (Wang *et al.*, 2014) which suggests that female sex hormones exert a tonic brake on PGE₂ levels and might explain why PGE₂ levels were not altered in APP/PS1 vs WT females.

In conclusion, neuroinflammatory hallmarks of AD are more precocious in double transgenic female mouse model of AD when compared to age-matched males. These histopathological

alterations are concomitant of strong and specific impairment of hippocampal-dependent memory task together with non-synaptic and synaptic modifications at the hippocampal PP-DG circuitry. Hence, our results are clear evidence that 1 - results originated from studies using males and females together must be taken with extreme caution as males and females clearly behave differently, 2 - depending on the hippocampal region and on the gender, mechanisms responsible for LTP impairment are different and 3 - more studies aiming at deciphering mechanisms on mouse models of AD in females are most wanted.

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No conflict of interest, financial or otherwise, are declared by the author(s).

Author contributions

T.A. conception and design of research; N.J., D.C., N.G., S.D. and T.A. performed experiments and analysed data; D.C., S.D., N.G. and T.A. interpreted results of experiments; T.A. prepared figures; T.A. drafted manuscript; T.A. and C.M. edited and revised manuscript; N.J., D.C., N.G., S.D., C.M. and T.A. approved final version of manuscript.

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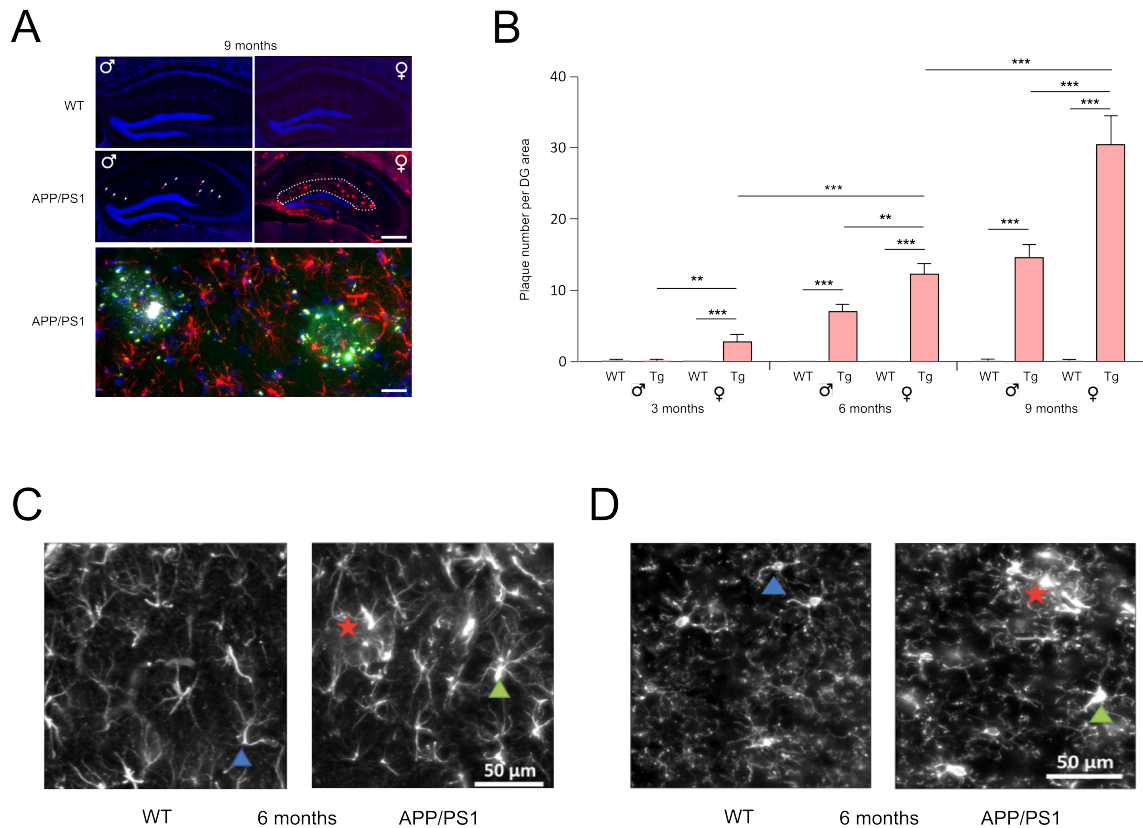


Figure 1: APP/PS1 mice display hippocampal A β plaques and glial activation earlier in female than male. **A.** Representative micrographs of a hippocampus in WT and APP/PS1 male vs female at 9 months of age. Slices were double stained for nuclei with 4',6-diamidino-2-phenylindole (DAPI, blue signal) and for A β plaques with 2,5-Bis(2-(4-hydroxyphenyl)vinyl) anisole (methoxy-X04, red signal). Note the heavy load of A β plaques in the *stratum moleculare* (delineated by the white dotted-line located above the *stratum granulare*) of the DG of female mice. Bar scale = 250 μ m. Lower panel, representative micrograph of A β plaques (anti- A β ₁₋₁₆ antibody, white signal) surrounded by activated astrocytes (GFAP, red signal) and microglia (Iba-1, green signal). Bar scale = 10 μ m. **B.** Bar graphs represents the number of A β plaques in the DG *stratum moleculare* (as shown in A) at different ages for male and female mice. Note the significant higher deposit of A β plaques for APP/PS1 female vs male mice at all age. (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). **C.** Magnification of a representative DG *stratum moleculare* region of WT and APP/PS1 female mice. Note the morphological changes in GFAP positive cells (astrocytes) in APP/PS1 (red star and green triangle) vs WT female mice (blue triangle). **D.** Magnification of a representative DG *stratum moleculare* region of WT and APP/PS1 female mice. Note the morphological changes in Iba-1 positive cells (microglia) in APP/PS1 (red star and green triangle) vs WT female mice (blue triangle).

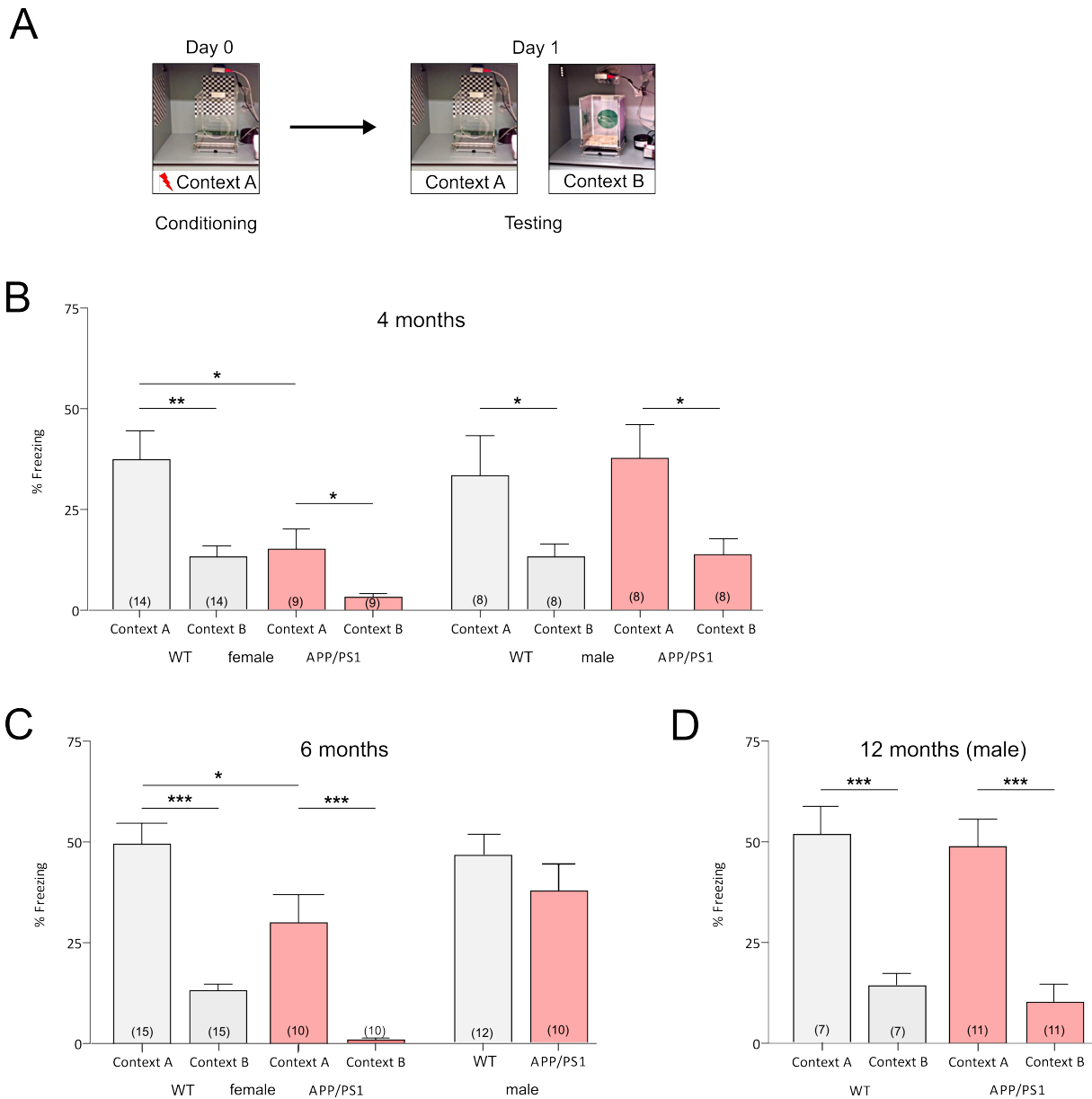


Figure 2: APP/PS1 female mice develop contextual memory deficits earlier than male. **A.** At day 0, WT and APP/PS1 male and female mice (4, 6, 12 months) were placed in a training chamber (context A) where they received an electric footshock (2s, 0.75 mA). At day 1, mice were reexposed to the conditioning context A then to an entirely new, therefore neutral, context (context B). Freezing percentage was calculated for each animal. **B.** At 4 months of age, when compared to WT mice, APP/PS1 female mice show impairment of contextual fear conditioning when reexposed to context A whereas APP/PS1 male mice does not. **C.** At 6 months of age, APP/PS1 male mice does not still show impaired contextual fear conditioning. **D.** At 12 months of age, APP/PS1 male mice still do not display impaired contextual fear conditioning. (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$)

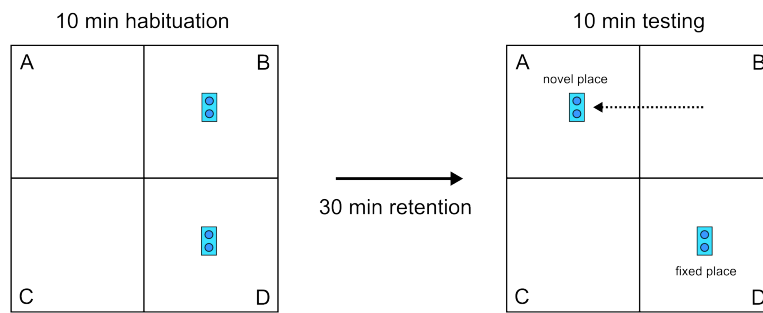
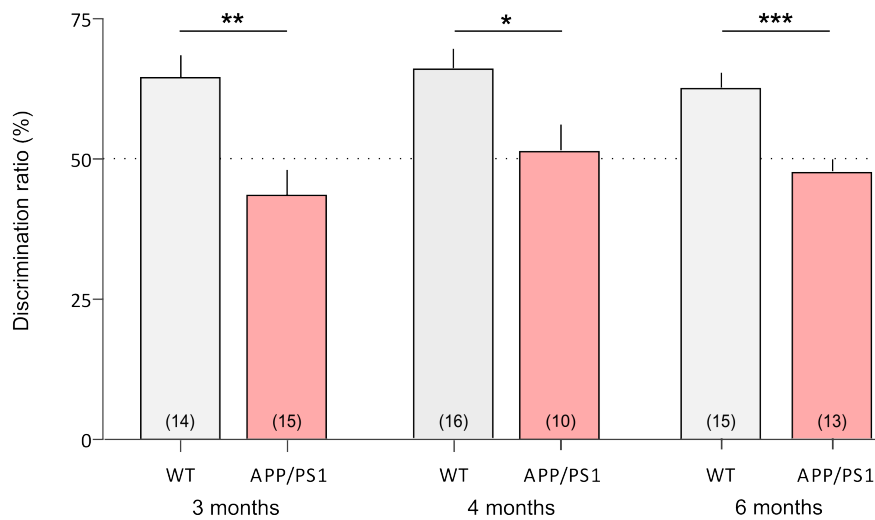
A**B**

Figure 3: APP/PS1 female mice show impaired object location task. **A.** During the habituation phase (10 min), mice are allowed to explore an open field arena with two identical objects. During the retention phase (30 min) mice were placed back to their home cage. During the test phase, the location of one object is changed and the time spent exploring the two objects was assessed. **B.** As early as 3 months of age, APP/PS female mice show impaired object place recognition. Discrimination ratio = time spent to explore the displaced object / total time spent to explore both objects. (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$)

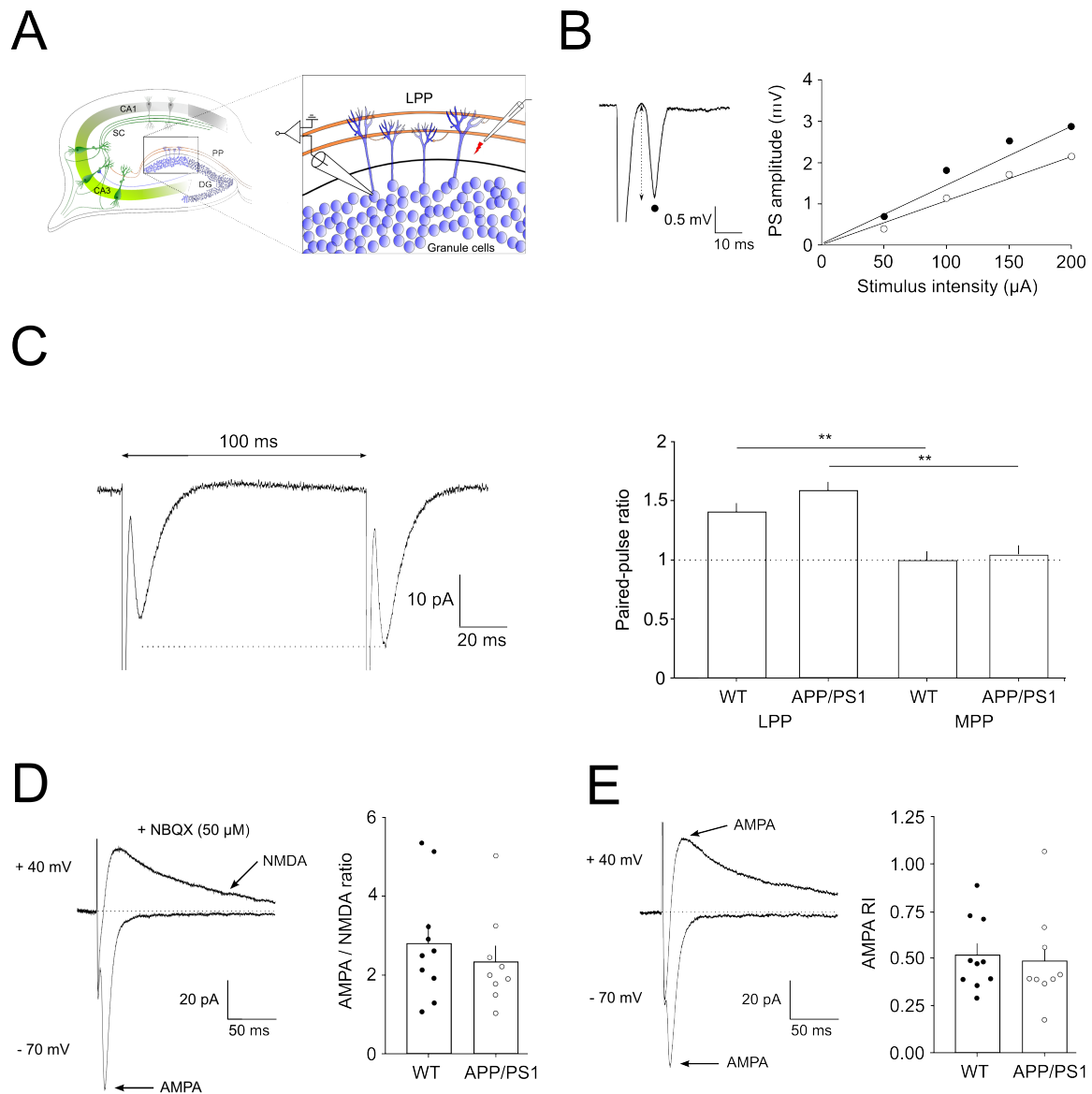


Figure 4: Electrophysiological characterization of the lateral perforant path (LPP) inputs to the hippocampal dentate gyrus (DG). **A.** Diagram shows the positions of the stimulating electrode (located in the outer DG molecular layer) and of the recording electrode (either onto a DG cell for patch-clamp recordings or near the crest of the suprapyramidal blade for field recordings). **B.** Input-output relationship between the population spike amplitude (PS amplitude) and the stimulus intensity. For each intensity, WT (black circles) and APP/PS1 (white circles) relationship were significantly different (50 μA : WT=0.68 \pm 0.12; APP/PS1=0.40 \pm 0.07, 100 μA : WT=1.79 \pm 0.19; APP/PS1=1.14 \pm 0.10, 150 μA : WT=2.54 \pm 0.22; APP/PS1=1.71 \pm 0.17, 200 μA : WT=2.85 \pm 0.26; APP/PS1=2.14 \pm 0.16, p <0.0001, 2-way ANOVA). **C.** Electrophysiological dissection of the LPP and medial

perforant path (MPP) by paired-pulse experiments. Whatever the gender, the paired-pulse ratio was significantly higher than 1 (facilitation) for the LPP, whereas it was lower than 1 for the MPP. **D.** Representative traces from whole-cell patch clamp experiments showing AMPA-receptor- and NMDA-receptor evoked currents (in the presence of 50 μ M NBQX) in DG cells. Histogram showing the AMPA to NMDA ratio for WT and APP/PS1 mice (WT:2.82 \pm 0.45, n=10; APP/PS1:2.35 \pm 0.39, n=9, $p=0.452$). **E.** Representative traces from whole-cell patch clamp experiments showing AMPA-receptor evoked currents from -70 and +40 mV in DG cells. Histogram showing the AMPA rectification index (AMPA RI) of DG cells for WT and APP/PS1 mice (WT:0.51 \pm 0.06, n=10; APP/PS1:0.49 \pm 0.08, n=9, $p=0.794$).

