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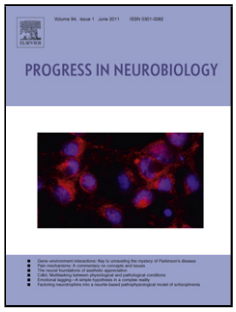

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Title: Role of cellular prion protein in interneuronal amyloid transmission.

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Highlights

- PrP^C can bind to different amyloid (β-sheet-rich) proteins.
- Amyloid-interacting PrP^C domains comprise two charged cluster domains (CC1 and 2).
- PrP^C participates in the expansion of amyloid (at least α-synuclein) deposits in wild-type mice.

Author contributions.

JADR and IF wrote most of the chapters. RG wrote Chapter 4 and prepared the figures.

Competing financial interest

The authors declare no conflicts of interest.

Abstract

Several studies have indicated that certain misfolded amyloids composed of tau, β-amyloid or α-synuclein can be transferred from cell to cell, suggesting the contribution of mechanisms reminiscent of those by which infective prions spread through the brain. This process of a 'prion-like' spreading between cells is also relevant as a novel putative therapeutic target that could block the spreading of proteinaceous aggregates throughout the brain which may underlie the progressive nature of neurodegenerative diseases. The relevance of β-amyloid oligomers and cellular prion protein (PrP^C) binding has been a focus of interest in Alzheimer's disease (AD). At the molecular level, β-amyloid/PrP^C interaction takes place in two differently charged clusters of PrP^C. In addition to β-amyloid, participation of PrP^C in α-synuclein binding and brain spreading also appears to be relevant in α-synucleopathies. This review summarizes current knowledge about PrP^C as a putative receptor for amyloid proteins and the physiological consequences of these interactions.

Abbreviations

AA = Amino acid
Aβo = Oligomeric β-amyloid
Aβ = β-amyloid
AD = Alzheimer's disease
α7nAChR = α7 Nicotinic acetylcholine receptor
ALS = Amyotrophic lateral sclerosis
APLP1 = Amyloid beta precursor-like protein 1
APP = Amyloid precursor protein
β-ARs = β-Adrenergic receptors
BSE = Bovine spongiform encephalopathy
CamKII = Calmodulin-dependent protein kinase II
CC1 = Charged cluster 1 (23-28 aa) of the PrP^C molecule
CC2 = Charged cluster 2 (95-110 aa) of the PrP^C molecule
CD = Central domain (94-133 aa) of the PrP^C molecule
CJD = Creutzfeldt-Jakob disease
CNS = Central nervous system
CNO = Clozapine-N-oxide
CREB = cAMP response binding protein
CWD = Chronic wasting disease
DLB = Dementia with Lewy bodies

DREADD = Designer receptors exclusively activated by designer drugs
EGCG = (-)-Epigallocatechin-3-gallate
EF2 = Elongation factor 2
ERK1/2 = Extracellular-regulated kinase 1 and 2
FFI = Fatal familial insomnia
FcγRIIb = Fcγ receptor II-b
Fyn = Proto-oncogene tyrosine-protein kinase
GPI = Glycosylphosphatidylinositol
GRP78 = Glucose regulated protein 78
GSK3 = Glycogen synthase kinase 3
GSS = Gerstmann-Straüssler-Scheinker syndrome
HE = Hematoxylin and eosin
HEK293 = Human embryonic kidney cell line
HD / HR = Hydrophobic region (110 / 113 -133 aa) of the PrP^{C} molecule
HSPGs = Heparan sulfate proteoglycans
IPs = Induced pluripotent stem
LB = Lewy bodies
LN = Lewy neurites
LAG3 = Lymphocyte-activation gene 3
MAPK = Mitogen-activated protein kinase
Mdm2 = Mouse double minute 2 or E3 ubiquitin-protein ligase
mGluR5 = Metabotropic glutamate receptor 5
MPTP = 1-Methyl-4-phenylpyridinium ion (MPP+)/1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MSA = Multiple system atrophy
NADPH = Nicotinamide adenine dinucleotide phosphate
NFT = Neurofibrillary tangles
N2a = Neuroblastoma cell line
NMDA = N-methyl-D-aspartate receptor subunit
NR2B = N-methyl-D-aspartate receptor subunit NR2B
OR = Octarepeat region (51-90 aa) of the PrP^{C} molecule
p75NTR = p75 Neurotrophin receptor
PirB = Paired immunoglobulin-like receptor B
LilrB2 = PirB's human orthologue receptor
PADK = Z-Phe-Ala-diazomethylketone
PD = Parkinson's disease
PHF = Paired helical filaments
PKA = Protein kinase A
PrP^{C} = Cellular prion protein
PS = Proteinaceous species
PrP^{res} = Proteinase K-resistant misfolded form of cellular prion protein
PrP^{Sc} = Scrapie prion protein
Pyk2 = Non-receptor tyrosine kinase of the focal adhesion kinase family
ROS = Reactive oxygen species
sCJD = Sporadic Creutzfeldt-Jakob disease
SOD1 = Superoxide dismutase 1
STI1 = Stress-inducible phosphoprotein 1
TNT = Tunnelling nanotubes
vCJD = Variant CJD

Keywords

Cellular prion protein, amyloid, proteinaceous species, 'prion-like' spreading, neurodegeneration.

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1. Introduction

Neurodegenerative diseases are characterised by the progressive degeneration of subsets of neurons accompanied by complex glial reactions in specific brain regions (Ferrer, 2017). Most neurodegenerative diseases develop and progress in parallel with a characteristic intra- or extra-cellular accumulation of misfolded PS including i) tau-containing neurofibrillary tangles (NFT) and β-amyloid plaques in Alzheimer's disease (AD) (Fig. 1) (Braak and Del Trecidi, 2015), ii) Lewy bodies (LB) and Lewy neurites (LN) containing α-synuclein in Parkinson's disease (PD) (Braak and Del Tredici, 2009) and dementia with Lewy bodies (DLB) (Goedert *et al.*, 2013), and α-synuclein oligodendroglial and neuronal deposits in multiple system atrophy (MSA) (Goedert *et al.*, 2013) (Fig. 2), and iii) TDP-43-positive skein-like and other inclusions in amyotrophic lateral sclerosis (ALS) (Guerrero *et al.*, 2016).

Clinical, molecular, cellular and biochemical studies have shown that these diseases are progressive disorders, and PS-associated pathologies spread from diseased to healthy cells, thus contributing to disease worsening (Costanzo and Zurzolo, 2013; Goedert *et al.*, 2017; Holmes and Diamond, 2017)for recent reviews). The spatiotemporal progression seems to correlate with brain propagation of PS-associated neuropathology through predictable anatomical pathways determined for each disorder, thereby suggesting a synaptic spreading of the disease (Bertrand *et al.*, 2004; Braak and Del Tredici, 2009; Costanzo and Zurzolo, 2013; Goedert *et al.*, 2017; Saper *et al.*, 1987) (Fig. 2). However, putative participation of astrocytes and mediators of PS

spreading in the neurodegenerative brain is also considered (Cavaliere *et al.*, 2017). The combined involvement of neurons and glia displaying PS inclusions in most neurodegenerative diseases (Ferrer *et al.*, 2015; Kovacs *et al.*, 2016) in PS spreading is currently undergoing in-depth study (Kuan *et al.*, 2016; Lee *et al.*, 2010). At the cellular level, involvement of cell-surface receptors in PS expansion has been described (see below), but other routes of cellular spreading have also been proposed such as extracellular vesicles, including exosomes and tunnelling nanotubes (TNTs) (Abounit *et al.*, 2016; Campana *et al.*, 2005; Costanzo and Zurzolo, 2013; Dieriks *et al.*, 2017; Okuda *et al.*, 2017; Tardivel *et al.*, 2016; Zeinabad *et al.*, 2016). In parallel, according to evidence demonstrating the ability of these misfolded proteins to propagate protein misfolding from diseased cells to recipient (healthy) cells, a 'prion-like' hypothesis has been postulated (Aguzzi and Lakkaraju, 2016; Ashe and Aguzzi, 2013; Collinge, 2016). Indeed, cell-spread of PS can act as self-propagating templates disrupting cellular homeostasis and eventually leading both to the death of recipient cells and to the advance of the neurodegenerative disorder (Aguzzi and Lakkaraju, 2016; Ashe and Aguzzi, 2013; Collinge, 2016; Holmes and Diamond, 2017).