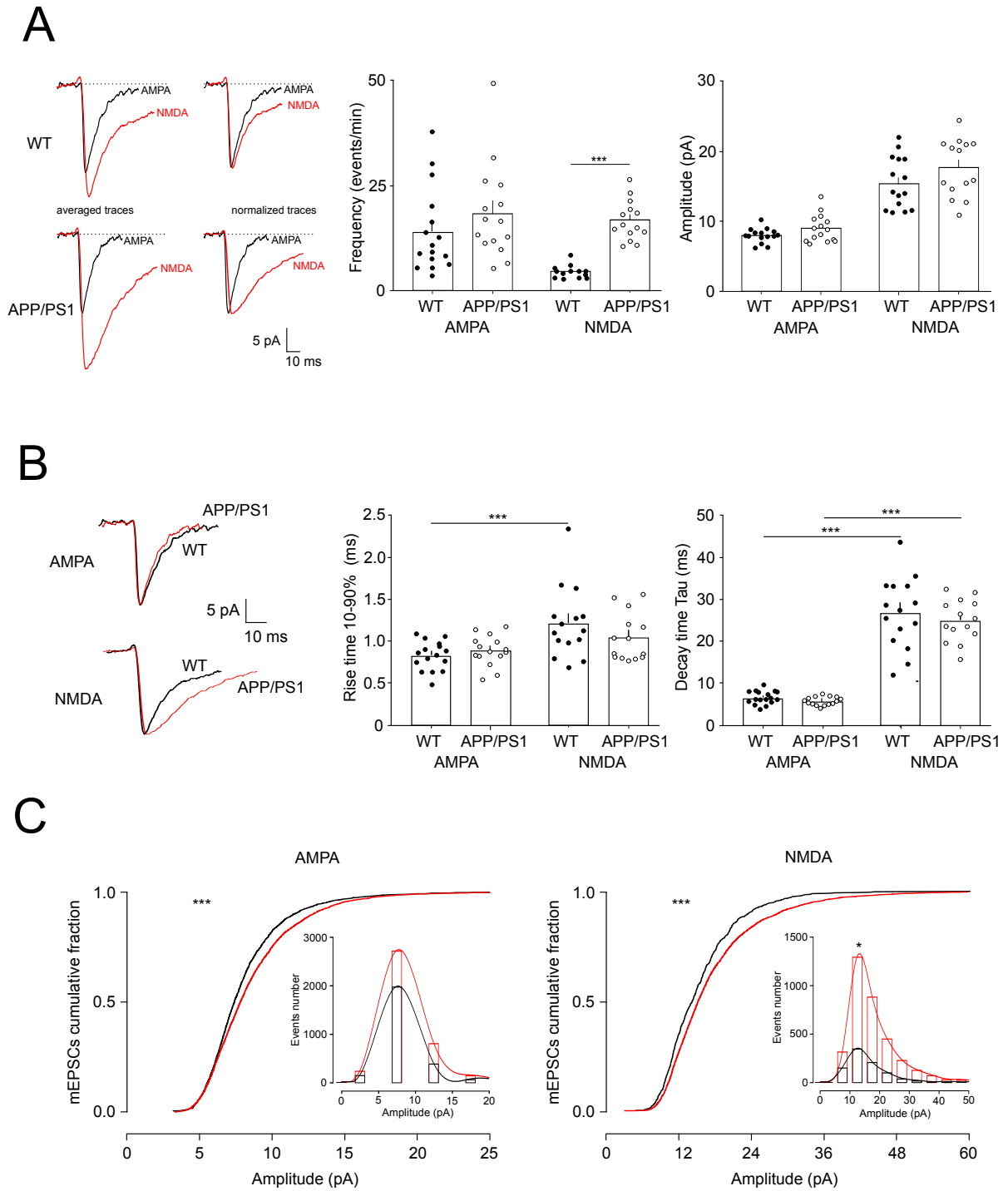


Figure 5: AMPA-receptor and NMDA-receptor mediated mEPSCs in DG cells. **A.** Superimposed representative traces of AMPA and NMDA mEPSCs (AMPA traces are the average of 2556 and 3884 mEPSCs events for WT and APP/PS1 respectively; NMDA traces are the average of 861 and 3461 mEPSCs events for WT and APP/PS1 respectively). DG cells were held at a holding potential of -70 mV in the presence of bicuculline (10 μ M), NBQX (10

μM) and TTX ($0.5 \mu\text{M}$) and recorded for 15 minutes. NMDA mEPSCs were recorded in nominal free Mg^{2+} aCSF, 4 mM Ca^{2+} and D serine ($50 \mu\text{M}$). The average frequency of NMDA mEPSCs was significantly increased for APP/PS1 mice vs WT mice (WT: 4.60 ± 0.43 , $n=13$; APP/PS1: 17.12 ± 1.29 , $n=14$, *** $p < 0.001$), whereas the amplitude was not (WT: 15.46 ± 0.94 , $n=15$; APP/PS1: 17.55 ± 1.09 , $n=14$, $p=0.166$). Neither the frequency (WT: 14.00 ± 2.50 , $n=16$; APP/PS1: 18.50 ± 2.91 , $n=15$, $p=0.249$) nor the amplitude (WT: 8.19 ± 0.27 , $n=15$; APP/PS1: 9.22 ± 0.51 , $n=15$, $p=0.088$) of AMPA mEPSCs were different. **B.** Kinetic analysis of AMPA and NMDA mEPSCs. Normalized AMPA and NMDA mEPSCs were superimposed for both genotypes. The rise time (10-90%) and decay time (Tau) were similar for AMPA mEPSCs between both genotypes (Rise time AMPA: WT 0.81 ± 0.04 , $n=15$; APP/PS1 0.88 ± 0.04 , $n=15$, $p=0.238$; Tau AMPA: WT 6.67 ± 0.34 , $n=16$; APP/PS1 6.00 ± 0.25 , $n=15$, $p=0.130$). Rise time and Tau were also similar for NMDA mEPSCs between both genotypes (Rise time: WT 1.22 ± 0.12 , $n=13$; APP/PS1 1.04 ± 0.07 , $n=14$, $p=0.237$; Tau: WT 27.86 ± 2.39 , $n=13$; APP/PS1 24.94 ± 1.34 , $n=14$, $p=0.287$). **C.** Cumulative probability analysis of AMPA and NMDA mEPSCs amplitude. The cumulative fraction for AMPA and NMDA mEPSCs amplitudes were significantly different between genotypes (AMPA, KS test, *** $p > 0.0001$; 2556 events of 16 cells for WT [black curve] and 3884 events of 15 cells for APP/PS1 [red curve] mice; NMDA, KS test, *** $p > 0.0001$; 861 events of 15 cells for WT [black curve] and 3461 events of 14 cells for APP/PS1 mice [red curve]). The distribution histogram (insets) was not statistically different between genotypes for AMPA mEPSCs amplitude (paired t test, $p=0.126$) and different between genotypes for NMDA mEPSCs amplitude (paired t test, * $p < 0.05$).

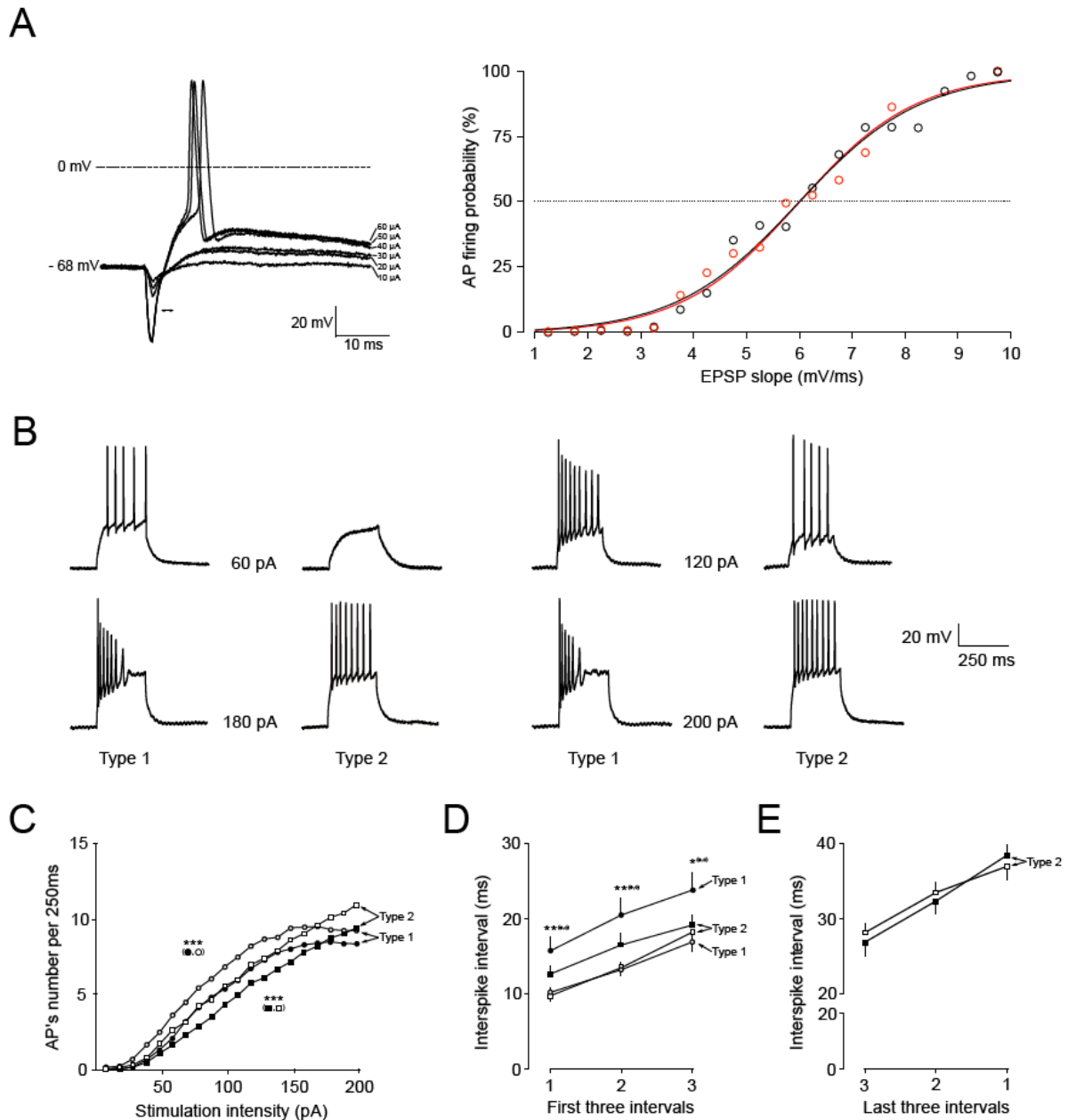


Figure 6: Characterization of firing properties of DG granule cells. **A.** Representative action potentials (APs) elicited in response to 0.2 ms depolarizing current steps with 10 μ A increments in a DG granule cell from WT mouse. For each genotype (WT, black circles; APP/PS1, red circles) EPSP-slope values were calculated for the first two milliseconds and plotted versus the probability to fire action potentials. No shift in the E-S curves was observed between both genotype (WT: $EC_{50}=5.97$, Hill slope=0.37; APP/PS1: $EC_{50}=5.98$, Hill slope=0.39). **B.** Representative trains of APs elicited by long depolarizing current steps (250 ms) of increasing intensity in a WT DG cell. Whatever the genotype, type 1 and type 2 cells

were not significantly distributed (Type 1: WT = 46.7 %, n=14, APP/PS1 = 54.8 %, n=17; Type 2: WT = 53.3 %, n=16, APP/PS1 = 45.2 %, n=14; $p=0.366$). **C.** Input-output curves for type 1 and type 2 DG cells. The number of spikes elicited in response to 250 ms-long depolarizing current steps with 10 pA increments was significantly higher in APP/PS1 vs WT DG cells for both type 1 and type 2 (type 1: WT, black circles; APP/PS1, open circles, *** $p<0.0001$, 2-way ANOVA; type 2: WT, black squares, APP/PS1, open squares, *** $p<0.0001$, 2-way ANOVA). Whatever the genotype the number of spikes elicited in response to 250 ms-long depolarizing current steps with 10 pA increments increased linearly for type 2 but reached a plateau for type 1. **D.** Interspike interval (ISI) plotted as a function of the first three intervals. ISI was significantly higher for type 1 (black circles) cells from WT mice compared to APP/PS1 mice (type 1, white circles) (* $p<0.05$; ** $p<0.01$) but not for type 2 (black and white squares, $p=0.102$; $p=0.132$ respectively). **E.** Spike frequency adaptation estimated by the interspike interval (ISI) for the last three intervals. No differences between genotypes were observed for any ISI.

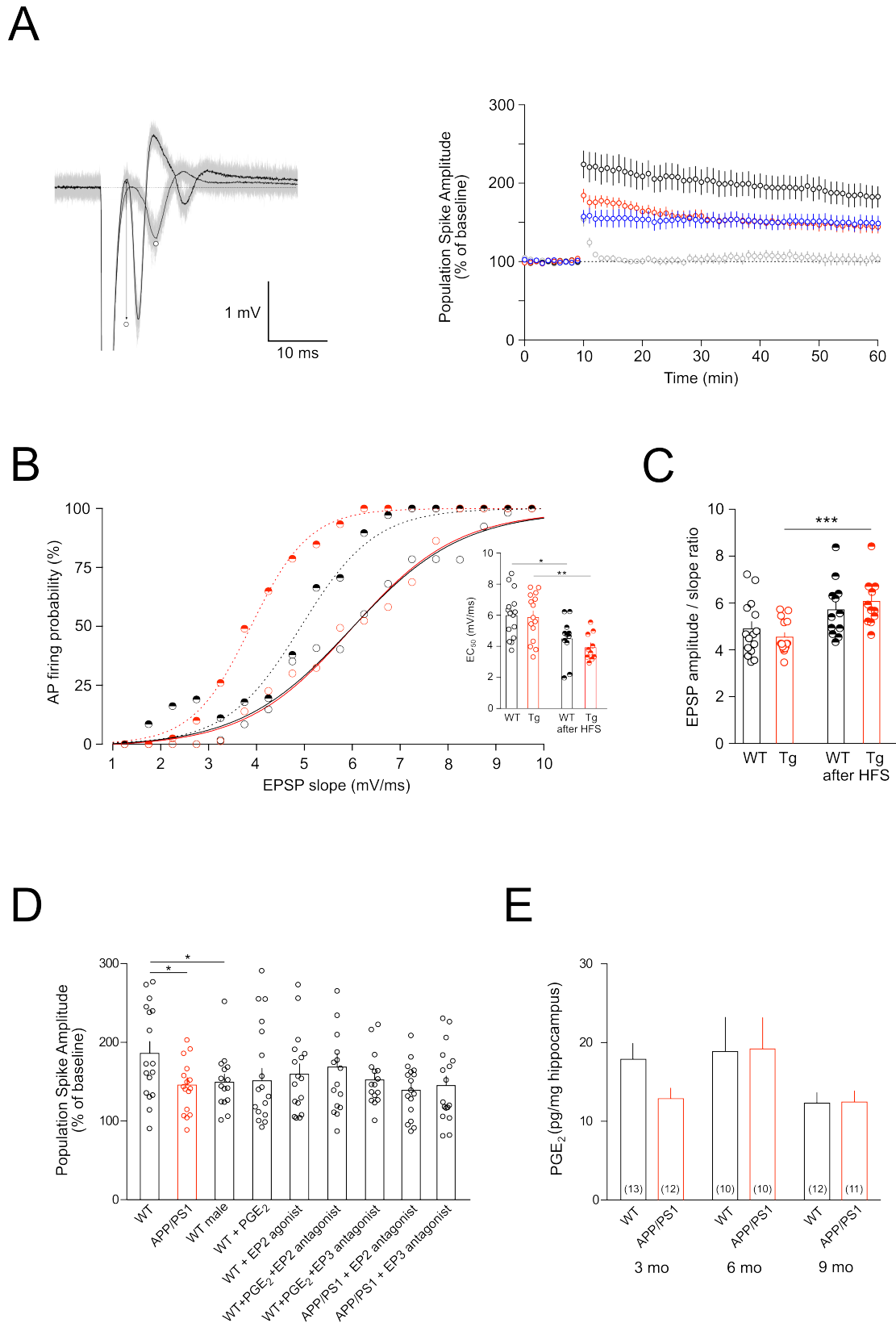


Figure 7: Population spike amplitude potentiation in DG granule cells. **A.** Left panel shows representative traces of population spike amplitude (PSA) before and after HFS. Potentiation

of PSA was induced by 3 bursts of 100 stimuli at 100 Hz with a 10-second interburst interval (HFS). Light grey traces are individual traces and black traces are the averaged traces of 60 sweeps before and after HFS. Right panel shows time course of potentiation of PSA (WT black circles, APP/PS1 red circles, WT male mice blue circles). Potentiation of PSA was completely blocked by AP-V (50 μ M, light grey circles) (n=9). **B.** E-S curves before and after HFS. Shifts to the left of E-S curves before and after HFS were significant for both genotype (WT: -1.45 ± 0.57 mV/ms, * $p=0.018$; APP/PS1: -1.93 ± 0.53 mV/ms, ** $p=0.0014$) but larger for APP/PS1 vs WT. A significant EC_{50} shift in the E-S relation was induced by HFS for both WT (WT: 5.96 ± 0.38 , n=15, $WT_{after\ HFS}$: 4.51 ± 0.41 , n=11, * $p<0.05$) and APP/PS1 (APP/PS1: 5.85 ± 0.39 , n=15, $APP/PS1_{after\ HFS}$: 3.92 ± 0.27 , n=10, ** $p<0.01$) although with a larger amplitude for the APP/PS1 genotype. **C.** EPSP amplitude/slope ratio before and after synaptic potentiation induced by high frequency stimulation (HFS). The amplitude/slope ratio was not different between WT and APP/PS1 mice before (WT: 4.90 ± 0.30 , n=15; APP/PS1: 4.55 ± 0.18 , n=15, $p=0.332$) or after synaptic potentiation induced by HFS ($WT_{after\ HFS}$: 5.72 ± 0.33 , n=12; $APP/PS1_{after\ HFS}$: 6.08 ± 0.31 , n=11, $p=0.406$) whereas it was significantly increased by HFS in APP/PS1 mice (APP/PS1: 4.55 ± 0.18 , n=15; $APP/PS1_{after\ HFS}$: 6.08 ± 0.31 , n=11, *** $p<0.001$) but not for WT (WT: 4.90 ± 0.30 , n=15; $WT_{after\ HFS}$: 5.72 ± 0.33 , n=12, $p=0.332$). **D.** Bar graph represents average of normalized PSA between 50 and 60 minutes after HFS protocol in different conditions (WT: $187 \pm 14\%$, n=17; APP/PS1: $146 \pm 8\%$, n=16; WTmale: 150 ± 9 , n=15; WT+PGE₂ (10 μ M): $161 \pm 15\%$, n=17; WT+EP2 agonist (ONO-AE1-259-01, 1 μ M): $160 \pm 12\%$, n=17; WT+PGE₂ (10 μ M) +EP2 antagonist (PF, 10 μ M): $169 \pm 16\%$, n=16; APP/PS1+EP2 antagonist (PF, 10 μ M): $140 \pm 8\%$, n=17. * $p<0.05$, *t* test with Welch's correction. **E.** Bar graph represents levels of PGE₂ in whole hippocampus in WT and APP/PS1 female mice at different ages. Note the lack of significant differences of levels of PGE₂ whatever the genotype or the age (3 months: WT: 17.90 ± 2.06 pg/mg, n=13; APP/PS1: 13.07 ± 1.31 pg/mg, n=12, $p=0.073$, 6 months: WT: 18.98 ± 4.31 pg/mg, n=10; APP/PS1: 19.31 ± 4.02 pg/mg, n=10, $p=0.705$, 9 months: WT: 12.44 ± 1.40 pg/mg, n=12; APP/PS1: 12.57 ± 1.36 pg/mg, n=11, $p=0.951$).

	Amplitude (mV)	Overshoot (mV)	Peak time (ms)	RMP (mV)	AP threshold (mV)	t ₅₀ (ms)	R _{in} (MΩ)
WT	132.2 ± 2.1 (12)	62.5 ± 2.1 (12)	2.4 ± 0.1 (12)	-72.7 ± 0.9 (23)	-33.2 ± 2.7 (10)	1.76 ± 0.09 (12)	323 ± 15 (32)
APP/PS1	125.4 ± 2.8 (13)	55.7 ± 2.7 (13)	2.4 ± 0.1 (13)	-75.4 ± 0.7 (15)	-32.1 ± 2.2 (10)	1.74 ± 0.09 (13)	335 ± 14 (31)

Table 1. Comparison between action potentials properties in DG granule cells from WT and APP/PS1 female mice. Data are mean ± SEM. Values between brackets are n values. *p* values were obtained with Student's *t*-test (amplitude: *p*=0.070; overshoot: *p*=0.064; peak time: *p*=0.885; RMP: * *p*<0.05; AP threshold: *p*=0.764; t₅₀: *p*=0.848; R_{in}: *p*=0.552). RMP: resting membrane potential. t₅₀: time to get half amplitude of AP. R_{in}: input resistance

	WT				APP/PS1			
	Type 1	p (I vs II)	Type 2	p (Type 1 WT vs APP/PS1)	Type 1	p (I vs II)	Type 2	p (Type 2 WT vs APP/PS1)
1 st AP latency (ms)	160 ± 9.1 (13)	0.178	177.4 ± 8.5 (17)	0.809	163 ± 8.2 (17)	0.746	158.4 ± 12.1 (14)	0.198
ADP (mV)	8.2 ± 0.7 (13)	0.244	9.5 ± 0.8 (16)	0.217	9.4 ± 0.7 (17)	0.824	9.7 ± 0.8 (14)	0.897
fast AHP (mV)	18 ± 0.9 (13)	0.086	15.3 ± 1.1 (17)	0.525	17.2 ± 0.8 (17)	0.018 *	14.4 ± 0.8 (14)	0.561
slow AHP (mV)	10.3 ± 1 (13)	0.209	8.6 ± 0.9 (16)	0.318	9.2 ± 0.7 (17)	0.343	8.2 ± 0.7 (12)	0.764
I_{thr} (pA)	53.1 ± 3.4 (13)	0.493	58.2 ± 5.9 (17)	0.001 **	37.1 ± 2.9 (17)	0.078	44.3 ± 2.5 (14)	0.053
R_{in} (MΩ)	368 ± 20 (14)	0.003 **	279 ± 19 (16)	0.993	368 ± 16 (17)	0.004 **	294 ± 17 (14)	0.562

Table 2. Comparison of action potentials properties of type 1 and type 2 DG granule cells between WT and APP/PS1 female mice. Data are mean ± SEM. Values between brackets are n values. p values were obtained with Student's t -test. ADP: after depolarisation. AHP: after hyperpolarisation. I_{thr} : AP current threshold (minimal current intensity inducing an AP)

Supplements

This part is presenting results which have not been embedded in the manuscript for publication.

A. Contextual Fear Conditioning in old male mice (18 months)

As demonstrated by our results, APP/PS1 males were spared at least up to 12 months of age. We decided to investigate if males were still spared at even older ages, *i.e.* 18 months, and tested the ability of WT and APP/PS1 male mice at 18 months of age to discriminate between two highly similar contexts, context A vs context B, or between two very different contexts, context A vs context C. When testing the two highly similar contexts (A vs B), WT and APP/PS1 did not show differences in freezing percentages between context A vs context B (WT: $F_{\text{context A}}$: day 1, $65.64 \pm 5.89\%$, $n=28$; day 2, $70.41 \pm 6.38\%$, $n=27$; day 3, $71.21 \pm 4.91\%$, $n=27$; day 4, $81.24 \pm 3.37\%$, $n=27$; day 5, $79.58 \pm 3.69\%$, $n=27$; day 6, $72.53 \pm 4.40\%$, $n=27$; day 7, $77.51 \pm 3.62\%$, $n=27$; $F_{\text{context B}}$: day 1, $50.66 \pm 5.73\%$, $n=28$; day 2, $77.92 \pm 4.55\%$, $n=27$; day 3, $78.33 \pm 3.47\%$, $n=27$; day 4, $75.55 \pm 3.79\%$, $n=27$; day 5, $75.17 \pm 4.6\%$, $n=27$; day 6, $71.59 \pm 4.15\%$, $n=27$; day 7, $70.64 \pm 5.08\%$, $n=27$; APP/PS1: $F_{\text{context A}}$: day 1, $57.78 \pm 6.19\%$, $n=28$; day 2, $80.25 \pm 3.97\%$, $n=26$; day 3, $87.00 \pm 2.15\%$, $n=25$; day 4, $86.41 \pm 2.11\%$, $n=26$; day 5, $85.95 \pm 2.62\%$, $n=26$; day 6, $83.55 \pm 2.68\%$, $n=26$; day 7, $84.17 \pm 3.52\%$, $n=27$; $F_{\text{context B}}$: day 1, $52.98 \pm 6.11\%$, $n=28$; day 2, $81.95 \pm 4.26\%$, $n=27$; day 3, $89.85 \pm 3.45\%$, $n=24$; day 4, $86.12 \pm 3.53\%$, $n=25$; day 5, $89.60 \pm 2.31\%$, $n=25$; day 6, $87.86 \pm 3.34\%$, $n=26$; day 7, $82.54 \pm 4.42\%$, $n=27$) (Fig. 1A, 1B). When testing the two very different contexts (A vs C), WT and APP/PS1 did discriminate equally well (WT: $F_{\text{context A}}$: $68.77 \pm 3.52\%$, $n=13$; $F_{\text{context C}}$: $4.10 \pm 1.10\%$, $n=11$; *** $p<0.0001$; APP/PS1: $F_{\text{context A}}$: $72.91 \pm 4.84\%$, $n=14$; $F_{\text{context C}}$: $25.91 \pm 6.02\%$, $n=14$, *** $p<0.0001$) (Fig. 1C, 1D). These results indicated a lack of context fear generalization in old male mice.

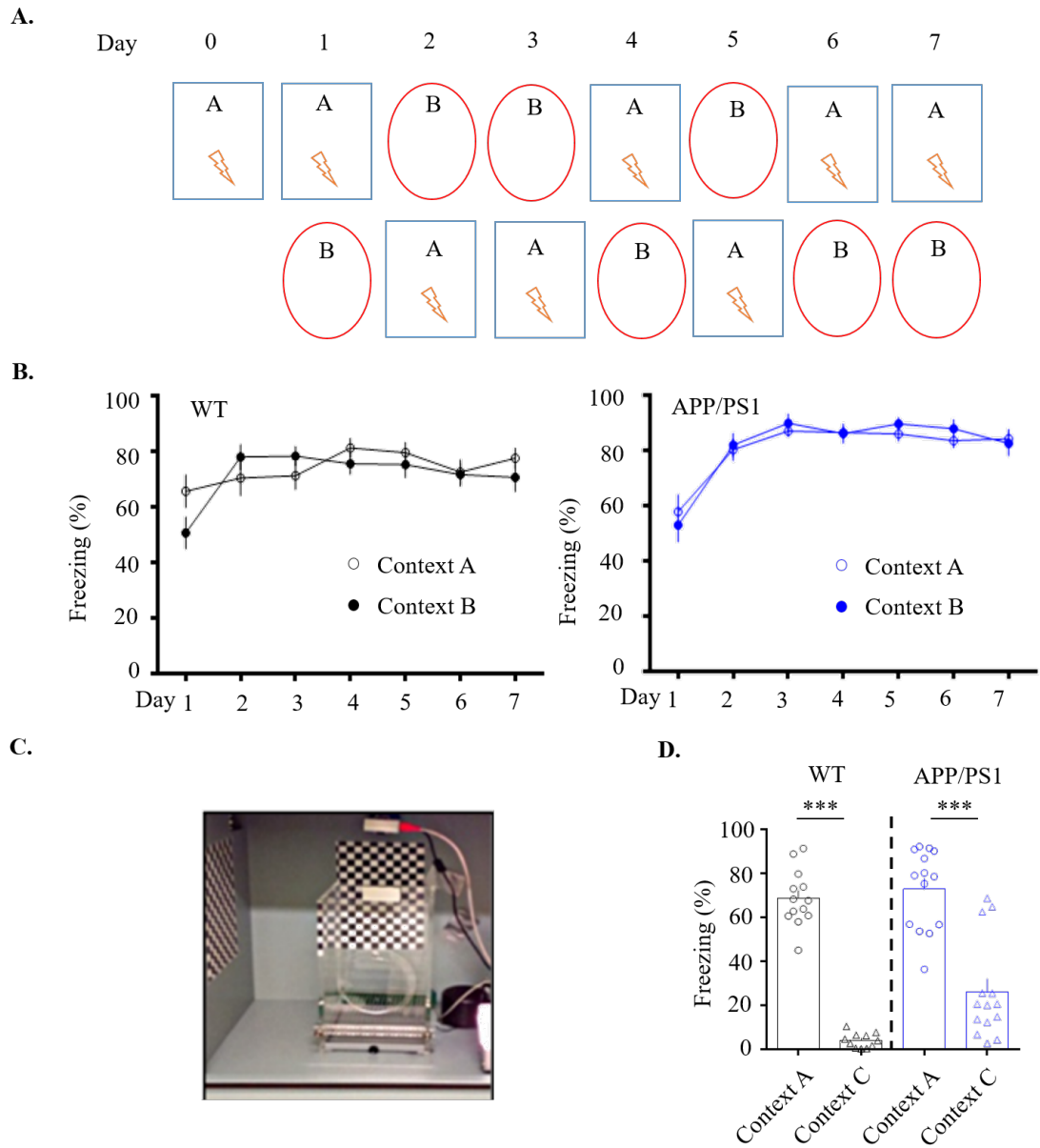


Figure 1: Contextual fear conditioning in 18 months old males

A. Experimental design for context A and context B. **B.** Similar freezing behavior of WT (black) and APP/PS1 (blue) mice in two highly similar contexts (context A and context B). **C.** CFC box. **D.** Contextual discrimination of WT (black) and APP/PS1 (blue) mice in very different contexts (context A and context C). *** $p < 0.0001$

B. APP/PS1 female mice do not present impaired presynaptic LTP at Mf-CA3 synapses

In a previous study we reported that presynaptic LTP at hippocampal MF/CA3 synapses was impaired in APP/PS1 males (Maingret *et al.*, 2017). We then investigated if the presynaptic LTP at Mf-CA3 synapses was also impaired in APP/PS1 female mice by recording fEPSPs. fEPSPs were evoked by minimal stimulation intensity at low frequency (0.1 Hz). The fEPSP slope is taken as a proxy to measure the excitatory drive to CA3 neurons.

Basal fEPSP slope were measured as 99.99 ± 3.75 % for WT female mice and 99.99 ± 3.75 % for APP/PS1 female mice at 4 months and were not significantly different. Long term synaptic potentiation (LTP) was triggered by high frequency tetanic stimulation (HFS, 3 x 100 Hz, every 10 s). The relative change in the fEPSP slope was 168.8 ± 13.59 % (n=8) of baseline during 30-40 min after HFS for WT and 154.3 ± 17.4 % (n=8, $p > 0.05$; Fig. 2A) for APP/PS1. The total blockade of fEPSPs by mGluR agonist LCCG-1 confirmed the recording of Mf-CA3 synaptic transmission (Fig. 2A).

Then we questioned whether LTP was altered in female mice at 6 months, an age for which both molecular and cellular hallmarks of AD were present, together with behavioral deficits. LTP was 163.8 ± 6.3 % (n=8) for WT and 148.78 ± 5.52 % (n=8, $p > 0.05$) for APP/PS1 (Fig. 2B).

Therefore, Mf-CA3 synaptic plasticity was not impaired in APP/PS1 female mice at 4 and 6 months of age despite prominent AD's alterations.

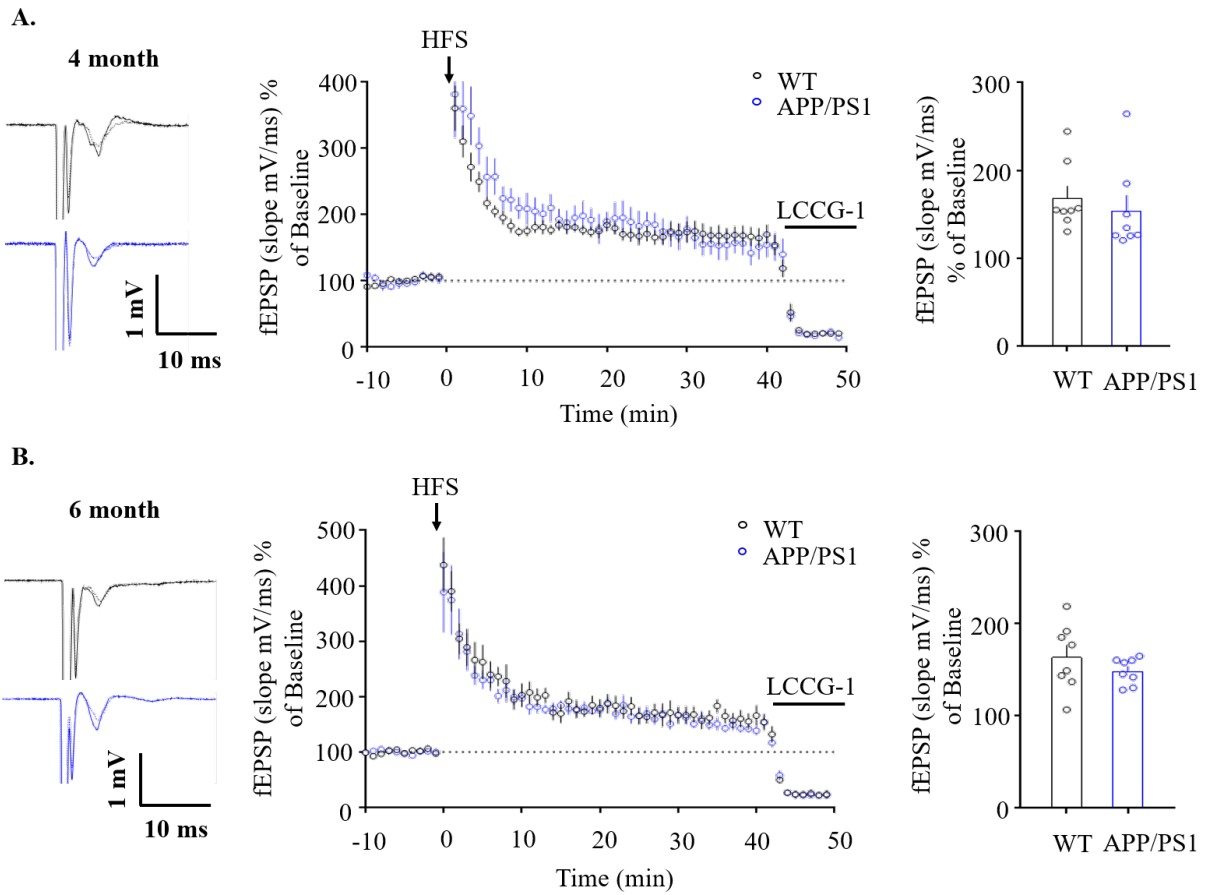


Figure 2: Presynaptic LTP at MF-CA3 synapses

A. Representative traces of WT and APP/PS1 female mice and time course of fEPSPs recorded in CA3 pyramidal neurons with low frequency (0.1 Hz) stimulation of Mf before (dotted line) and after HFS (solid line) in WT mice (black) and APP/PS1 mice (blue) at 4 months. Slope of fEPSPs were normalized as percentage of average baseline fEPSPs slope. Each point is an average of 6 subsequent fEPSPs. Scale bar is 10 ms for horizontal line and 1 mV for vertical line. Histograms show averaged relative fEPSPs slope recorded between 30 and 40 minutes (WT: 168.8 ± 13.59 %, $n = 8$; APP/PS1: 154.3 ± 17.4 %, $n = 8$; ns). **B.** Representative traces of WT and APP/PS1 female mice and time course of fEPSPs recorded in CA3 pyramidal neurons at 6 months. Histograms show averaged relative fEPSPs slope recorded between 30 and 40 minutes (WT: 163.8 ± 6.3 %, $n = 8$; APP/PS1: 148.78 ± 5.52 %, $n = 8$; ns).

C. Impaired LTP of population spikes in APP/PS1 mice at LPP/DG synapses

Then we investigated if synaptic potentiation was affected in APP/PS1 female mice at another synapse. We chose to investigate the lateral perforant pathway - DG synapse because of its location in the *stratum moleculare* of the DG, a region displaying a massive load of A β plaques at 6 months of age. To do so, we measured the population spike amplitude (PSA), which reflects APs discharge, between the upper positive deflection and the lower negative deflection (Fig. 3A). The basal stimulation intensity was chosen to evoke 25-30% of maximal PSA. This allowed us to compare the response of neurons in DG to the same excitatory drive before and after HFS (Fig. 3A). The relative potentiation of PSA was $186.6 \pm 14.33\%$ (n=17) for WT and significantly impaired in APP/PS1 ($146.5 \pm 8.52\%$, n=16, * $p < 0.05$; Fig. 3B). Interestingly, LTP in WT male mice was significantly lower than in age-matched WT female mice (male: $149.8 \pm 9.39\%$, n=15; female: $186.6 \pm 14.33\%$, n=17, * $p < 0.05$). LTP was purely NMDA-dependent as completely blocked by AP-V (Fig. 3B).

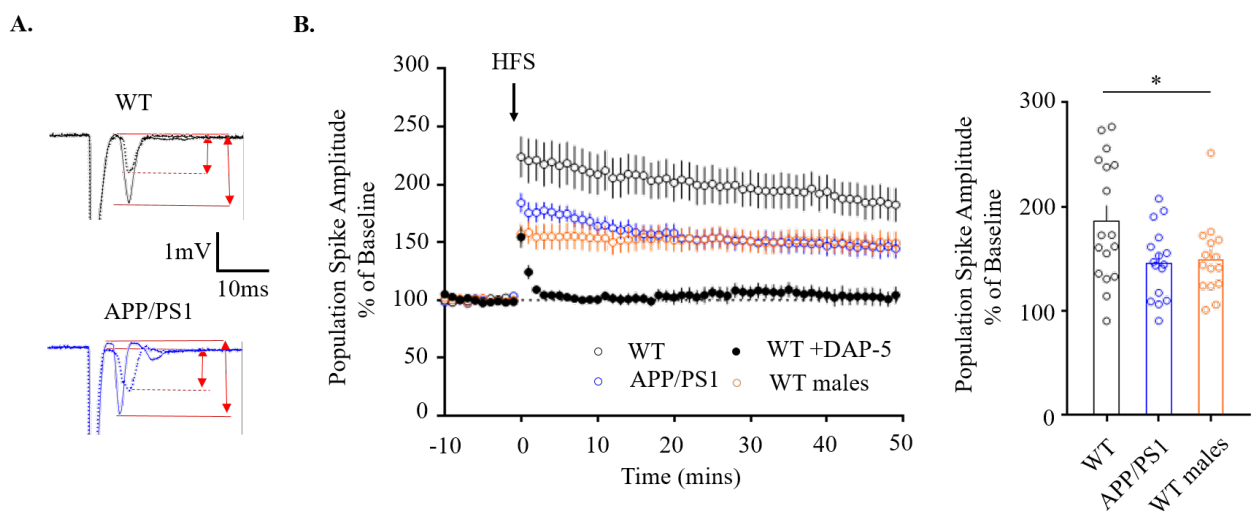


Figure 3: Population spike amplitude potentiation at LPP/DG synapses

A. Left panel shows representative traces of population spike amplitude (PSA) before and after HFS. Potentiation of PSA was induced by 3 bursts of 100 stimuli at 100 Hz with a 10-second inter burst interval (HFS). **B.** Middle panel shows time course of potentiation of PSA at 6 months (WT females, black circles; APP/PS1 females, blue circles; WT males, orange circles). Potentiation of PSA was completely blocked by AP-V (50 μ M, solid black spot) (n=9). Bar graph represents average of normalized PSA between 40 and 50 minutes after HFS protocol in different conditions (WT: $186.6 \pm 14.33\%$, n=17; APP/PS1: $146.5 \pm 8.52\%$, n=16, * $p < 0.05$).

D. PGE₂ and LTP at LPP-DG synapses

a. PGE₂

PGE₂ is a key neuroinflammatory molecule and of particular interest in AD studies (McGeer and McGeer, 2007). Having shown that PGE₂ impaired synaptic plasticity at the Mf-CA3 synapse (Maingret *et al.*, 2017), we investigated the effects of PGE₂ at LPP-DG synapse during the process of the neuroinflammation in APP/PS1 female mice.

Bath application of PGE₂ (10 μM) had no effect on synaptic basal transmission in WT female mice at LPP-DG synapses (baseline: $99.9 \pm 1.77\%$, n=8; PGE₂: $99.58 \pm 1.04\%$, n=8, $p > 0.05$; Fig. 4). These data indicated that exogenous application of PGE₂ did not affect basal transmission (Fig. 4 B).

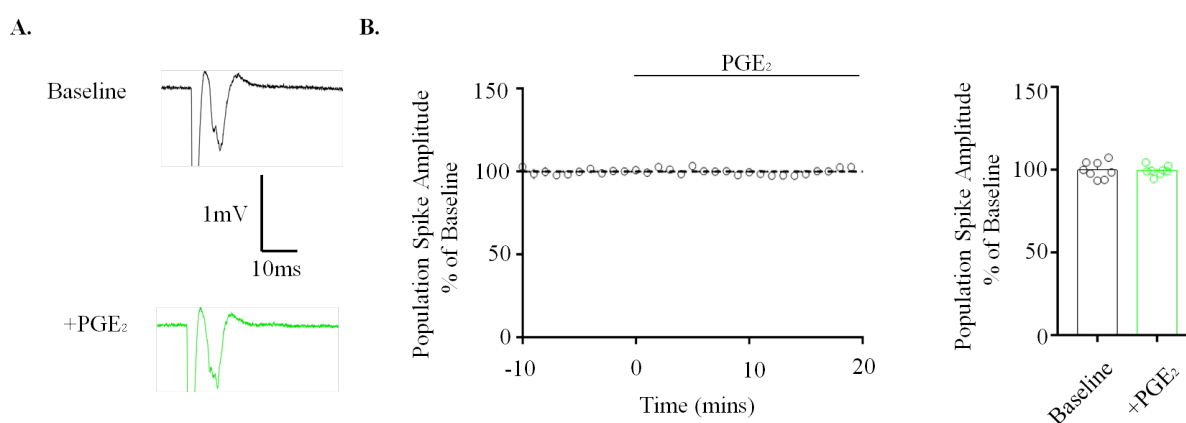


Figure 4: PGE₂ has no effect on basal transmission

A. Averaged traces of baseline (black) and application of PGE₂ (green). **B.** Representative time course of PS recorded in DG granule cells in response to 0.1 Hz stimulation of LPP in baseline (black circles) and after the application of PGE₂ (10μM, green circles). PS amplitude was normalized as percentage of average baseline of PS amplitude. Each dot corresponds to average of 6 consecutive PS amplitude recorded. PGE₂ (10μM) was performed after 10 mins of baseline and PS were acquired for 20 minutes. Scale bar is 10 ms for horizontal line and 1 mV for vertical line. Bar graph represent average amplitude of PS recorded between 10 and 20 minutes (baseline: $99.9 \pm 1.77\%$, n=8; PGE₂: $99.58 \pm 1.04\%$, n=8; $p > 0.05$).

b. EP4 receptor

NB: the putative involvement of EP2 and EP3 receptors is presented in **III. Results** section. Last we investigated the putative involvement of EP4 receptor. Pre-incubation of 10 μ M ONO-AE1-329, a selective EP4 receptor agonist, had neither an effect on LTP in WT (WT: 186.6 \pm 14.33 %, n=17; WT + PGE₂: 161.7 \pm 15.41%, n=17; WT + ONO-AE1-329: 149.2 \pm 13.72 %, n=14; $p > 0.05$) (Fig. 5A, 5B) nor ONO-AE2-227, a selective EP4 receptor antagonist, rescued impaired LTP in APP/PS1 mice (APP/PS1: 146.5 \pm 8.52 %, n=16; APP/PS1 + ONO-AE2-227, 166.8 \pm 9.2 %, n=10; $p > 0.05$) (Fig. 5A, 5B).