Cellular prion protein (PrP^C) is a cell surface protein expressed in a variety of different tissues with high levels in the central and peripheral nervous system (Aguzzi and Miele, 2004; del Rio and Gavin, 2016; Linden *et al.*, 2008; Prusiner *et al.*, 1998). PrP^C is best known for its crucial role as a molecular substrate in the pathogenesis of prion diseases such as Creutzfeldt-Jakob disease (CJD), Gerstmann–Straüssler–Scheinker (GSS) syndrome, and familial fatal insomnia

(FFI) in humans, scrapie in sheep and goats, bovine spongiform encephalopathy (BSE) in cattle, and chronic wasting disease (CWD) in deer (Prusiner and DeArmond, 1994). In these diseases, PrP^{C} is converted into an aberrantly folded, β-sheet-rich isoform, designated scrapie prion protein (PrP^{Sc}) (Collinge, 2016; Prusiner, 1989; Prusiner and DeArmond, 1994). PrP^{Sc} is found in extracellular deposits in diseased brains, and it is the essential constituent of infectious prions (Collinge, 2016; Prusiner, 1989; Prusiner and DeArmond, 1994; Prusiner *et al.*, 1998)(Fig. 3). Increasing knowledge about the participation of PrP^{C} in prion infection contrasts with the elusive, and to some extent controversial, data regarding its physiological role, probably related to its molecular pleiotropy or specific interactions. Their interacting partners remain uncertain. However, what in our opinion is more relevant in the present context is the absence, until a few years ago, of appropriate knockout mouse models to dissect its physiology and biological relevance in specific processes of the central nervous system (CNS) (del Rio and Gavin, 2016; Nuvolone *et al.*, 2016; Onodera *et al.*, 2014; Steele *et al.*, 2007; Wulf *et al.*, 2017).

PrP^{C} is considered, in some studies, to be a neuroprotective molecule (Carulla *et al.*, 2015; Resenberger *et al.*, 2011b; Roucou *et al.*, 2004). On the other hand, PrP^{C} overexpression increases susceptibility to neurotoxicity and cell death (Paitel *et al.*, 2003; Paitel *et al.*, 2004; Rangel *et al.*, 2009). Changes in PrP^{C} and *PRNP* mRNA have been reported during disease progression in AD, DLB and certain tauopathies (Llorens *et al.*, 2013a; Vergara *et al.*, 2015). Moreover, PrP^{C} interacts with oligomeric β-amyloid (Aβo) neurotoxic species. Along this line, the discovery that PrP^{C} was a high-affinity binding partner of

Aβo is relevant to identify early triggering agents of disease (Dohler *et al.*, 2014; Fluharty *et al.*, 2013; Freir *et al.*, 2011; Ganzinger *et al.*, 2014; Lauren *et al.*, 2009; Zou *et al.*, 2011). Resenberger *et al.* showed that the N-terminal domain of PrP^C can bind to β-rich peptides including Aβ (Resenberger *et al.*, 2011a; Resenberger *et al.*, 2012). Amino acid (aa) residues 95-110 of PrP^C are indeed the major residues responsible for binding of Aβo (Chen *et al.*, 2010; Lauren *et al.*, 2009). Yet although interaction between PrP^C and Aβo has been demonstrated (Dohler *et al.*, 2014; Fluharty *et al.*, 2013; Freir *et al.*, 2011; Ganzinger *et al.*, 2014; Lauren *et al.*, 2009; Zou *et al.*, 2011), it is still unclear whether PrP^C is a mediator of the neurotoxic effects of Aβo (Calella *et al.*, 2010; Haas *et al.*, 2016; Kessels *et al.*, 2010; Kostylev *et al.*, 2015; Wulf *et al.*, 2017). From these results, several laboratories started to analyse whether PrP^C might also be a cellular partner of other PS (Resenberger *et al.*, 2011a), and whether PrP^C may participate in or regulate the spreading of particular misfolded aggregates and associated neuropathologies. Recent results suggest that membrane-anchored PrP^C may also bind to α-synuclein (Aulic *et al.*, 2017; Ferreira *et al.*, 2017; Urrea *et al.*, 2017) and may participate in its neuronal spreading (Aulic *et al.*, 2017; Urrea *et al.*, 2017). In this review, we discuss neuronal cell surface molecules with high affinity for disease-associated PS, particularly β-amyloid and α-synuclein, with a focus on the role of PrP^C in this process. The interaction of PrP^C with tau is also discussed.