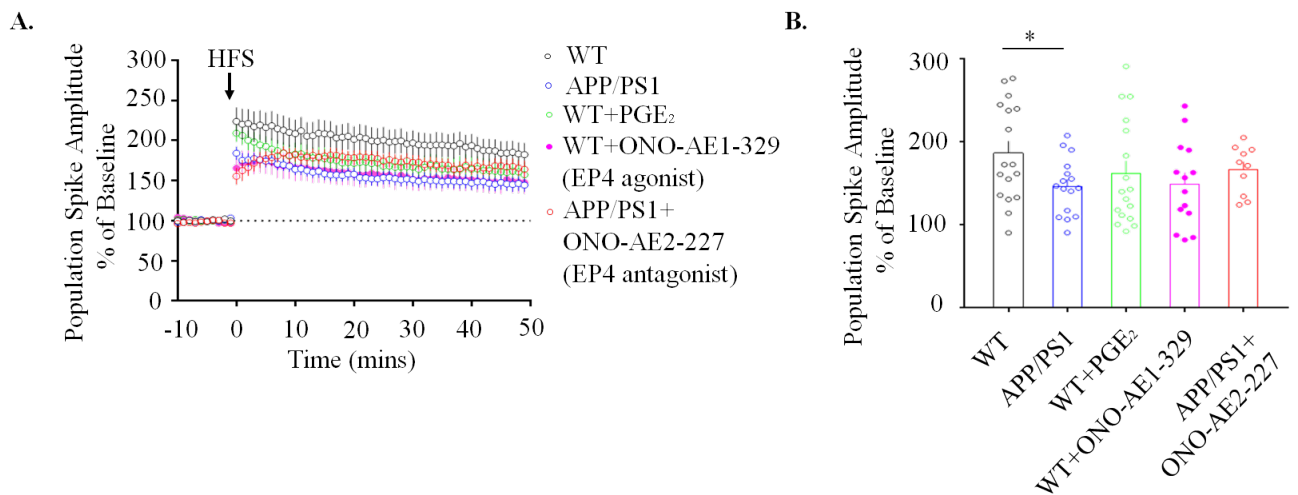


Figure 5: EP4 receptor has no effect at LPP-DG synapse

A. Average time course of LPP-DG LTP in WT and APP/PS1 female mice at 6 months. PSA LTP was induced by 3 bursts at 100 Hz with a 10s interburst interval. **B.** Bar graph represents average of normalized PSA between 40 and 50 minutes after the HFS protocol (WT: 186.6 \pm 14.33 %, n=17; WT + PGE₂: 161.7 \pm 15.41 %, n=17; WT + ONO-AE1-329: 149.2 \pm 13.72 %, n=14; APP/PS1: 146.5 \pm 8.52 %, n=16; APP/PS1 + ONO-AE2-227, 166.8 \pm 9.2 %, n=10).

E. APs number in APP/PS1 vs WT female mice

At the maximal current intensity (200 pA) and for a 250 ms step, we studied the number of spikes during the first and the last 50 ms of the step (Fig. 6A). During the first 50 ms, type I DG cells (WT: 2.92 \pm 0.21, n=13; APP/PS1: 3.70 \pm 0.20, n=17; * $p < 0.05$) but not type II DG cells (WT: 2.94 \pm 0.24, n=17; APP/PS1: 3.50 \pm 0.20, n=14) showed a significant increase of spikes number in APP/PS1 vs WT mice (Fig. 6B). For the last 50 ms, both type I and type II DG cells did not show significant spikes number between WT and APP/PS1 mice

(type I WT: 0.53 ± 0.14 , $n=13$; type I APP/PS1: 1 ± 0.24 , $n=17$; type II WT: 1.23 ± 0.13 , $n=17$; type II APP/PS1: 1.57 ± 0.17 , $n=14$) (Fig. 6C). However, WT type II DG cells significantly fired more spikes than type I DG cells (Type I: 0.53 ± 0.14 , $n=13$; Type II: 1.23 ± 0.13 , $n=17$; $** p < 0.05$) (Fig. 6C).

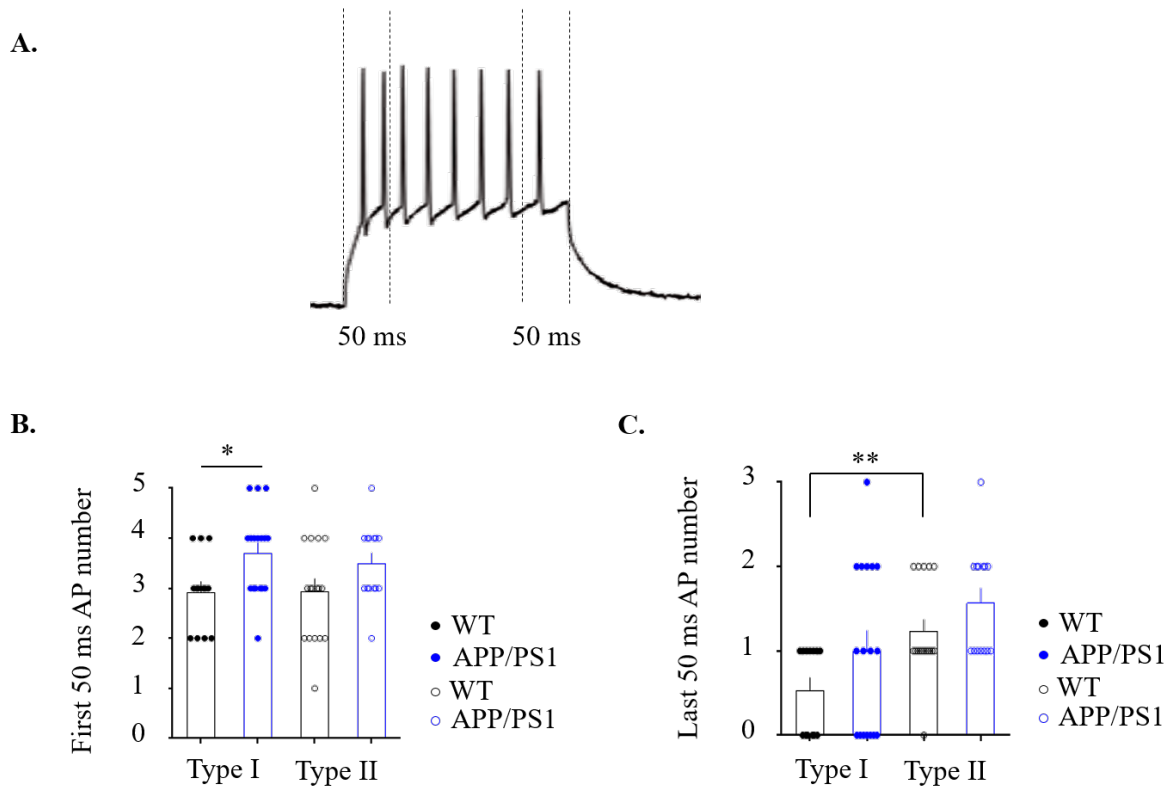


Figure 6: Increased spikes number in APP-PS1 mice

A. Spikes evoked in response to 250 ms prolonged depolarizing current at 200 pA. **B** and **C**: Spikes firing number for the first and last 50 ms (Type I, solid: WT: 2.92 ± 0.21 , $n=13$; APP/PS1: 3.70 ± 0.20 , $n=17$; $* p < 0.05$. WT: Type I: 0.53 ± 0.14 , $n=13$; Type II, circle: 1.23 ± 0.13 , $n=17$; $** p < 0.05$).

IV. Discussion - Conclusion

Gender Differences in LTP of population spike amplitude (PSA)

The physiological basis underlying gender differences is still elusive, but it is known for a long time that estrogen modulates neuronal cell activity in the hippocampus (Teyler *et al.*, 1980; Foy and Teyler, 1983; Wong and Moss, 1992). As such, the significant larger increase in LTP in WT female mice vs male mice could be related to estrogen levels.

Interestingly, in female rats, a large LTP was observed during proestrus when estrogen was expressed at a high level (Warren *et al.*, 1995). Furthermore, HFS did not induce LTP in males as large as in females when estrogen levels were low. In ovariectomized rats, administration of 17- β -estradiol promoted induction of LTP, showing that estrogen could indeed modulates LTP (Cordoba *et al.* 1997). Our results showed that WT female mice displayed significantly larger LTP when compared to males. Because all experiments have been done without consideration of the estrous cycle, we cannot conclude on a possible effect of estrogen but such effect is plausible and may deserve further investigation.

A β impaired LTP at LPP-DG synapse

In APP/PS1 female mice, DG region displayed a massive load of A β plaques when compared to other hippocampal regions. Entorhinal cortex is the main input to hippocampus via DG for new memory encoding, and mostly prone to dysfunction in early AD. Evidences suggest that A β may affect neuronal activity (Cirrito *et al.*, 2005) and affect LTP (Wang *et al.*, 2004; Gong *et al.*, 2006; Ahmed *et al.*, 2010). Our results showed the partial impairment of LTP in APP/PS1 female mice of 6 months of age at LPP-DG synapses. Many factors could contribute to this impairment, a decrease in the probability of transmitter release, a decrease in the reliability or the strength of individual synapses, a decrease in the number of functional synapses or receptors.

PGE₂

PGE₂ is of particular interest because its level is increased in the CSF of AD patients (Montine *et al.*, 1999; Combrinck *et al.*, 2006). PGE₂ can bind to four subtypes of receptors (EP1-4) which are all expressed in the hippocampus and its effects may vary accordingly. PGE₂ is produced mostly from COX-2 signaling pathway (Brock *et al.*, 1999; Vidensky *et al.*, 2003; Chen and Bazan, 2005) and it is an important modulator of hippocampal synaptic transmission and plasticity (Bazan, 2001; Chen *et al.*, 2002; Chen and Bazan, 2005).

Here we show that PGE₂ via the activation of EP3 receptors inhibits a presynaptic form of LTP at Mf-CA3 synapse whereas it had no effects on LTP at LPP-DG synapse whatever the subtype of EP receptor. Furthermore, to our surprise, hippocampal levels of PGE₂ were not increased with time in APP/PS1 females vs WT females.

Aβ and intrinsic excitability

Series of studies show that Aβ accumulation and abnormal excitatory neuronal activity are associated (Buckner *et al.*, 2005; Mintun *et al.*, 2006; Seeley *et al.*, 2009; Sperling *et al.*, 2009) but the molecular mechanisms are still illdefined.

It has been demonstrated that over activation of NMDA receptor has a crucial effect on increased excitability, which is thought to play a key role in AD. More specifically, Aβ oligomers has been illustrated to enhance NMDA receptor mediated currents in rat DG (Wu *et al.*, 1995) and to enhance NMDA receptor mediated hippocampal neuronal firing *in vivo* (Molnár *et al.*, 2004). The interaction of Aβ oligomers with NMDA receptor promotes a Ca²⁺ influx (Felice *et al.*, 2007) which induces an increase of intracellular calcium stocks and in turn the activation of ryanodine receptor (RyR) mediated Ca²⁺ induced Ca²⁺ release (CICR) from endoplasmic reticulum stores (Lima *et al.*, 2011). Ultimately, this lead to persistant neuronal depolarization and the activation of synaptotoxic calcium associated pathways (Ferreira and Klein, 2011).

In conclusion, the amyloid hypothesis of AD postulates that accumulation of Aβ in the brain is the primary influence driving AD pathogenesis (Selkoe *et al.*, 2000; Cirrito *et al.*, 2005; Hu *et al.*, 2018). The formation of Aβ is the result of cellular process via successive enzymatic cuts of APP by β-secretase and γ-secretase (Querfurth *et al.*, 2010). In the present work we have highlighted the presence of Aβ plaques in the hippocampus in both APP/PS1 male and female mice and demonstrated that the deposition was occurring earlier and heavier in female mice than in age-matched male mice.

Numerous publications have reported that Aβ was inducing synaptic dysfunction through changes of neuronal intrinsic excitability in AD brain (Zhang and Linden, 2003). Hence, application of soluble Aβ to cultured neurons (Cuevas *et al.*, 2011), brain slices (Minkeviciene *et al.*, 2009; Varga *et al.*, 2014; Ren *et al.*, 2014) or *in vivo* (Orbán *et al.*, 2010; Busche *et al.*, 2012), induced neuronal hyperexcitability in the hippocampus. These data are consistent with some AD mouse models for which increased Aβ levels are involved in altered neuronal activity, spontaneous activity and hyperexcitability activity (Palop *et al.*, 2007;

Palop and Mucke, 2010; Brown *et al.*, 2011; Kerrigan *et al.*, 2014). Alterations of synaptic activity is considered as an early event leading to cognitive deficits appearing in the course of AD (Mesulam *et al.*, 1999; Srivareerat *et al.*, 2009, 2011; Alkadhi *et al.*, 2010, 2011).

In the hippocampus, DG granule cells are susceptible to AD pathology (A.T. Dao *et al.*, 2016). Following these findings, our data showed that in the DG area, the deposition of A β plaques paralleled a decreased excitability of APP/PS1 female DG granule cells detected shown by the input/output (I/O) relationships. Hippocampal LTP is generally accepted to be an important molecular mechanism related to learning and memory processes (Abraham *et al.*, 2003; Malenka *et al.*, 2004). It has been proposed that depression of basal synaptic transmission and LTP reported in mouse models of AD could be related to the activation of presynaptic Ca²⁺ activated K⁺ channels, which modifies in turn excitability and transmitter release (Ye *et al.*, 2010).

The present study showed an increased intrinsic excitability and LTP impairment at LPP/DG synapse in a mouse model of AD. Although increased excitability activity and its cellular and functional consequences are typically reported in mouse models of AD, the mechanisms of A β -induced neuronal hyperexcitability remain to be clarified. Neuronal brain activity is also tightly controlled via a balance between excitation and inhibition neurotransmission and an imbalance may lead to hyperexcitability. NMDARs play an essential role in the hyperexcitability, which is also considered to be a potential target of AD.

Abnormal firing activity can seriously weaken or impair LTP *in vitro* (Schubert *et al.*, 2005; Salmani *et al.*, 2011). Interestingly, LTP and increased excitability activity may crossover. Therefore, attenuating excessive neuronal excitability activity could reverse HFS induced LTP impairment (Albensi *et al.*, 2007). According to these reports, administration of levetiracetam prevented aberrant network activity in hAPP mice and restored learning and memory function (Sanchez *et al.*, 2012) and had similar actions in patients with mild cognitive impairments (MCI) that have significant brain A β deposition (Bakker *et al.*, 2012). Collectively, our data show that A β -induced excessive excitatory activity in the hippocampus may have a crucial role in the development of early synaptic functional deficits in AD.

V. Perspectives

Although gender differences have been reported for many years, our understanding of the potential bases underlying these differences is relatively poor (Joseph *et al.*, 1978; Williams *et al.*, 1990; Roof *et al.*, 1993; Gresack and Frick, 2003; Clinton *et al.*, 2007; Richetin *et al.*, 2017). Clearly, more research is needed to investigate the etiology of sex differences in memory and synaptic functions.

Our studies may help to clarify that A β may affect hippocampal-dependent memory task and neuronal activity in a sex dimorphic manner. These findings could have significant benefits for a differential preventive treatment of the human pathology (Claxton *et al.*, 2013; Zissimopoulos *et al.*, 2017; Ferretti *et al.*, 2018). The complex network of gender differences should be taken into account when designing models for disease prevention and diagnosis as well.

To further address A β effects on neuronal activity, it would be necessary to evaluate it taking into consideration distances between recorded neuron and A β plaques (Busche *et al.*, 2008; Ovsepiyan *et al.*, 2016). In this respect, Ab form is an important issue (oligomers *vs* plaques) as soluble A β oligomers are described to impact more severely synaptic and cognitive dysfunction than plaques (Selkoe *et al.*, 2008; Shankar *et al.*, 2008; Mucke and Selkoe, 2012). Therefore, it will be relevant to assay A β 40/A β 42 levels in hippocampal homogenates from APP/PS1 females *vs* males and to compare them to the number of Ab plaques.

Although the putative effects of A β on LTD have not been studied in the present work, it is known that Ab may facilitate LTD (Snyder *et al.*, 2005; Li *et al.*, 2009; Chen *et al.*, 2013) by involving mGluR, p38MAPK and caspase-3 activities. Strikingly, same Ab - activated intracellular signaling pathways are involved in the blockade of hippocampal LTP (Wang *et al.*, 2004) which suggests that a crossover pathway could reinforce Ab induced LTP impairments (reviewed in Mucke and Selkoe, 2012).

Whereas we have evaluated how the intrinsic properties of type 1 and type 2 DG cells were affected in APP/PS1 female mice, we have not investigated if the impaired LTP was

different depending on the subtype of DG cells. This is an important issue as type 1 and type 2 are signatures of the degree of maturation and circuitry integration of DG cells which can be differentially impacted by the pathology.

So far, almost all clinical trials have failed and the number of AD patients is continuously growing. Therefore, there is an urgent need for new studies. Deciphering relationships between A β -associated aberrant excitatory neuronal activity and cognitive deficits in AD could present valuable insights into the mechanisms of early stages of AD to lead to new therapeutic methods.

Indeed, disruptions of the balance between excitatory and inhibitory activity are likely involved in the early processes of cognitive deficits in AD (Palop *et al.*, 2007; Leonard and McNamara, 2007). Here, we focused only on excitatory inputs onto granule cells in the DG but it would be necessary to study inhibitory activity that was also reported to be associated with impairments of both short and long term plasticity in DG (Palop *et al.*, 2007). As such, prominent increases of neuronal activity in hippocampal networks may possibly coexist with altered glutamatergic transmission in our mouse model of AD and the complex relationships between excitatory and inhibitory activity need to be further clarified.

VI. Annexes

PGE₂-EP3 signaling pathway impairs hippocampal presynaptic long-term plasticity in a mouse model of Alzheimer's disease

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ABSTRACT: Alzheimer's disease (AD) is a progressive neurodegenerative disease characterized by early cognitive deficits linked to synaptic dysfunction and loss. Considerable evidence suggests that neuroinflammation contributes to AD. Prostaglandin E₂ (PGE₂), a key neuroinflammatory molecule, modulates hippocampal synaptic transmission and plasticity. We investigated the effect of PGE₂ on synaptic transmission and presynaptic plasticity at synapses between mossy fibers from the dentate gyrus and CA3 pyramidal cells (Mf-CA3 synapse). These synapses are involved in mnemonic processes and consequently may be of relevance for AD. We provide evidence that although PGE₂ had no effect both on either basal transmission or short-term plasticity, it strongly impaired presynaptic Mf-CA3 long-term potentiation (LTP) by acting on PGE₂ receptor 3 (EP3) receptors. During aging, hippocampal levels of PGE₂ markedly increased in the APP/PS1 mouse model of AD and impaired specifically presynaptic LTP via a PGE₂-EP3 signaling pathway. In summary, the building up of PGE₂ during the progression of AD leads to specific impairment of hippocampal presynaptic plasticity and highlights EP3 receptors as a potential target to alleviate cognitive deficits in AD.