2. Cellular prion protein: protein structure, cellular processing and functions

PrP^C is a glycosylphosphatidylinositol (GPI)-anchored protein of 231 aa encoded by the *PRNP* gene located on chromosome 20 in humans and on chromosome 2 in mice (Aguzzi and Miele, 2004; del Rio and Gavin, 2016; Linden *et al.*, 2008; Prusiner *et al.*, 1998). As a GPI-anchored protein, PrP^C is rich in cholesterol-enriched lipid-raft domains of plasma membrane. In addition, PrP^C undergoes clathrin-dependent and/or -independent endocytosis with further degradation or recycling (Aguzzi and Miele, 2004; Linden *et al.*, 2008; Prusiner *et al.*, 1998). PrP^C is highly conserved in mammals. The sequence of PrP^C can be divided into 2 structurally well-defined regions: a long, N-terminal flexible tail (approximately the first 100 residues), present in most of the species assessed, except deer and elk, containing series of four or five repeats of eight amino acids (PHGGGWGQ), and a globular C-terminal domain containing 3 α-helices and 2 β-strands flanking the first α-helix. The flexible tail also has distinctive features: a small charged cluster (CC1), an octarepeat (OR) region and a central domain (CD), which in turn comprises a second charge cluster (CC2) and a hydrophobic region (HR). Additionally, two N-glycosylation sites are located at 180 and 196 aa, and there is one disulphide bond between 178 and 213 aa. PrP^c presents at least three distinct topological orientations: the fully extracellular form (Holscher *et al.*, 2001) and two transmembrane isoforms (called Ntm-PrP and Ctm-PrP) with opposite sequence orientations with respect to the lumen of the endoplasmic reticulum (Hegde *et al.*, 1998).

PrP^C is widely expressed in the CNS during early development, and in adult neurons and glial cells. In the adult brain, maximal *PRNP* mRNA expression is observed in the neocortex and cerebellum. In addition to the nervous system,

mammalian expression of PrP^C has been reported in several tissues including lymphoid organs and the heart (Ford *et al.*, 2002; Linden *et al.*, 2008; Miele *et al.*, 2003), and lower levels in the kidney and liver (Miele *et al.*, 2003; Tichopad *et al.*, 2003).

PrP^C is actively recycled in the plasma membrane. In addition, the protein can be proteolysed in a similar way to amyloid precursor protein (APP) by several proteases (α, β, and probably γ-cleavage). After protease activity, different fragments of PrP^C are generated: N1 + C1 after α-cleavage, N2 + C2 after β-cleavage and C3 fragments after γ-cleavage (Vincent *et al.*, 2000; Vincent *et al.*, 2001). Among other roles, α-cleavage plays a part in preventing PrP^{sc} generation; its failure induces cell death in affected cells (Vincent *et al.*, 2000; Vincent *et al.*, 2001). PrP^C function is far from fully understood. Here, some selected functions are addressed for practical purposes; more complete information is available in other reviews (Legname, 2017; Linden, 2017; Linden *et al.*, 2008; Nicolas *et al.*, 2009; Steele *et al.*, 2007; Wulf *et al.*, 2017).

2.1. An overview of the neural functions of PrP^C

The functions described for PrP^C cover a wide spectrum including ion balance homeostasis, control of cell proliferation and neural differentiation. However, it must be stressed that most of these functions have been reported using Zurich I (B6129 *Prnp$^{0/0}$*, outbred) mice carrying 129Sv-associated '*flanking genes*' (Nuvolone *et al.*, 2016; Steele *et al.*, 2007). Thus, analyses of the published roles using cell cultures or living mice with this background should be

interpreted with caution. One of these flanking genes, SIRPα, is responsible for a function previously associated with PrP^C in macrophages (Nuvolone *et al.*, 2013). The presence of these '*flanking genes*' promotes intrinsic susceptibility of B6.129 *Prnp*$^{0/0}$ mice to excitotoxic insults (Carulla *et al.*, 2015).