Keywords: Alzheimer's disease, Inflammation, PGE₂, Synaptic plasticity, Hippocampus, APP/PS1

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Introduction

Alzheimer's disease (AD) is a progressive and devastating neurodegenerative disease characterized by deficits in learning and memory processes. There is considerable evidence that neuroinflammation involving the activation of glial cells contributes to the disease progression and pathology (Akiyama *et al.*, 2000; Heneka and O'Banion, 2007). As such, epidemiological studies have revealed that chronic intake of nonsteroidal anti-inflammatory drugs (NSAIDs) reduced the prevalence of AD (McGeer and McGeer, 2007). Furthermore, clinical trials have revealed that NSAIDs, when given to asymptomatic patients, reduce AD incidence, whereas they have adverse effect on AD pathogenesis in its later stages (Breitner *et al.*, 2011).

When activated in neuroinflammatory conditions, glia release a plethora of neuroinflammatory molecules such as inflammatory cytokines, chemokines, and prostanoids (Eikelenboom *et al.*, 1994). The prostanoid prostaglandin E₂ (PGE₂) plays pivotal functions in inflammation (Bos *et al.*, 2004). PGE₂ is produced from arachidonic acid by the microsomal prostaglandin-E₂ synthase (mPGES) and by 2 rate-limiting enzymes, Cox-1 and Cox-2 (Smith *et al.*, 1991), which are expressed by hippocampal neurons (Yasojima *et al.*, 1999), astrocytes and microglia (Font-Nieves *et al.*, 2012). Although Cox-1 is constitutively expressed, Cox-2 and mPGES are strongly activated by and during neuroinflammation (Font-Nieves *et al.*, 2012). PGE₂ can bind to 4 different subtypes of G-protein-coupled receptors (EP1-4), which regulate adenylyl cyclase (AC) activity and/or phosphoinositol turnover and intracellular calcium mobilization (Breyer *et al.*, 2001).

PGE₂ plays an important role in the pathophysiology of AD. It is a primary target of NSAIDs, and its level is elevated together with the expression of Cox-2 in the brain of AD patients (Kitamura *et al.*, 1999; Montine *et al.*, 1999; Yasojima *et al.*, 1999). Both in vitro and in vivo, PGE₂ stimulates amyloid beta (A β) production by microglial cells, astrocytes, and neurons (Hoshino *et al.*, 2007). Selective inhibition of Cox-2 by NS398

acutely prevents the A β -induced impairment of long-term potentiation (LTP) of α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor-mediated synaptic transmission at CA3-CA1 synapses (Kotilinek *et al.*, 2008). Exogenous addition of PGE₂ did not impair by itself LTP in wild-type mice but prevented the ability of NS398 to restore LTP impairment induced by synthetic A β suggesting complex interactions between PGE₂, A β , and postsynaptic forms of LTP (Kotilinek *et al.*, 2008).

AD, at least in its early stage, is thought to involve synaptic dysfunction and loss (Jacobsen *et al.*, 2006; Selkoe, 2002; Sheng *et al.*, 2012). Connections between the dentate gyrus (DG) and the hippocampal CA3 region through mossy fiber (Mf) synapses have been proposed to participate in the rapid encoding of novel memory (Kesner, 2007), a mnemonic process particularly affected in AD conditions. Mf synapses onto CA3 pyramidal cells display a wide dynamic range of presynaptic plasticity, including prominent short-term plasticity and a form of LTP that is independent of N-methyl-D-aspartate (NMDA) receptors (Henze *et al.*, 2002; Salin *et al.*, 1996). Alterations in Mf synaptic function in the context of AD have only been studied in senescent (24- to 25-month old) Tg2576 mice that is long after a massive load of amyloid plaques (Witton *et al.*, 2010).

Here we thus studied Mf-CA3 synaptic function in the APP^{swe}/PS1 Δ E9 (APP/PS1) mouse model of AD in relation with PGE₂. APP/PS1 mice are characterized by an over-production of A β protein leading to a noticeable load of amyloid plaques around 12 months of age (Jankowsky *et al.*, 2004) and display synaptic dysfunction (Volianskis *et al.*, 2010) and learning deficits (Lagadec *et al.*, 2012; Reiserer *et al.*, 2007). We report that acute application of PGE₂ impairs presynaptic Mf-LTP in young mice through activation of EP3 receptors. In APP/PS1 mice, presynaptic Mf-LTP was noticeably impaired at 12 months of age in parallel with a building up of endogenous levels of PGE₂ in the hippocampus. Mf-LTP could be fully rescued by blockade of EP3 receptors. Considering the diverse physiological roles of PGE₂, directly targeting the EP3 receptor may prove to be a more specific

therapeutical strategy in AD than the global inhibition of prostaglandins by NSAIDs.

Materials and methods

2.1. Ethical approval

Animal anesthesia and euthanasia procedures were carried out in accordance with the Animal Protection Association of ethical standards and the French legislation concerning animal experimentation and were approved by the University of Bordeaux/CNRS Animal Care and Use Committee (#55).

2.2. Animals

The animals used in this study were male APP/PS1 mice obtained from Jackson Laboratory (Bar Harbor, ME, USA) and their wild-type (WT) littermates (C57BL6/J). The APP/PS1 mice express a chimeric mouse/human amyloid precursor protein APP_{swe} (mouse APP695 harboring a human A β domain and mutations K595N and M596L linked to Swedish familial AD) and a human presenilin 1 mutated in familial AD (PS1 Δ E9; deletion of exon 9). These bigenic mice were created by co-injection of both transgenes allowing for a co-segregation of the transgenes as a single locus (Jankowsky *et al.*, 2004). Mice were allowed free access to food and water and maintained in a 12 hours dark/light cycle. Mice were genotyped and systematically re-genotyped after each experiment using transcript-specific primers (Table S1).

2.3. Acute hippocampal slices

C57BL6/J mice (P19-P21) were sacrificed by cervical dislocation. The brain was quickly removed from the skull and chilled in ice-cold low-calcium artificial CSF containing the following (in mM): 87 NaCl, 2.5 KCl, 1.25 NaH₂PO₄, 0.5 CaCl₂, 7 MgCl₂, 10 glucose, 25 NaHCO₃ and 75 sucrose with pH 7.4 adjusted by saturating with carbogen (95% O₂ and 5% CO₂), and an osmolarity of 300-310 mOsm. Isolated brains were glued onto the stage of a vibratome (VT 1200S, Leica Microsystems, Nussloch, Germany), and parasagittal hippocampal slices (320 μ m) were cut. Slices were then transferred in an extracellular solution containing the

following (in mM): 129 NaCl, 2.5 KCl, 1.25 NaH₂PO₄, 2 CaCl₂, 1 MgCl₂, 11 glucose, 25 NaHCO₃ equilibrated with 95% O₂ and 5% CO₂ (310-320 mOsm) for 30 minutes at 33 °C and thereafter maintained at room temperature until required.

Because of the well-documented difficulty in preparing healthy acute brain slices from mature adult rodents and in accordance with Animal Protection Association of ethical standards and the French legislation, APP/PS1 and WT mice at 6, 9, and 12 months were deeply anesthetized with a mix of ketamine (80 mg/kg; intraperitoneal) and xylazine (16 mg/kg; intraperitoneal) and then intracardially perfused for 2-3 minutes with a protective solution at 4°C containing the following (in mM): 2 KCl, 1.25 NaH₂PO₄, 0.4 CaCl₂, 8 MgCl₂, 22 glucose, 26 NaHCO₃, 200 sucrose, 2.8 pyruvic acid, 0.4 ascorbic acid, equilibrated with 95% O₂ and 5% CO₂ (310-320 mOsm). When the solution coming out of the heart was free of blood, the mouse was sacrificed by decapitation, and the brain was quickly removed from the skull and stored in the same solution. Parasagittal brain slices (320 μ m) were cut in the protective solution for which sodium chloride was replaced by sucrose, and then incubated at 33 °C for 30 minutes in a resting solution containing the following (in mM): 103 NaCl, 2 KCl, 1.25 NaH₂PO₄, 0.27 CaCl₂, 8 MgCl₂, 22 glucose, 26 NaHCO₃, 0.4 ascorbic acid equilibrated with 95% O₂, and 5% CO₂ (300-310 mOsm). Slices were thereafter maintained at room temperature in the same solution until required.

2.4. Electrophysiological studies on acute hippocampal slices

For recording, slices were transferred into a recording chamber where they were submerged and continuously perfused with an oxygenated (95% O₂ and 5% CO₂) extracellular solution at 32 °C-34 °C. Whole-cell patch clamp recordings (3-4 M Ω electrodes, 70 mV holding potential) were made from hippocampal CA3 pyramidal neurons visualized by infrared videomicroscopy. Patch clamp electrodes were pulled out from borosilicate glass (GF 150 F-10) and filled with an internal solution containing the following (in mM): 140 CsCH₃SO₃, 2 MgCl₂, 4 NaCl, 5 phospho-creatine, 2 Na₂ATP, 0.2

EGTA, 10 HEPES, and 0.33 GTP (pH 7.3) adjusted with CsOH. Bicuculline (10 μ M) was added to the bath to inhibit g-aminobutyric acid-A receptors. Voltage-clamp recordings were performed on CA3 pyramidal neurons identified with a differential interference contrast microscope (Eclipse FN-1, Nikon, Champigny sur Marne, France) equipped with an infrared camera (VX 44, Till Photonics, Gräfelfing, Germany) using an Axopatch-200B amplifier (Axon Instruments, Sunnyvale, CA, USA). Signals were filtered at 2 kHz and digitized at 5 kHz via a DigiData 1322A interface (Axon Instruments). Series resistance (10-20 M Ω) was monitored during the recording using a 10 mV hyperpolarizing voltage step of 50 ms length occurring at the beginning of each recording. Neurons were rejected if more than a 20% change in series resistance occurred during the experiment. Neurons with a holding current exceeding 300 pA at a holding potential of -70 mV were also rejected. Data were collected and analyzed using pClamp software 9.2 (Axon Instruments).

Mf-CA3 excitatory postsynaptic currents (EPSCs) were evoked by minimal intensity stimulation (Marchal and Mulle, 2004). A glass microelectrode (~1 μ m tip diameter) was placed in the hilus of the DG to stimulate Mfs. The baseline stimulation frequency for all experiments was 0.1 Hz. Mf synaptic currents were identified according to the following criteria: robust low-frequency facilitation (ratio 1/0.1 Hz), low release probability at 0.1 Hz, rapid rise times of individual EPSC ((~1 ms), and EPSCs decays free of secondary peaks that may indicate the presence of polysynaptic contamination. The group II mGluR agonist (2S,1'S,2'S)-2-(Carboxycyclopropyl) glycine (LCCG)-1 (10 μ M) was applied routinely to confirm that Mfs were actually stimulated. Extracellular recordings were made from the stratum lucidum using glass electrodes (1-2 M Ω) filled with extracellular solution. Field excitatory postsynaptic potentials (fEPSPs) were evoked in response to electrical stimuli applied via another glass electrode (1-2 M Ω) filled with extracellular solution placed in the hilus of the DG. Signals were low-pass filtered at 20 kHz and digitally sampled at 50 kHz. All experiments were performed in the presence of D-AP5 (50 μ M) to block NMDA receptor activity, bicuculline (10 μ M), and (2S)-3-((1S)-1-[3,4-dichlorophenyl]ethyl)

amino-2 hydroxypropyl)(phenylmethyl)phosphinic acid hydrochloride (CGP) (3 μ M) to block, respectively, g-aminobutyric acid-A and -B receptors. Input-output curves were collected by varying stimulation strength from 40 μ A to 120 μ A in 40 μ A intervals. For subsequent experiments on basal transmission and synaptic plasticities, the stimulus strength was set to a level that produced 50% of the maximal responses.

2.5. Tissue processing and laser capture microdissection

C57/Bl6J mice (Jackson Laboratory, Bar Harbor, ME, USA) at P21 were anesthetized with intraperitoneal administration of pentobarbital (50 mg/kg body weight), and the brain was removed and rapidly frozen in heptane and stored at -80 °C until sectioning. For slicing, the frozen brain was mounted onto a chuck with Tissue-Tek O.C.T compound (Sakura Finetek France, Villeneuve-d'Ascq, France) and placed in a Leica CM3050S cryostat (Leica Microsystems, Wetzlar, Germany) for 20 minutes to equilibrate with the chamber temperature of 20 °C. Then, 60- μ m-thick sections of the hippocampus region were cut and placed on polyethylene naphthalate (PEN) Membrane Frame slides (Carl Zeiss, Munich, Germany) under RNAase-free conditions. Then, the slides were immediately placed on dry ice and stained on the same day using cresyl violet. Briefly, slides were transferred from dry ice into ice-cold 95% ethanol for 40 seconds and incubated in 75% ethanol for 30 seconds and in 50% ethanol for 30 seconds. Specimens were briefly stained in 1% cresyl violet solution. Tissue sections were dehydrated through 50% ethanol (30 seconds), 75% ethanol (30 seconds), 95% ethanol (30 seconds), followed by two 40 seconds incubations in anhydrous 100% ethanol. Slides were dried for 5 minutes at room temperature. Immediately after dehydration, laser capture microdissection (LCM) was performed using a P.A.L.M. MicroBeam microdissection system Version 4.8 equipped with a P.A.L.M. RoboSoftware (P.A.L.M. Microlaser Technologies AG, Bernried, Germany). Microdissection was performed at 5X magnification. Samples were collected in adhesives caps (P.A.L.M. Microlaser Technologies AG, Bernried, Germany).

2.6. RNA isolation, reverse transcription, and quantitative PCR

To limit RNA degradation during isolation, samples were collected for up to 30 minutes per slide, after which the LCM caps were placed on a sterile microcentrifuge tube containing 350 μ L of lysis buffer. The samples were stored at -80°C until extraction was done. Total RNA was extracted from microdissected tissues, i.e., CA3 and DG, using the RNeasy micro Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol and eluted with 14 μ L of RNase-free water. The concentration of RNA was determined using Nanodrop 1000 and the integrity of RNA was determined using RNA 6000 Pico Kit and Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). The RNA quality was assessed according to the RNA integrity number. A RNA integrity number threshold value of 7 was fixed, and samples below this value were discarded. 0.2 μ g of total RNA was reverse transcribed to cDNA using RevertAid Premium Reverse Transcriptase (Fermentas) and primed with oligo-dT primers (Fermentas) and random primers (Fermentas). Quantitative polymerase chain reaction (q-PCR) was performed using a LightCycler 480 Real-Time PCR System (Roche, Meylan, France). q-PCR reactions were done in duplicate for each sample, using transcript-specific primers (Table S2), cDNA (4 ng), and LightCycler 480 SYBR Green I Master (Roche) in a final volume of 10 μ L. The PCR data were exported and analyzed with Gene Expression Analysis Software Environment developed at the Neurocentre Magendie. For the determination of the reference gene, the geNorm method was used (Bustin *et al.*, 2009). Relative expression analysis was corrected for PCR efficiency and normalized against the housekeeping gene succinate dehydrogenase complex subunit A (Sdha). The relative level of expression was calculated using the comparative ($2^{-\Delta\Delta\text{CT}}$) method (Livak and Schmittgen, 2001).

2.7. Immunohistological staining and analysis

WT and APP/PS1 mice at 6, 9, and 12 months of age were anesthetized with intraperitoneal administration of pentobarbital (50 mg/kg body weight) and were fixed by transcardial perfusion with

buffered saline and 50 mL of 4% paraformaldehyde (PFA). Brains were removed, postfixed in 4% PFA overnight, and cut into 40 μ m-thick frontal sections on a vibratome. Sections were collected into PBS 0.1 M and stored at 4°C until the staining protocol. After a thorough wash in TBST (tris buffer sodium: 0.0384-M trisma base 0.1263-M NaCl, pH: 7.4 p 0.1% Tween 20), free-floating sections were incubated with TBST-10% Normal Goat Serum 0.3% Triton X-100, for 1 hour at room temperature. Then sections were incubated with primary antibody in TBST-3.5% Normal Donkey Serum overnight at 4°C (chicken anti-glial fibrillary acidic protein (GFAP): 1/1000 USBiologicals; rabbit anti-ionized calcium-binding adapter molecule (Iba)-1: 1/5000 Wako; mouse anti- $\text{A}\beta_{1-16}$: 1/150 Millipore). After washes in TBST, sections were incubated with secondary antibodies for 1 hour (Goat anti-chicken Alexa 647, anti-mouse Alexa 555, and anti-rabbit Alexa 488: 1/500, Invitrogen). Slices were finally washed and mounted in mounting medium (Fluoromount + DAPI, SouthernBiotech). Slices were imaged using the up-right widefield microscope Leica DM5000 (Leica Microsystems, Nanterre, France) using objectives HC PL Fluotar 20X NA 0.5. Fluorescence excitation was performed by a LED SOLA Light (Lumencor, Beaverton, USA). Images were obtained by the resolute cameras CoolSnap HQ2 (Photometrics, Tucson, USA) and a cooled QICAM (QImaging, Surrey, Canada). A galvanometric stage (Leica Microsystems) allowed the z stack reconstructions. The mosaics were made using a motorized stage Scan (Märzhäuser, Wetzlar, Germany). This system was controlled by MetaMorph software (Molecular Devices, Sunnyvale, USA). Analyses were performed with Image J software (National Institutes of Health, New York, USA) on the maximum intensity fluorescence projections of the 3D mosaics. The distribution of GFAP positive cells (number of astrocytes per mm^2), GFAP labelling, and Iba-1 positive cells (number of microglia per mm^2) were represented as box plot for which the top of each box indicates the 75th quartile (Q3), the bottom indicates the 25th quartile (Q1), and the bar inside the box is the median (Q2). The whiskers extend out to the most extreme data point that is at most 1.5 times the interquartile range above the third quartile ($Q3 + 1.5 \times [Q3 - Q1]$) or below the first quartile ($Q3 - 1.5 \times [Q3 - Q1]$).

2.8. Enzyme immunoassay determination of PGE₂

The concentration of PGE₂ was quantified both in WT and APP/PS1 at 6, 9, 12, and 15 months of age. After anesthesia induction by intraperitoneal administration of pentobarbital (50 mg/kg body weight), the hippocampus was rapidly dissected and immediately frozen into liquid nitrogen. The concentration of PGE₂ present in the hippocampus was quantified with a specific PGE₂ competitive EIA kit (ACETM EIA kit, Cayman Chemical, Ann Arbor, MI, USA) following the procedures detailed in the instructions. The detection limit of this EIA kit was 15 pg/mL (80% B/Bo). Absorbance was measured at 415 nm by a microplate reader model 680 (Bio-Rad, Marnes-la-Coquette, France).

2.9. Statistical analysis

To eliminate bias, experimenters were kept blind to the genotype of the mice until all analysis was completed. Values are presented as mean standard error of the mean of *n* experiments. Unless stated otherwise, a Student *t* test for 2 groups and a 1-way analysis of variance with Bonferonni's test for more than 2 groups were used for statistical comparison. The *p*-values given are 2-tailed and were considered significant if *p* < 0.05. Statistical analysis was performed with Prism 5.0 (GraphPad Software, La Jolla, CA, USA).

2.10. Drugs

LCCG-1, bicuculline, D-2-amino-5-phosphonopentanoate (D-AP5), and CGP55845 were obtained from Tocris biosciences. Sulprostone, PGE₂, and forskolin were obtained from SigmaAldrich. ONO compounds were a generous gift from ONO Pharmaceuticals Co Ltd (Japan).

Results

3.1. PGE₂ impairs presynaptic long-term potentiation at MfCA3 synapses

We tested the effects of PGE₂ on Mf-EPSCs recorded from CA3 pyramidal cells of P19-P21 wild-type mice using the whole-cell voltage clamp mode of the patch clamp technique (Fig. 1A). Mf-EPSCs evoked by minimal intensity stimulation at low

frequency (0.1 Hz) were not affected by bath application of 1-10 μM PGE₂ (Fig. 1B). Mf-CA3 synapses display prominent forms of presynaptic short-term plasticity (Nicoll and Schmitz, 2005), including paired-pulse facilitation (PPF: 2 stimulations separated by 10-200 ms), and frequency facilitation (FF: switching from 0.1 Hz to >1 Hz tonic stimulation). PGE₂ (10 μM) neither affected the pronounced PPF (Fig. 1C) nor FF (Fig. 1D). Last, we investigated possible effects of PGE₂ on post-tetanic potentiation, another form of presynaptic short-term plasticity triggered by a single train of 100 stimuli at 100 Hz and characterized by a robust increase in Mf-EPSCs amplitude lasting several minutes. As shown in Fig. 1E, PGE₂ (10 μM) did not affect post-tetanic potentiation.

Altogether, exogenous application of PGE₂ affected neither basal synaptic transmission nor different forms of presynaptic short-term plasticity at Mf-CA3 synapses in young (P19-P21) mice. We then investigated whether PGE₂ affected LTP of Mf-EPSCs (Mf-LTP), a presynaptic form of plasticity which is independent of NMDA receptors (Nicoll and Schmitz, 2005). In the control condition, a high-frequency stimulus (HFS: 3 trains of 100 stimuli at 100 Hz) triggered presynaptic Mf-LTP as measured by the mean amplitude of Mf-EPSCs recorded 30-40 minutes after HFS (Fig. 1F). Bath application of 10-μM PGE₂ starting 10 minutes before HFS fully prevented Mf-LTP (Fig. 1F). In contrast, when applied after the induction of Mf-LTP (PGE₂ AI), PGE₂ had no effect (Fig. 2A, right panel). These results indicate that PGE₂ impairs the induction of Mf-LTP but not its maintenance.

3.2. PGE₂ impairs Mf-LTP by acting on EP3 receptors

PGE₂ can bind to 4 subtypes of G-proteincoupled receptors, namely EP1, EP2, EP3, and EP4 (Breyer *et al.*, 2001). Markedly, the expression of messenger RNAs (mRNAs) coding for EP2 and EP3 receptors has been reported in mouse and rat hippocampus (Zhu *et al.*, 2005). Thus, we investigated which subtype of PGE₂ receptor was involved in the impairment of Mf-LTP. Sulprostone (10 μM), a potent EP3 receptor agonist (Coleman *et al.*, 1987) fully prevented Mf-LTP, exactly

mimicking the effect of PGE₂ (Fig. 2A). In contrast, ONO-AE1-259-01 (1 μM), a specific EP2 receptor agonist (Mori *et al.*, 2009) had no effect on Mf-LTP (142 ± 26, n = 5; p = 0.903; Fig. 2A). ONO-AE3-240, a specific EP3 receptor antagonist fully prevented the impairment of LTP induced by PGE₂ (Fig. 2A, left panel). Altogether these results are strong evidence that the EP3 receptor is solely responsible for the impairment of LTP. The lack of effect of the EP2 agonist rules out the involvement of the EP4 receptor since it is coupled to the same intracellular signaling pathway as EP2. Finally, because it is coupled to phosphatidylinositol-calcium second messenger system, the EP1 receptor does not regulate AC activity and therefore is unlikely to be involved in presynaptic plasticity at Mf-CA3 synapses. Therefore, we focused on EP3 receptors in the rest of the study.

3.3. Mechanisms of action of EP3 receptors at Mf-CA3 synapses

EP3 receptors are coupled to G proteins through a Gi/o subunit (Hatae *et al.*, 2002) which negatively regulates AC activity. Presynaptic 3',5'-cyclic adenosine monophosphate (cAMP) is crucial for the regulation of presynaptic mechanisms at Mf-CA3 synapses; presynaptic receptors coupled to Gs subunits, such as the 5-hydroxytryptamine 4 receptor (5-HT₄) (Kobayashi *et al.*, 2008) or dopamine receptor 1 (D1) (Kobayashi *et al.*, 2012) potentiate Mf-CA3 synaptic transmission. To test the hypothesis that PGE₂ negatively modulates AC activity through the activation of EP3 receptors, we investigated the effect of sulprostone on a moderate increase of AC activity induced by forskolin (2.5 μM). Forskolin progressively increased Mf-EPSC amplitude (Fig. 2B) and sulprostone (10 μM) markedly reduced this potentiation (Fig. 2C).

Both the reduction of forskolin-induced potentiation of synaptic transmission and the action on presynaptic Mf-LTP by PGE₂ are consistent with a presynaptic locus of action for PGE₂. Unfortunately, there is currently no suitable antibody to ascertain a presynaptic localization of the EP3 receptor in Mf terminals by immunocytochemistry. Commercially available antibodies for EP3 receptors lead to a strong background staining in brain sections in both EP3^{+/+} and EP3^{-/-} mice (data not shown), which

precluded any relevant conclusion (see also Shi *et al.*, 2012). We thus investigated the expression of mRNA coding for EP3 receptors (EP3 mRNA) using LCM of hippocampal neurons from the CA3 region and from the granule cell layer of the DG in brain slices followed by quantitative real-time reverse transcriptase quantitative polymerase chain reaction (RT-qPCR). Slices were first stained with cresyl violet to visualize better CA3 and DG regions (Fig. 3A). Gene expression level of Ptger3, which codes for the EP3 receptor, was normalized to the housekeeping gene succinate dehydrogenase subunit A (Sdha) and mRNA expression was presented relative to that in the CA3 region of P19-P21 mice. RTqPCR indicated the expression of EP3 mRNA by DG granule cells (mean threshold cycle [Ct]: 31.54 ± 0.15, n = 3) further supporting the localization of the EP3 receptor in Mfs. EP3 mRNA was also expressed, although at a much lower level, in the pyramidal cell layer of CA3 (mean Ct: 32.74 ± 0.55, n = 3), and the relative expression of EP3 mRNA was 2 fold in DG as compared to CA3 (2.1 fold 0.3, n = 3; p < 0.05) (Fig. 3B). Altogether, these results strongly suggest that PGE₂ acting on presynaptic EP3 receptors controls the modulation of Mf-CA3 synaptic transmission by cAMP.

3.4. Neuroinflammatory status of wild type versus APP/PS1 mice

It is well established that at the clinical diagnosis stage, AD brains display extracellular deposits of Aβ plaques accompanied by a prominent inflammatory reaction characterized by periplaque microgliosis and astrogliosis (Akiyama *et al.*, 2000). We characterized the neuroinflammatory status of the hippocampus in WT and APP/PS1 mice at different ages (6, 9, and 12 months of age). Aβ plaques were stained by an antibody raised against Aβ₁₋₁₆. Until 9 months of age, Aβ plaques were barely detectable in the hippocampus of APP/PS1 mice. At 12 months of age, Aβ plaques were virtually absent in WT mice (Fig. 4A), whereas they were abundant in APP/PS1 mice (per hippocampus, WT: 0.3 ± 0.5 plaques; APP/PS1: 21 ± 5 plaques, n = 4, p < 0.001; Fig. 4B). Strikingly, Aβ plaques appeared to be nonuniformly distributed throughout the hippocampus but rather concentrated in the stratum lacunosum moleculare and the molecular layer of the

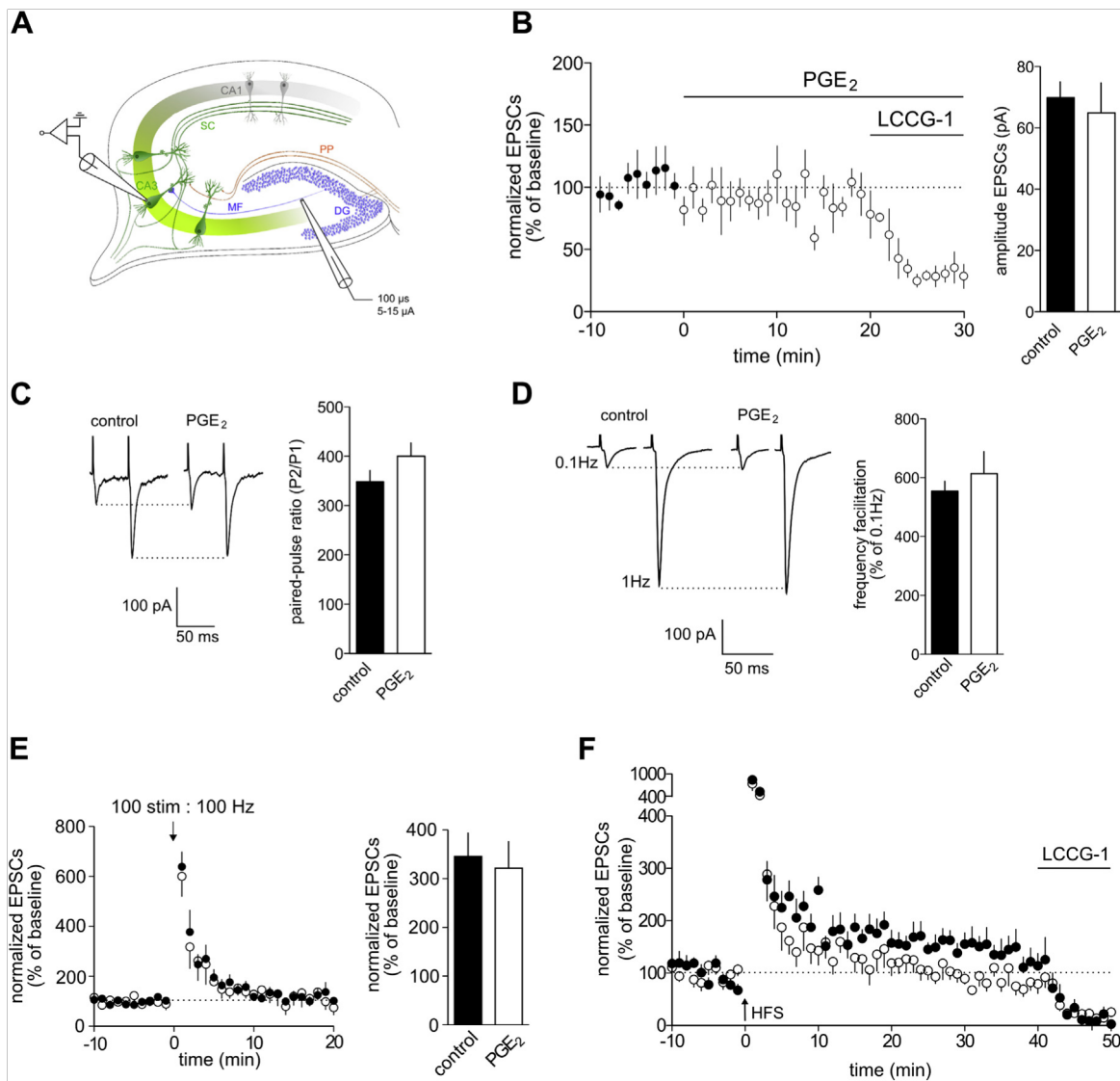


Fig. 1. PGE₂ impairs presynaptic long-term potentiation at Mf-CA3 synapses. (A) Diagram shows the positions of the stimulating electrode (located in the mossy fiber tract) and of the recording electrode (CA3 neuron, whole-cell configuration of the patch clamp technique). (B) Representative time course of EPSCs recorded in CA3 pyramidal neurons (P19-P21) in response to low frequency (0.1 Hz) stimulation of mossy fiber in control conditions (black circles) and in the presence of PGE₂ (10 μM; white circles). Note that EPSCs were evoked by minimal intensity stimulation (100 ms, 5-15 mA). EPSCs amplitude was normalized as percentage of average baseline EPSCs amplitude. Each dot corresponds to average of 5 consecutive EPSCs recorded for 8 cells. PGE₂ (10 mM) was added after 10 minutes of baseline, and EPSCs were recorded for the following 30 minutes. The complete inhibition of EPSCs by group II mGluR agonist LCCG-1 (10 μM) confirmed that they were Mf-EPSCs. Bar graph represents average amplitude of EPSCs recorded between 10 and 20 minutes (control: 69.6 ± 5.6 pA, n = 8; PGE₂: 64.1 ± 10.1 pA, n = 8; p = 0.28). (C) Average of 30 traces (left) illustrating paired-pulse facilitation (PPF) at 40 ms of Mf-EPSCs in control condition or in presence of PGE₂ (10 mM). Bar graph represents average paired pulse ratio at 40 ms interstimulus interval (control: 348 ± 23%, n = 12; PGE₂: 400 ± 28%, n = 12, p = 0.17). (D) Average of 50 traces (left) illustrating frequency facilitation (FF) of Mf-EPSCs when increasing the simulation frequency from 0.1 Hz to 1 Hz in control condition or in presence of PGE₂ (10 μM). Bar graphs of mean FF from 0.1 Hz to 1 Hz (control: 554 ± 34%, n = 12; PGE₂: 613 ± 76%, n = 12, p = 0.096). (E) Representative time course of post-tetanic potentiation induced by stimulation at 100 Hz during 1 second. Bar graph represents average of normalized EPSCs during the 5 first minutes following the tetanic stimulation (control: 346 ± 48 %, n = 8; PGE₂: 322 ± 55 %, n = 7, p = 0.742). (F) Time course of the effect of PGE₂ (10 μM) on mossy fiber presynaptic LTP. Mf-LTP was induced by 3 bursts of 100 stimuli at 100 Hz with a 10-second interburst interval (HFS). PGE₂ was bath applied 10 minutes before HFS. Note the complete prevention of LTP induced by PGE₂ (10 μM). Each dot corresponds to average of 6 consecutive EPSCs recorded for n cells. Data are presented as mean standard error of the mean. Abbreviations: DG, dentate gyrus; EPSCs, excitatory postsynaptic currents; LTP, long-term potentiation; Mf, mossy fiber; PGE₂, prostaglandin E₂; PP, perforant pathway; SC, shaffer collaterals.

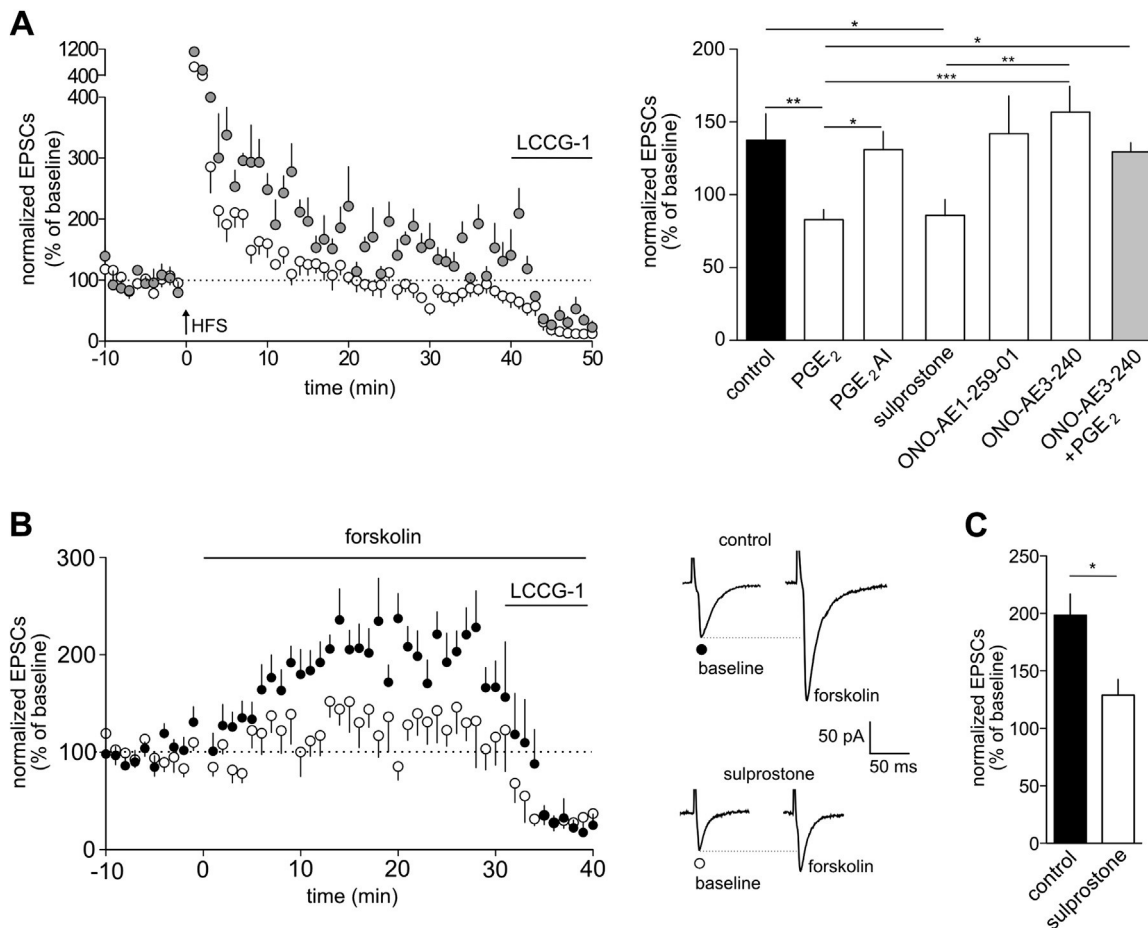


Fig. 2. PGE₂ impairs Mf-LTP by acting on EP3 receptors. (A) Left panel: ONO-AE3-240 (1 μM) (light gray symbols) rescued impairment by PGE₂ of presynaptic Mf-LTP. Right panel: Bar graph represents average of normalized EPSCs between 30 and 40 minutes after the HFS protocol in different conditions (PGE₂ AI stands for PGE₂ after induction) (control: 137 ± 18%, n = 10; PGE₂: 83 ± 7%, n = 11; **p < 0.01; PGE₂ AI: 136 ± 12%, n = 5, *p < 0.05; sulprostone: 86 ± 11%, n = 7, *p < 0.05; ONO-AE1-259-01: 142 ± 26%, n = 5; ONO-AE3-240: 159 ± 18%, n = 5; **p < 0.01 vs. sulprostone and ***p < 0.001 vs. PGE₂; ONO-AE3-240 + PGE₂: 134 ± 6%, n = 5). Note that when applied after the induction of LTP, PGE₂ had no effect. Note that sulprostone (EP3 receptor agonist) fully mimicked PGE₂ induced blockade of LTP, and ONO-AE3-240 fully prevented the blockade of LTP induced by PGE₂. ONO-AE1-259-01 (1 μM; EP2 receptor agonist) had no effect on LTP. (B) Time course of Mf-EPSCs potentiation induced by bath application of forskolin (2.5 μM) in control condition and in presence of sulprostone (10 μM). Mf-EPSCs were averaged every minute and normalized to the mean amplitude of the 10 minutes preceding bath application of forskolin. Representative currents (averages of 60 EPSCs) recorded between 20 and 30 minutes after forskolin application illustrating the effect of sulprostone on forskolin-induced potentiation of Mf-EPSCs. (C) Bar graph represents the effect of sulprostone on forskolin-induced potentiation of Mf-EPSCs (control: 198 ± 18%, n = 9; sulprostone: 128 ± 13%, n = 6, *p < 0.05). The complete inhibition of EPSCs by group II mGluR agonist LCCG-1 (10 μM) in A, B, and C confirmed that they were Mf-EPSCs. Data are presented as mean standard error of the mean. Abbreviations: EPSCs, excitatory postsynaptic currents; HFS, high-frequency stimulus; PGE₂, prostaglandin E2.

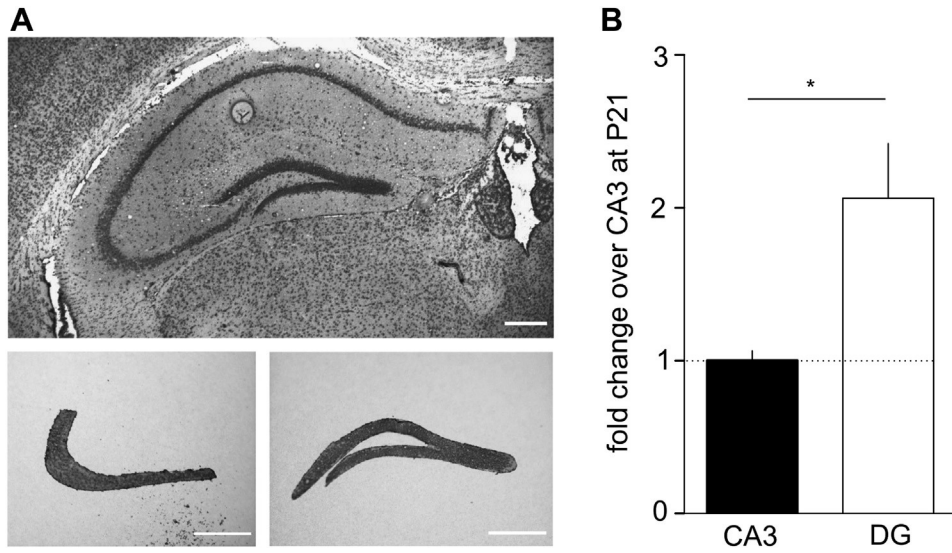


Fig. 3. EP3 receptor expression in dentate gyrus (DG) and CA3 pyramidal layer. (A) Laser capture microdissection (LCM) of DG and CA3 neurons. Hippocampii were stained with cresyl violet. Upper panel: intact hippocampus before LCM. Left lower panel: LCM captured CA3 neuronal layer. Right lower panel: LCM captured DG. Bar = 300 μ m. (B) Quantitative real-time polymerase chain reaction of hippocampal EP3 receptor mRNA derived from wild-type mice (P21) in DG and CA3 pyramidal layer. Bar graph illustrates the increase of expression in DG compared to CA3 pyramidal layer (2.1 fold \pm 0.3, $n = 3$, per group; * $p < 0.05$).