The octarepeat region of PrP^c binds Cu^{2+} and Cu^{+}, thus modulating their intraneuronal levels (Hornshaw *et al.*, 1995a; Hornshaw *et al.*, 1995b). Along this line, lower levels of copper are found in the brain parenchyma of *Prnp*$^{0/0}$ compared to wild-type mice (Herms *et al.*, 1999), although this observation was not reproduced in other studies (Waggoner *et al.*, 2000). Importantly, Cu^{2+} level regulation by binding to PrP^c has been associated with: i) superoxide dismutase-1 (SOD-1) activity (Brown *et al.*, 1997b; Sorenson, 2001), ii) neural transmission (Brown *et al.*, 1997a; Herms *et al.*, 1999), and iii) clathrin-mediated endocytosis (Cheng *et al.*, 2006; Haigh *et al.*, 2005; Pauly and Harris, 1998). In short, control of intracellular copper points to a homeostatic function of PrP^C (Sakudo *et al.*, 2004). Several studies have addressed the putative stress-protective properties of this protein. Pioneering studies reported PrP^C protection of cultured neurons from serum deprivation (Kim *et al.*, 2004; Nishimura *et al.*, 2007). Later on, several studies showed that PrP^C protects cells against oxidative stress in several cell types (Watt *et al.*, 2007; Zanetti *et al.*, 2014), a function that requires the integrity of the octapeptide repeats of the protein (Watt *et al.*, 2005; Zeng *et al.*, 2003). PrP^C also protects primary hippocampal neurons and neuroblastoma cell lines from staurosporine-mediated cell death, possibly through an interaction with stress-inducible phosphoprotein 1 (STI1) by activating protein kinase A (PKA) (Zhang *et al.*, 2006). In contrast,

overexpression or PrP^C sensitizes the HEK293 and Rov9 cell lines to cell death in the presence of staurosporine (Paitel *et al.*, 2002). Subsequent studies have shown that this sensitization occurs through a p53-dependent caspase 3-mediated activation controlled by mouse double minute 2 (Mdm2) and p38 mitogen-activated protein kinase (MAPK) (Paitel *et al.*, 2003; Paitel *et al.*, 2004). Increased caspase 3 activation after PrP^C overexpression has also been reported in other studies (Nicolas *et al.*, 2007; Vilches *et al.*, 2016). Thus, it is reasonable to consider that PrP^C may be maintained at a physiological level, since increase or reduction may strongly interfere with other cellular processes that might induce cell death (Llorens *et al.*, 2013b; Vergara *et al.*, 2015).

Other studies have shown that aggregation of PrP^C in cell membrane *in vitro* (Mouillet-Richard *et al.*, 2000) and *in vivo* (Solforosi *et al.*, 2004) triggers cell death which is associated with increased production of reactive oxygen species (ROS), and activation of the proto-oncogene tyrosine-protein (Fyn) kinase and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. However, overexpression of PrP^C alone does not increase ROS production (Vilches *et al.*, 2016), thus reinforcing the opinion that PrP^C-mediated p53 activation is not associated with Fyn activation (Paitel *et al.*, 2003; Paitel *et al.*, 2004).To sum up, PrP^C-mediated sensitization and aggregation may regulate cell death by different mechanisms.

Finally, mice expressing truncated forms of PrP^C present an exacerbated lethal ataxic syndrome which is counteracted by the introduction of the complete PrP^C sequence (Baumann *et al.*, 2007; Li *et al.*, 2007; Shmerling *et al.*, 1998). These

studies suggest that the central region of PrP^C has neuroprotective properties, and that its absence triggers neurotoxic pathways that promote cell death, cerebellar atrophy and white-matter degeneration (Li *et al.*, 2007). Another study suggests that the C-terminal domain of the protein plays a role in modulating the neurotoxic properties of the N-terminal domain (Wu *et al.*, 2017) by the blockage of this domain with specific antibodies or by introducing deletions which induce the generation of spontaneous ion currents in cultured neurons (Wu *et al.*, 2017). This last study is in line with our proposed model of PrP^C-mediated neurotoxicity (del Rio and Gavin, 2016). Following this rationale, it is postulated that putative binding of PrP^C with PS interferes with neuroprotective functions, leading, in turn, to cell death (Pietri *et al.*, 2006).