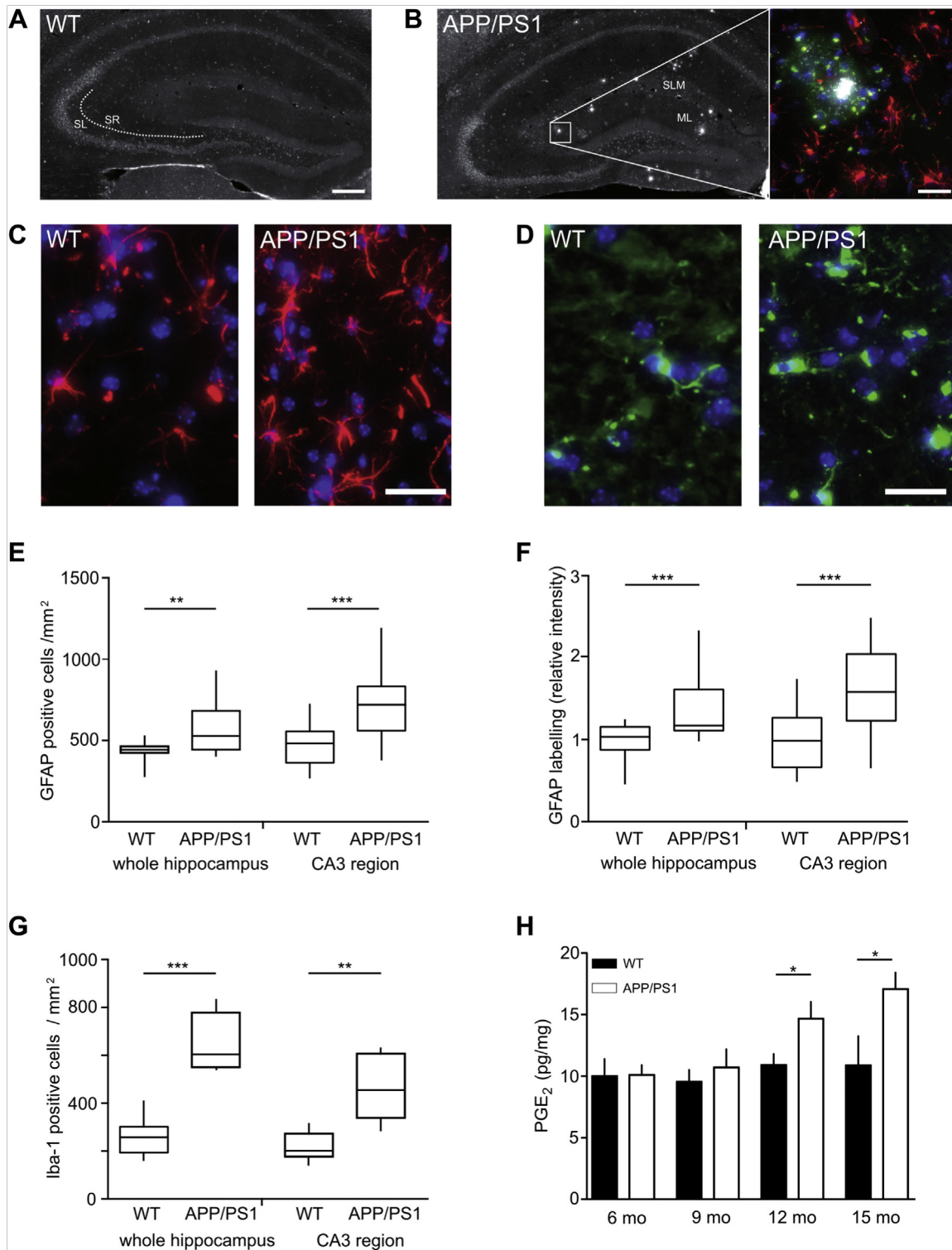


Fig. 4. Neuroinflammatory status in APP/PS1 mice. (A) Representative micrograph of a hippocampus in a WT mouse (12 months of age) double stained for nuclei with 40,6-diamidino-2-phenylindole (DAPI) and for A β plaques (anti-A β ₁₋₁₆ antibody). No A β plaques were detected. Bar scale = 250 mm. (B) Representative micrograph of a hippocampus in a APP/PS1 mouse (12 months of age). Note the presence of A β plaques (white signal) mainly distributed in the stratum lacunosum moleculare and the molecular layer (ML) of the DG. Bar scale = 250 mm. Right panel: close up of an A β plaque (white signal) surrounded by glial cells. Note the large cell bodies with short and thick processes of astrocytes (GFAP, red signal) in the vicinity of the plaque. Microglia (Iba-1, green signal) are also present. (C) High-magnification images of GFAP (red) and nuclei (blue) in WT and APP/PS1 mice. Scale bar = 50 mm. (D) High-magnification images of Iba-1 (green) and nuclei (blue) in WT and APP/PS1 mice. Scale bar = 50 mm. (E) Box plot showing GFAP positive cells /mm² in the whole hippocampus and CA3 region for WT and APP/PS1 mice. Significant differences are indicated by ** (p < 0.01) and *** (p < 0.001). (F) Box plot showing GFAP labelling (relative intensity) in the whole hippocampus and CA3 region for WT and APP/PS1 mice. Significant differences are indicated by *** (p < 0.001). (G) Box plot showing Iba-1 positive cells /mm² in the whole hippocampus and CA3 region for WT and APP/PS1 mice. Significant differences are indicated by *** (p < 0.001) and ** (p < 0.01). (H) Bar graph showing PGE₂ (pg/mg) levels in WT (black bars) and APP/PS1 (white bars) mice at 6, 9, 12, and 15 months of age. Significant differences are indicated by * (p < 0.05).

green signal) walling off the plaque displayed round cell bodies with retracted processes. Bar scale = 10 mm. (C) Magnification of representative hippocampal area in the CA3 region (stratum lucidum and part of stratum radiatum) of WT and APP/PS1 mice (12 months of age). Note the increase in the number of GFAP positive cells (astrocytes, red signal) in APP/PS1 versus WT mice. Nuclei are stained with DAPI (blue signal). Bar scale = 10 mm. (D) Iba-1 positive cells (microglia, green signal) are more numerous in APP/PS1 versus WT mice. Nuclei are stained with DAPI (blue signal). Bar scale = 10 mm. (E) Box plot showing the distribution of GFAP positive cells (number of astrocytes per mm) in whole hippocampus and CA3 region in WT and APP/PS1 mice. Whole hippocampus: WT: Q1 = 426, Q2 = 443, Q3 = 465; APP/PS1: Q1 = 447, Q2 = 528, Q3 = 681; CA3 region: WT: Q1 = 365, Q2 = 468, Q3 = 554; APP/PS1: Q1 = 559, Q2 = 720, Q3 = 829. (F) Box plot showing the distribution of GFAP labeling of astrocytes in whole hippocampus and CA3 region in WT and APP/PS1 mice. Whole hippocampus: WT: Q1 = 0.87, Q2 = 1.03, Q3 = 1.15; APP/PS1: Q1 = 1.11, Q2 = 1.17, Q3 = 1.60; CA3 region: WT: Q1 = 0.67, Q2 = 0.98, Q3 = 1.26; APP/PS1: Q1 = 1.22, Q2 = 1.58, Q3 = 2.02. (G) Box plot showing the distribution of Iba-1 positive cells (number of microglia per mm²) in whole hippocampus and CA3 region in WT and APP/PS1 mice. Whole hippocampus: WT: Q1 = 195, Q2 = 259, Q3 = 301; APP/PS1: Q1 = 549, Q2 = 604, Q3 = 770; CA3 region: WT: Q1 = 176, Q2 = 202, Q3 = 272; APP/PS1: Q1 = 338, Q2 = 452, Q3 = 602. A 1-way analysis of variance (ANOVA) followed by a KruskalWallis post hoc test was used in D, E, and G (*p < 0.05, **p < 0.01, ***p < 0.001). (H) Bar graph represents levels of PGE₂ in whole hippocampus in WT and APP/PS1 at different ages. Note the significant increase of PGE₂ levels at 12 and 15 months of age in APP/PS1 mice versus WT mice. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

PGE₂ is a key neuroinflammatory molecule linked to AD in epidemiological studies (McGeer and McGeer, 2007) and in transgenic mouse models of AD (Kotilinek *et al.*, 2008; Liang *et al.*, 2005; Shi *et al.*, 2012). We then quantified PGE₂ within the hippocampus during aging for both genotypes. No significant difference in PGE₂ levels was observed between WT and APP/PS1 mice (Fig. 4H) at ages up to 9 months (6 months: WT: 10.0 ± 1.4 pg/mg, n = 8; APP/PS1: 10.1 ± 0.84 pg/mg, n = 9; p = 0.957; 9 months: WT: 9.54 ± 0.98 pg/mg, n = 14; APP/PS1: 10.71 ± 1.51 pg/mg, n = 12; p = 0.511). In contrast, PGE₂ levels were increased in APP/PS1 mice compared to WT mice at 12 and 15 months of age (12 months: WT: 10.91 ± 0.9 pg/mg, n = 18; APP/PS1: 14.67 ± 1.36 pg/mg, n = 17; *p < 0.05; 15 months: WT: 10.87 ± 2.38 pg/mg, n = 6; and APP/PS1: 17.07 ± 1.42 pg/mg, n = 13; Fig. 4H). Interestingly, the expression of hippocampal EP3 mRNA was reported to increase for the same APP/PS1 mouse model (Shi *et al.*, 2012). These results show that APP/PS1 mice displayed, together with the load of Aβ plaques and a marked activation of astrocytes and microglia, increased hippocampal levels of PGE₂.

3.5. Presynaptic Mf-LTP is impaired in 12-month-old APP/PS1 mice

Since PGE₂ alters synaptic plasticity at Mf-CA3 synapses in WT mice, we investigated what could be the impact of increased levels of PGE₂

caused by neuroinflammation in APP/PS1 mice. Because of the very low rate of success in obtaining patch-clamp recordings from 6, 9, and 12-month-old mice, we recorded fEPSP in the remainder of the study. We did not observe any differences between WT and APP/PS1 mice in input-output relationships for fiber volley amplitude versus fEPSP slope at 6, 9 (data not shown), and 12 months of age (Fig. 5A). Similarly, neither PPF nor FF was differentially affected between the 2 genotypes (Table 1). No difference in the extent of Mf-LTP was observed between APP/PS1 mice and their WT littermates at 9 months of age (Fig. 5B). In contrast, presynaptic Mf-LTP was impaired in APP/PS1 mice at 12 months of age (Fig. 5C).

3.6. Role of EP3 receptors in Mf-LTP deficits in APP/PS1 mice

Strikingly, the alteration of Mf-LTP was concomitant with the increased levels of PGE₂ in APP/PS1 mice (see Fig. 4H). We thus tested whether the building up of ambient PGE₂ produced within the course of the pathology in APP/PS1 mice was involved in the impairment of presynaptic Mf-LTP observed at 12 months of age. On the one hand, blocking EP3 receptors with ONO-AE3-240 (1 μM) added 30 minutes before the HFS burst rescued Mf-LTP in APP/PS1 mice to a similar extent as Mf-LTP in WT mice but had no effect per se on Mf-LTP in WT mice (Fig. 5E). On the other hand, activating EP3 receptors with sulprostone (1 μM)

did not significantly impair further Mf-LTP in APP/PS1 mice (Fig. 5C). Last, presynaptic Mf-LTP was strongly impaired by PGE₂ (10 μM) an effect faithfully mimicked by 1 μM sulprostone (Fig. 5D), demonstrating the functionality of the PGE₂-EP3 pathway in WT littermates.

Taken together, these results provide strong evidence that PGE₂ acting via EP3 receptors is responsible for the impairment of Mf-LTP in 12-month-old APP/PS1 mice, an age where the building up of endogenous PGE₂ due to neuroinflammation, presumably reaches a critical level to impact EP3 receptors.

Discussion

Our findings demonstrate that PGE₂, acting through the EP3 receptor subtype, inhibits presynaptic long-term plasticity at Mf-CA3 synapses. We further provide evidence that in the APP/PS1 mouse model of AD, the PGE₂-EP3 receptor signaling pathway is responsible for the impairment of presynaptic LTP at Mf-CA3 synapses by showing that the blockade of EP3 receptors fully rescues the LTP at 12-month-old, an age characterized by a neuroinflammatory reaction and chronically elevated levels of the neuroinflammatory molecule PGE₂. These results highlight EP3 receptors as a potential therapeutical target to alleviate synaptic dysfunction, and possibly also cognitive deficits associated with AD.

4.1. PGE₂ inhibits Mf-LTP by acting on EP3 receptors likely localized presynaptically

PGE₂- and Cox-2-signaling pathways modulate hippocampal synaptic transmission and/or plasticity (Chen and Bazan, 2005; Sang *et al.*, 2005; Yang *et al.*, 2009). Depending on the concentration, PGE₂ can either be beneficial or detrimental to hippocampal transmission and/or plasticity. Endogenous basal levels of PGE₂ regulate membrane excitability, synaptic transmission, and plasticity in hippocampal CA1 pyramidal neurons (Chen and Bazan, 2005; Chen *et al.*, 2002) and are necessary for memory acquisition (Rall *et al.*, 2003),

consolidation (Teather *et al.*, 2002), and retention (Cowley *et al.*, 2008; Sharifzadeh *et al.*, 2006; Shaw *et al.*, 2003). In the course of neuroinflammation, Cox-2 and microsomal PGE₂ synthase expression levels are increased in different mouse models of AD (Akitake *et al.*, 2013; Ferretti *et al.*, 2012) in parallel with impairments of synaptic plasticity and cognitive deficits. These data are coherent with a key role of PGE₂, which is further supported by the induction of cognitive deficits in transgenic mice overexpressing Cox-2 in hippocampal neurons (Andreasson *et al.*, 2001).

As a part of a complex system, PGE₂ can bind to 4 different subtypes of receptors (EP1-4). Depending on the subtype, the activation of EP receptors leads to changes in the production of cAMP and/or in phosphoinositol turnover and intracellular calcium mobilization (Breyer *et al.*, 2001). Our data show that ONO-AE1-259-01, a specific EP2 receptor agonist (Mori *et al.*, 2009), had no effect on presynaptic Mf LTP, therefore ruling out any significant involvement of this receptor. Furthermore, as the EP4 receptor is coupled to the same intracellular signaling pathway as the EP2 receptor, namely the G_s-AC-cAMP pathway leading to an increase of cAMP production, our data also rule out a significant involvement of the EP4 receptor in PGE₂-induced impairment of Mf LTP.

Here we show that activation of EP3 receptors by either exogenous PGE₂ in WT mice or endogenous PGE₂ in APP/PS1 mice inhibits a presynaptic form of LTP at Mf-CA3 synapses. EP3 receptors couple with the G_i-AC-cAMP pathway leading to inhibition of cAMP generation (Breyer *et al.*, 2001; Irie *et al.*, 1994). Two isoforms of AC are expressed by hippocampal neurons; AC1 and AC8 (Wang *et al.*, 2003; Xia *et al.*, 1991). AC8 activity is exclusively dependent on intracellular Ca²⁺ (Nielsen *et al.*, 1996), whereas AC1, which is highly expressed in DG cells (Xia *et al.*, 1991), is both regulated by G proteins and intracellular Ca²⁺ (Willoughby and Cooper, 2007). Therefore, it is likely that only AC1 may be linked to the PGE₂-EP3 signaling pathway. Our results are consistent

with other studies showing that simple deletion of AC1 (Villacres *et al.*, 1998) or double deletion of AC1/AC8 (Wang *et al.*, 2003) do not affect basal properties at Mf-CA3 synapses. However, we observed a normalization of Mf-EPSCs amplitude following application of a moderate concentration of forskolin, an activator of AC which is known to enhance Mf-CA3 synaptic transmission. Hence, the activation of PGE₂-EP3 signaling pathway does not directly modulate synaptic basal transmission; however, it may interact with pathways linked to AC, when previously activated. In keeping with this, we found that PGE₂ impaired presynaptic Mf-LTP, an effect mimicked by sulprostone (EP3 agonist) and blocked by ONO-AE3-240 (EP3 antagonist), whereas ONO-AE1-259-01 (EP2 agonist) had no effect. These data provide strong evidence for an effect of PGE₂ mediated by EP3 receptors, but not EP2/EP4 receptors, and indicate that PGE₂ acts by downregulating AC activity triggered during the HFS bursts. Because of the impact of EP3 receptor activation on presynaptic mechanisms, we propose that EP3 receptors are localized in presynaptic terminals. Indeed, many lines of evidence coherently point at a presynaptic localization of EP3 receptors at Mf-CA3 synapses. First, PGE₂ modulates the release of neurotransmitters by Mf through a EP3 receptor G_i-AC-cAMP pathway. Second, the PGE₂-EP3 receptor signaling pathway specifically impairs the presynaptic form of LTP at Mf-CA3 synapses. Third, DG cells express mRNA coding for EP3 receptor. Last, the PGE₂-EP3 receptor signaling pathway regulates the hippocampal expression of presynaptic proteins such as synaptophysin, synapsin 1, SNAP-25, or VAMP-2 but not the expression of proteins belonging to the postsynaptic density (Shi *et al.*, 2012). However, due to the lack of a suitable antibody against EP3 receptors (this study; Shi *et al.*, 2012), we were not able to ascertain its cellular localization. All the data are consistent with EP3 receptors being expressed at Mfs, but they cannot rule out a putative glial expression of EP3 receptors (Slawik, 2004). However, glial process has a restricted access to Mf-CA3 synaptic cleft in physiological conditions (Rollenhagen *et al.*, 2007),

which seems to preclude a major role for glial EP3 receptor in the modulation of Mf synaptic plasticity.

4.2. Elevation of PGE₂ during AD-linked neuroinflammatory processes is responsible for impairment of synaptic plasticity at Mf-CA3 synapses

Most relevant to our study, PGE₂-EP3 signaling seems to be a key component in the progression of the pathology in the APP/PS1 mouse model of Alzheimer's disease (Shi *et al.*, 2012). The present finding that PGE₂-EP3 negatively controls presynaptic Mf-LTP in WT mice prompted us to investigate if this plasticity was altered in APP/PS1 mice during the course of the disease and if the PGE₂-EP3 signaling pathway was involved. So far, most of the studies focussed on synaptic failure in mouse models of AD have not addressed whether and how progressive neuroinflammatory processes are linked with synaptic dysfunction. Here, we showed that presynaptic Mf-LTP was impaired in APP/PS1 mice starting at 12 months of age, whereas presynaptic short-term plasticity was not. Strikingly, this impairment paralleled the increase in hippocampal levels of PGE₂ in APP/PS1 mice that we could detect starting at 12 months of age. Furthermore, EP3 receptor blockade by ONO-AE3-240 fully rescued impaired Mf-LTP in 12-month-old APP/PS1 mice clearly supporting a key role of the PGE₂-EP3 signaling pathway in the development of synaptic dysfunction at the Mf-CA3 synapse in neuroinflammatory processes linked to AD. Worthy to note, ONO-AE3-240 had no effect on Mf-LTP in 12-month-old WT mice suggesting that the amount of PGE₂ produced during normal aging was not sufficient to activate tonically EP3 receptors and therefore impair plasticity. Interestingly, a time point of 8 months in the APP/PS1 mouse was proposed for the expression onset of inflammatory genes IL-1 β , iNOS, and TNF α , which is driven by an increased expression of hippocampal EP3 mRNA (Shi *et al.*, 2012). In our study, the time course of impairment of presynaptic Mf-LTP correlates well with the progressive development of neuroinflammation characterized by a mild activation of microglia and astrocytes and

elevated levels of PGE₂. Mf-CA3 synapses seem therefore relatively spared by the pathology in terms of presynaptic plasticity, at least up to 12 months of age. In contrast, postsynaptic long-term potentiation at associative/commissural CA3 synapses is completely abolished starting at 6 months of age in APP/PS1 mice (Viana da Silva *et al.*, 2016). These data suggest that early learning deficits involving short-term working memory (Lagadec *et al.*, 2012) or long-term spatial memory (Reiserer *et al.*, 2007), which occur at 6-7 months of age may not involve presynaptic Mf-CA3 plasticity but rather may be related to alterations of postsynaptic plasticity at CA3-CA1 synapses (Volianskis *et al.*, 2010) and/or CA3-CA3 synapses (Viana da Silva *et al.*, 2016).

4.3. Pathophysiological relevance of PGE₂-EP3 signaling at Mf-CA3 synapses in the context of AD

Potential alterations of Mf-CA3 synaptic function and plasticity in the context of AD have only received limited attention, despite the key role of DG-CA3 circuits in the encoding of new spatial information and complex mnemonic processes (Kesner, 2007). Our study demonstrates for the first time that presynaptic Mf-LTP is specifically altered through a PGE₂-EP3 signaling pathway in APP/PS1 mice at 12 months of age and most importantly can be entirely rescued by the pharmacological blockade of the EP3 receptor. Therefore, in addition to being instrumental in neuroinflammation processes (Shi *et al.*, 2012), PGE₂-EP3 signaling is also linked to synaptic alterations. Further work is necessary to elucidate how the engagement of the PGE₂-EP3 signaling pathway during neuroinflammatory processes contributes to AD-related cognitive deficits. Interestingly, contextual fear conditioning is altered in APP/PS1 mice (Kilgore *et al.*, 2010), and PGE₂ has been shown to be necessary and sufficient to induce CFC when injected in the dorsal hippocampus (Hein *et al.*, 2007).

In conclusion, our data demonstrate that PGE₂ signaling via EP3 receptors impairs a presynaptic form of LTP at Mf-CA3 synapses, hence identifying a potential mechanism to explain

deficits in 1-trial memory in AD (Kilgore *et al.*, 2010). This pathway, implicated in neuroinflammatory processes in the course of AD (Shi *et al.*, 2012), may identify a presynaptic form of LTP as a new target and leads to the promising idea of a therapeutic targeting of EP3 receptors instead of an upstream global inhibition of the production of PGE₂, which may have deleterious effects.

Disclosure statement

The authors have no conflicts of interest to disclose.

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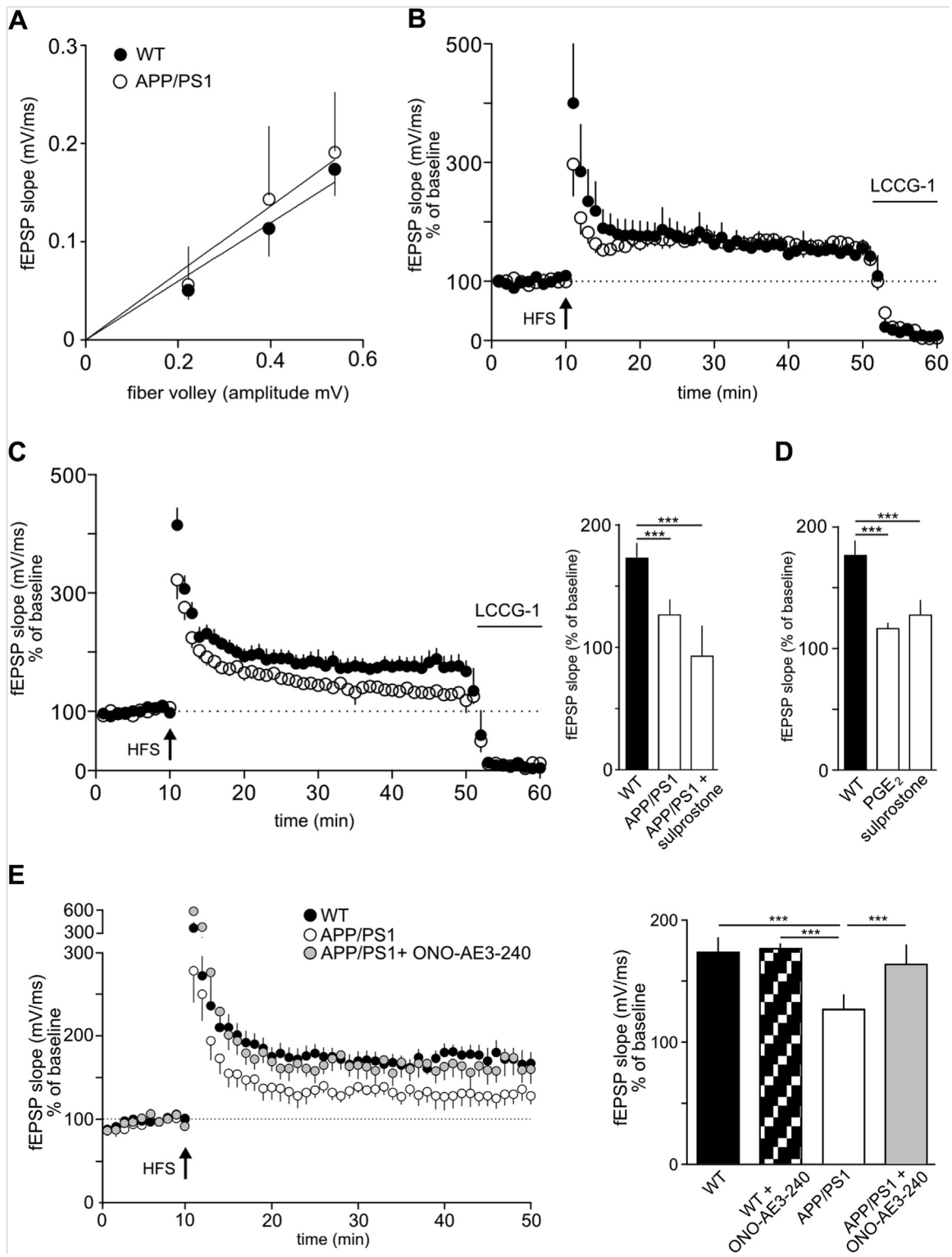


Fig. 5. PGE₂-EP3 signaling pathway impairs Mf presynaptic plasticity in APP/PS1 mice aged 12 months. (A) Inpuoutput relationship between the amplitude of fiber volley and the slope of fEPSP for gradually increasing stimulation intensities in wild type animals (black circles) and APP/PS1 animals (white circles) at 12 months of age. Both genotypes (WT and APP/PS1) displayed similar inpuoutput relationships for fiber presynaptic LTP (Mf-LTP) in WT and APP/PS1 mice at 9 months of age. Mf-LTP was induced by 3 bursts of 100 stimuli at 100 Hz fiber volley amplitude versus fEPSP slope (WT: 0.21 mV/ms ± 0.08 mV/ms, n = 8; APP/PS1: 0.28 mV/ms ± 0.12 mV/ms, n = 6; p = 0.624). (B) Average time course of mossy with a 10-second interburst interval (HFS). There was no difference between the 2 genotypes for normalized EPSCs measured between 40 and 50 minutes (WT: 152 ± 14%; APP/PS1: 160 ± 11%, p = 0.67, n = 5 for both conditions). (C) Average time course of Mf-LTP in WT and APP/PS1 mice aged 12 months. Note the impairment of Mf-LTP in APP/PS1 mice (WT: 173 ± 12%, n = 15; APP/PS1: 126 ± 13%, n = 11, p < 0.001). Bar graph represents average of normalized slopes from fEPSPs. Sulprostone had no further significant effect on LTP reduction in APP/PS1 mice (APP/PS1 + sulprostone: 92 ± 26%, n = 4, p < 0.001). (D) Bar graph represents average of normalized slopes from fEPSPs in WT mice at 12 months of age either in the presence of PGE₂ or sulprostone. Note that sulprostone or PGE₂ induced similar decrease of LTP (WT: 173 ± 12%, n = 15; PGE₂: 114 ± 5%, n = 9; sulprostone: 125 ± 12%, n = 7; p < 0.001). (E) Average time course of Mf-LTP illustrates the rescue of Mf-LTP in presence of ONO-AE3-240 (1 mM) in APP/PS1 mice at 12 months of age. Bar graph illustrates the average of fEPSPs slopes (WT: 173 ± 12%, n = 15; WT + ONO-AE3-240: 180 ± 4%, n = 7; APP/PS1: 126 ± 13%, n = 11; APP/PS1 + ONO-AE3-240: 163 ± 17%, n = 10; p < 0.001). In these series of experiments (not shown due to the time scale), the complete inhibition of EPSCs by group II mGluR agonist LCCG-1 (10 mM) confirmed that they were Mf-EPSCs. ***p < 0.001. Abbreviations: EP3, prostaglandin E2 receptor 3; fEPSP, field excitatory postsynaptic potential; HFS, high-frequency stimulus; Mf, mossy fiber; PGE₂, prostaglandin E2.

Table 1

Short-term plasticity in WT and APP/PS1 mice at 9 and 12 months of age

Short-term plasticity	WT (9 mo)	APP/PS1 (9 mo)	WT (12 mo)	APP/PS1 (12 mo)
PPF	381 ± 43%	369 ± 59%, p = 0.869	383 ± 75 %	282 ± 37%, p = 0.296
FF	673 ± 100%	484 ± 61% , p = 0.148	532 ± 88 %	418 ± 123%, p = 0.432

For PPF, average of 30 traces at 40 ms interstimulus interval. For FF, average of 50 traces when increasing the stimulation frequency from 0.1 Hz to 1 Hz. Data are mean ± standard error of the mean, n = 6-8 mice per group; Student t test. Key: FF, frequency facilitation; PPF, paired-pulse facilitation; WT, wild-type mice.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.neurobiolaging.2016.10.012>.

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La maladie d'Alzheimer est une maladie neurodégénérative dépendante de l'âge qui est principalement caractérisée par des déficits cognitifs dans les stades précoces liés à un dysfonctionnement synaptique et à une perte de synapses (Selkoe, 2002; Jacobsen *et al.*, 2006; Scheff *et al.*, 2006; Sheng *et al.*, 2012). De nombreuses études de la littérature montrent qu'il existe un dimorphisme sexuel important s'exprimant par un dépôt de plaques amyloïdes plus important et une apparition plus précoce de troubles de la mémoire chez les souris femelles par rapport aux souris mâles (Gresack et Frick, 2003; Clinton *et al.*, 2007; Taniuchi *et al.*, 2007; Gallagher *et al.*, 2013; Richetin *et al.*, 2017).

Le peptide β -amyloïde ($A\beta$) revêt une importance primordiale dans la pathogenèse de la maladie d'Alzheimer (Hardy *et al.*, 1992). Dans ce travail, nous avons montré que les plaques $A\beta$ apparaissent plus tôt et sont plus nombreuses chez les souris femelles par rapport aux souris mâles et que parallèlement, des troubles cognitifs dépendants de l'hippocampe (conditionnement de la peur contextuelle et tâche de localisation de l'objet) apparaissent dès l'âge de 4 mois chez les souris femelles APP / PS1 alors que les souris mâles ne montrent aucun déficits jusqu'à l'âge de 12 mois.

D'autre part, de nombreux travaux montrent que la neuroinflammation, portée par l'activation des cellules microgliales, des astrocytes et des neurones à proximité des plaques amyloïdes (Hoozemans *et al.*, 2008), est une composante importante de la maladie d'Alzheimer notamment en stimulant en retour la production d' $A\beta$ (Hoshino *et al.*, 2007, 2009). Dans cette étude, nous avons montré une forte activation des astrocytes et des cellules de la microglie au voisinage des plaques amyloïdes.

Parmi les molécules neuroinflammatoires produites au cours de la maladie d'Alzheimer, la prostaglandine E_2 (PGE_2) joue un rôle majeur (Combrinck *et al.*, 2006; Wei *et al.*, 2010; Shi *et al.*, 2012). La PGE_2 est produite à partir de l'acide arachidonique par deux enzymes limitantes, Cox-1 et Cox-2, et agit sur quatre sous-types de récepteurs, à savoir EP1, EP2, EP3 et EP4. Dans une étude précédente, nous avons démontré que la Potentialisation présynaptique à Long Terme (PLT) des synapses fibres moussues - neurones CA3 de l'hippocampe était altérée chez les souris mâles APP/PS1 en raison d'une augmentation de la concentration hippocampale de PGE_2 et de l'engagement d'une voie de signalisation PGE_2 -EP3 (Maingret *et al.*, 2017). L'hippocampe est l'une des premières régions du cerveau touchées par la maladie d'Alzheimer (Arriagada *et al.*, 1992). Dans l'hippocampe, les synapses fibres moussues - neurones CA3 sont des composantes importantes de la circuiterie excitatrice de l'hippocampe. Ces synapses ont une taille relativement importante et expriment plusieurs propriétés plastiques et de transmission uniques, notamment la facilitation par stimulation pairées, la facilitation fréquence-dépendante et une PLT d'origine présynaptique. Il est généralement accepté que la PLT est un substrat moléculaire de la mémoire et soit impliquée dans le codage rapide de nouvelles mémoires (Kesner, 2007), un processus mnémonique particulièrement affecté dans la maladie d'Alzheimer. Par conséquent, nous avons étudié la PLT présynaptique aux synapses fibres moussues - neurones CA3 chez les souris femelles APP/PS1 à 4 et 6 mois, un âge pour lequel des changements cellulaires et comportementaux sont déjà présents. De manière inattendue, la PLT présynaptique à cette synapse n'est pas altérée chez les souris femelles APP/PS1.

Ayant observé un dépôt accru de plaques amyloïdes à proximité du *gyrus dentelé* chez les souris femelles APP/PS1, nous avons alors étudié la transmission synaptique et la

plasticité aux synapses voie perforante - neurones granulaires du *gyrus dentelé*. Dans l'hippocampe de rongeur, les projections de la voie perforante en provenance du cortex entorhinal dans le *gyrus dentelé* sont impliquées dans les phénomènes de séparation des traces mnésiques, de l'information spatiale et du codage de la mémoire (Rolls et Kesner, 2006; Leutgeb *et al.*, 2007; Hunsaker *et al.*, 2007; Sahay *et al.*, 2011). La voie perforante est subdivisée entre la voie latérale et la voie médiale (McNaughton, 1980). Sur la base de l'emplacement de l'électrode de stimulation et des critères électrophysiologiques (facilitation vs dépression de stimulations paires), il est possible de distinguer la voie latérale de la voie médiale. Nous avons focalisé notre étude à la synapse voie latérale - neurones granulaires du *gyrus dentelé* sur les souris femelles sauvages vs APP/PS1 âgées de 6 mois.

Nous avons d'abord observé que la facilitation par stimulations paires (plasticité présynaptique liée à la probabilité de libération du neurotransmetteur dans la fente synaptique) était similaire pour les souris femelles sauvages et APP/PS1 à 6 mois.

La relation entrée/sortie (amplitude du "Population Spike" (PS) par rapport à l'intensité de la stimulation) est affectée chez les souris APP/PS1 par rapport aux souris sauvages. Un protocole de stimulation à haute fréquence déclenche une potentialisation à long terme (PLT) de l'amplitude du PS chez les souris sauvages. Cette PLT est totalement bloquée par le D-AP5 (antagoniste des récepteurs NMDA). De manière remarquable, la PLT est partiellement réduite chez les souris femelles APP/PS1 à 6 mois. Cependant ni PGE₂ ni l'activation sélective des différents sous-types de récepteurs EP (EP2, EP3, EP4) ne réduisent la PLT à cette synapse chez les souris sauvages et d'autre part ne sont impliqués dans la réduction de la PLT chez les souris femelles APP/PS1 à 6 mois.

Nous avons alors étudié l'hypothèse de changements dans les propriétés intrinsèques des cellules granulaires du *gyrus dentelé* (mécanismes non synaptiques) pouvant être impliqués dans la réduction de la PLT chez les souris femelles APP/PS1. Pour tester cette hypothèse, nous avons enregistré par la technique de "patch clamp" les propriétés intrinsèques des cellules granulaires du *gyrus dentelé*. Un échelon de potentiel dépolarisant de longue durée (250 ms) déclenche un train de potentiels d'action (PAs) qui présente un plus grand nombre de PAs pour les souris femelles APP/PS1 par rapport aux souris femelles sauvages ce qui révèle une augmentation de l'excitabilité des cellules granulaires du *gyrus dentelé*. De nombreux travaux font état d'une augmentation de l'excitabilité neuronale liée à une accumulation d'A β (Buckner *et al.*, 2005; Mintun *et al.*, 2006; Seeley *et al.*, 2009; Sperling *et al.*, 2009).

Nous avons également caractérisé deux types (type 1 et type 2) de cellules granulaires du *gyrus dentelé* en fonction du patron de décharge des PAs et de la résistance d'entrée spécifique et ceci pour les deux génotypes (sauvage vs APP/PS1). En revanche d'autres propriétés intrinsèques (potentiel membranaire de repos, amplitude du PA, seuil de déclenchement du potentiel d'action...) des cellules granulaires du *gyrus dentelé* sont similaires pour les deux génotypes.

Au niveau synaptique, nous avons étudié la courbe E-S, qui relie la pente du Potentiel Excitateur Post Synaptique (PEPS) à la probabilité de déclenchement d'un potentiel d'action. Nous n'avons observé aucune modification significative de la courbe E-S entre les souris femelles sauvages et APP/PS1 qui pourrait expliquer les modifications de

l'excitabilité intrinsèque. Cependant, l'application d'une stimulation haute fréquence qui déclenche une PLT provoque un déplacement de la courbe E-S qui traduit une augmentation supérieure d'excitabilité pour les souris femelles APP/PS1 vs sauvages. Ces résultats indiquent que la potentialisation synaptique induit un changement d'excitabilité intrinsèque des cellules granulaires du *gyrus dentelé* dépendant du génotype.

Nous avons donc ensuite analysé la possibilité que l'augmentation d'excitabilité présentée par les cellules granulaires du *gyrus dentelé* puisse résulter de modifications de l'intégration dendritique. Une façon d'étudier l'intégration dendritique consiste à établir le rapport amplitude/pente du PEPS au cours des deux premières millisecondes, c'est-à-dire lorsqu'il reflète principalement la composante rapide de la transmission synaptique des récepteurs AMPA (Lopez *et al.*, 2016).

Le rapport amplitude/pente PEPS est similaire pour les deux génotypes mais nous avons observé une différence significative dans le rapport amplitude/pente PEPS avant et après PLT pour les souris femelles APP/PS1 mais pas pour les souris femelles sauvages. Ces résultats suggèrent que des modifications du traitement dendritique des entrées synaptiques dans les cellules granulaires du *gyrus dentelé* chez les souris femelles APP/PS1 pourraient sous-tendre des modifications de l'excitabilité.

La diminution de PLT observée chez les souris femelles APP/PS1 pourrait être due à une diminution de la probabilité de libération du neurotransmetteur, à une diminution de la fiabilité ou du poids des synapses individuelles, à une diminution du nombre de synapses ou de récepteurs fonctionnels. De façon remarquable les oligomères A β activent une voie de signalisation dépendante des récepteurs NMDA qui conduit à une diminution de la PLT hippocampale par internalisation des récepteurs synaptiques AMPA et NMDA (Kamenetz *et al.*, 2003; Kessels *et al.*, 2013; Snyder *et al.*, 2005). Nous avons donc mesuré le rapport AMPA/NMDA pour évaluer cette possibilité, mais n'avons observé aucun changement de ce rapport entre les souris femelles sauvages et APP/PS1 à 6 mois.

Nous avons également étudié les courants synaptiques excitateurs miniatures des cellules granulaires. Il est généralement décrit que l'amplitude des courants miniatures est reliée à des événements postsynaptiques quand leur fréquence est attribuée à des mécanismes présynaptiques (Turrigiano *et al.*, 1998; Kim and Tsien, 2008; Ardiles *et al.*, 2012) bien qu'une diminution de la fréquence puisse également être liée à une diminution du nombre de synapses fonctionnelles. Les résultats montrent une augmentation de la fréquence des courants miniatures médiés par les récepteurs NMDA dans les cellules granulaires du *gyrus dentelé* chez les souris femelles APP/PS1 par rapport aux souris femelles sauvages. Cette augmentation de la fréquence des courants miniatures NMDA pourrait être due à un nombre de synapses silencieuses (c'est à dire dépourvues de récepteurs AMPA) supérieur chez les souris femelles APP/PS1 par rapport aux souris femelles sauvages. En revanche, l'amplitude des courants miniatures AMPA ou NMDA n'est pas significativement modifiée par le génotype.

En conclusion, l'ensemble de ce travail montre qu'il existe des différences importantes liées au sexe avec un dépôt plus fort et précoce de plaques amyloïdes, une activation gliale neuro-inflammatoire plus importante associée à des déficits précoces de la

mémoire dépendante de l'hippocampe chez les souris femelles APP/PS1 vs souris mâles APP/PS1. En parallèle de ces altérations moléculaires, cellulaires et comportementales, nous observons des déficits de la transmission et de la plasticité synaptique à la synapse voie perforante - cellules granulaires du *gyrus dentelé* (voie d'entrée des informations en provenance du cortex entorhinal) ainsi qu'une modification des propriétés intrinsèques des cellules granulaires.