3. Axonal transport and cell-to-cell PS transmission

Most *in vivo* studies aimed at determining cell-to-cell transmission and propagative properties along axonal tracts of Aβ, tau and α-synuclein (among others) have been developed in mice using stereotaxic injections of: i) viral particles encoding recombinant proteins, ii) recombinant proteins in several assembly stages, and iii) brain extracts from mutant mouse models or from human brain samples. The term ‘prion-like’ is widely used to name the type of seeding and propagation of PS in different settings. However, only a few of these PS strictly display all the molecular features of infective prions described in transmissible spongiform encephalopathies (TSEs) (Erana *et al.*, 2017). Infective prions in TSEs show three key features: i) two distinct conformational states of the protein are present in the same neuron/cell and one shows a

tendency to generate insoluble aggregates; ii) the formed aggregate is self-propagating, meaning that it can influence the conversion of the natural conformation into a misfolded aberrant conformation; and iii) this propagation occurs at the cellular level and, more importantly, between different individuals. In addition, the neurotoxic potential progressively increases during the propagation process. It has recently been stressed (Harbi and Harrison, 2014) that 'prion-like activity' or 'prion-like propagation' also refers to self-propagating protein aggregates not meeting a strict 'prion' definition in certain circumstances in which the transmission between organisms is not clearly demonstrated. The term 'prion-like' describing the propagation activities of misfolded proteins will be used in the present review. Misfolded Aβo, tau and α-synuclein are hereafter considered putative 'prion-like' proteins.

3.1. *In vitro* studies

Direct observation of axonal transport and cell-to-cell transmission of PS is obtained in primary neurons growing in microfluidic devices which physically isolate the perikaryon and the axonal compartments into two neuronal populations growing in different reservoirs interconnected with numerous microgrooves (Neto *et al.*, 2016; Taylor *et al.*, 2005) (Fig. 4). Using these (or similar) devices, axonal transport and cell-to-cell propagation of α-synuclein (Brahic *et al.*, 2016; Danzer *et al.*, 2011; Freundt *et al.*, 2012), Aβ (Song *et al.*, 2014) and tau (Wu *et al.*, 2016) have been demonstrated. Aggregates (fibrils of extracts) are typically added to the cell media at concentrations (within the range of a microgram) higher than those occurring in real diseased human cells.

With respect to α-synuclein, misfolded α-synuclein fibrils (or isolated LB) can be captured by neurons and transported between donor and recipient neurons in both retrograde and anterograde manners (Brahic *et al.*, 2016; Freundt *et al.*, 2012). After transport, misfolded α-synuclein spreads between synaptically connected neurons growing in the second (Fig. 4) or additional reservoirs, leading to increased p-α-synuclein content (Freundt *et al.*, 2012). Although both retrograde and anterograde transports have been described, there is a tendency toward retrograde transport of α-synuclein in microfluidic devices as also observed *in vivo*. This seems to be somewhat different from what is reported for Aβ since Aβ is absorbed by axonal processes and transported in a retrograde manner towards the neuronal soma. It should be mentioned that the transport cannot be blocked by Dynasore™ suggesting propagation mechanisms other than endocytosis (Song *et al.*, 2014). Misfolded fibrillar tau can be transported in an anterograde manner between post-mitotic neurons (Ki67-negative) *via* its extracellular release and further neuronal update by micropinocytosis linked to heparan sulfate proteoglycans (HSPGs) in second level neurons (Wu *et al.*, 2016).

In summary, better understanding of the mechanisms of uptake and axonal transport, either linked to kinesin or dynein, vesicle coating, and selective retrograde *vs.* anterograde transport is needed to overcome the present limitation in delineating effective therapeutic approaches targeting PS transport and disease spreading.

3.2. *In vivo* studies

Several studies using mouse models have shown that assembled normal and mutated P301L tau behave like 'prion-like' molecules (Clavaguera *et al.*, 2013; Clavaguera *et al.*, 2009; Iba *et al.*, 2015; Soto, 2012). Moreover, tau extracted from AD patients is able to transmit tau pathology in non-transgenic mice (Guo *et al.*, 2016). Injection of Aβ aggregates derived from AD patients or from aged transgenic mice into young TgAPP23 animals initiates the deposition of endogenous Aβ (Meyer-Luehmann *et al.*, 2006). Additional studies have shown the self-propagating properties of recombinant Aβ *in vivo* (Stohr *et al.*, 2012). Regarding α-synuclein, pioneer studies described the presence of α-synuclein inclusions in foetal grafted neurons in PD patients (Kordower *et al.*, 2008). Indeed, recombinant α-synuclein fibrils spread among neurons in wild-type mice (Luk *et al.*, 2012; Masuda-Suzukake *et al.*, 2014; Urrea *et al.*, 2017). Intranigral or intrastriatal inoculation of PD-derived LB extracts in monkeys results in progressive nigrostriatal neurodegeneration (Recasens *et al.*, 2014). Recent studies have shown the spreading of mouse α-synuclein in marmoset (Shimozawa *et al.*, 2017). In most cases, α-synuclein is transported in a retrograde manner after injection from the striatum into the neocortex and amygdala.

3.3. Some aspects to be considered in analysing PS transport *in vivo* and *in vitro*

New methods and platforms aimed at modulating or monitoring neural physiology *in vitro* and *in vivo* have improved our knowledge of intercellular PS

transport and regulatory factors. However, the results obtained in *in vivo* experiments using patient-derived extracts largely depend on the protocol used to isolate PS extracts (McCormack *et al.*, 2016). Moreover, the molecular composition of these proteinaceous extracts has not been fully determined (i.e., (Shults, 2006) for LB). Specific conformations (also termed 'strains') of PS oligomers may occur in different brain regions as observed for α-synuclein in A53T mice (Tsika *et al.*, 2010). Patient-derived PS extracts show different seeding properties compared to recombinant proteins (Guo *et al.*, 2016) which argue against the use of recombinant proteins as the best PS model. The isolation process is also relevant since, for example, the presence of traces of endotoxin during the production of aggregates in bacteria may influence not only the process of spreading but also the formation of different species of aggregates with divergent biochemical properties (Kim *et al.*, 2016).

Since neural activity facilitates axonal transport of proteins and other molecules, recent studies have used cell-directed optogenetic activation (Wu *et al.*, 2016; Yamamoto *et al.*, 2015). Alternatively, designer receptors exclusively activated by designer drugs (DREADD) and clozapine-N-oxide (CNO)-dependent activation are also used in mouse models (Wu *et al.*, 2016). Although described for tau (Wu *et al.*, 2016) and Aβ (Yamamoto *et al.*, 2015) but not for α-synuclein, the specific stimulation of projecting neurons by optogenetics or CNO increases PS formation, transport and deposition in appropriate mouse models after PS inoculation. Nonetheless, neuronal activation mediated by optogenetic stimulation with blue light (2 seconds' stimulation per minute for 240 minutes) (Yamamoto *et al.*, 2015) or 2 seconds on/off for 30 minutes (Wu *et al.*, 2016) is

far from being a 'physiological' activation. Thus, more adequate 'physiological' stimulation is needed to rule out seizure-mediated effects on interneuronal PS transport. In addition, although DREADD expression in neurons is driven by neuron-specific promoters (Wu *et al.*, 2016), the particular roles of different neuronal types in PS formation and spreading needs to be addressed using neuron-subtype specific promoters (i.e., interneuron *vs.* projecting neuron). This is mandatory considering that global neuronal activity (i.e., gamma frequency waves) controlled by cortical fast-spiking interneurons (Cardin *et al.*, 2009) also modulates microglial activation and amyloid plaque formation (at least in AD mouse models) (Iaccarino *et al.*, 2016).

Regarding microfluidic design devices, three aspects must be addressed in future studies: i) clear definition of anterograde and retrograde PS transport; Newly developed 'diode' microfluidic devices displaying only unidirectional neuronal connections should be used (Peyrin *et al.*, 2011); ii) the real absence of fluid (media) transport between reservoirs using at least three reservoir devices (i.e., (Cirrito *et al.*, 2005)); and iii) determination of whether the spreading of the PS might also affect synaptic activity in recipient cells. Most studies analyse the effects of PS incubation on neuronal activity using Ca^{2+} wave analysis and spike generation experiments in treated neurons but not in recipient neurons (i.e., (Volpicelli-Daley *et al.*, 2011)).

4. Interactions between α-synuclein, tau and Aβo

Strong interplay among α-synuclein, tau and Aβ may synergistically promote shared seeding (Kotzbauer *et al.*, 2004; Tsigelny *et al.*, 2008). As a matter of

fact, neurofibrillary tangles enriched in hyperphosphorylated tau and Aβ deposits surrounding α-synuclein deposits in LB or LBN are frequent in α-synucleinopathies (Fig. 5) (Fujishiro *et al.*, 2008; Ishizawa *et al.*, 2003; Nagaishi *et al.*, 2011; Piao *et al.*, 2001). Fig. 6 illustrates a particular MSA case displaying both α-synuclein and AD-associated pathology (Fig. 6 A, B). α-synuclein and p-tau deposits co-localise in a subgroup of neurons (Fig. 6 C, D), thus suggesting cross-seeding interactions between the two proteins. Likewise, an exogenous supply of α-synuclein by viral delivery or by inoculating aggregated forms to cultured cells can induce tau phosphorylation (Badiola *et al.*, 2011). AD-like mouse models (APP/PS1) also expressing human P301L tau in entorhinal cortex-projecting neurons have shown that increased Aβ deposits are associated with increased spreading of p-tau in the hippocampus (Pooler *et al.*, 2015). Similar results have been observed using 3D *in vitro* culture models of AD (Choi *et al.*, 2014). Conversely, elevated levels of tau increase α-synuclein aggregation (Badiola *et al.*, 2011).

4.1. Cross-seeding activity between amyloids

It is well known that tau promotes microtubule assembly and stabilization at low concentrations, inhibiting microtubule polymerization at high concentrations and under aggregating conditions. The presence of α-synuclein fibrils potentiates tau aggregation in some *in vitro* assays (Giasson *et al.*, 2003; Riedel *et al.*, 2009) but not in others (Nonaka *et al.*, 2010). This aggregation inhibits tau-dependent microtubule assembly *in vitro* (Oikawa *et al.*, 2016). Moreover, α-synuclein aggregates a decrease in length but an increase in number in the

presence of tau (Badiola *et al.*, 2011; Giasson *et al.*, 2003). The same study reported a reduction in the more insoluble forms of α-synuclein and increased toxicity of these α-synuclein aggregates in the presence of tau (Badiola *et al.*, 2011). Further studies are needed to improve understanding of the molecular mechanisms involved in this binding.

4.2. Interplay in controlling protein phosphorylation

In addition to aggregation, several studies have focused on the potentiating properties of α-synuclein in tau phosphorylation (at Ser396/404 identified with the PHF1 antibody or at Ser199/202 recognized with the AT8 antibody). This likely occurs by the modulation of tyrosine phosphorylation of glycogen synthase kinase 3β (GSK3β) which leads to increased kinase activity (Khandelwal *et al.*, 2010; Waxman and Giasson, 2011). Inoculation of α-synuclein fibrils increases tau phosphorylation *in vivo* (Masuda-Suzukake *et al.*, 2014). However, hyperphosphorylated tau recognized as puncta-like deposits rarely co-localises with p-α-synuclein after fibril injections (Masuda-Suzukake *et al.*, 2014). α-Synuclein-mediated tau hyperphosphorylation may occur in an α-synuclein concentration-dependent manner. Variable amounts of intracellular α-synuclein can be modulated under cellular stress conditions such as in the presence of ROS (Kawakami and Ichikawa, 2015). Modified α-synuclein plays an active pro-aggregation role for tau *in vivo*, and it is considered by several authors to be a regulator of tau protein phosphorylation. Similar alterations can be observed following unrelated insults such as treatment with rotenone (Chaves *et al.*, 2010) or 1-methyl-4-phenylpyridinium ion (MPP+)/1-methyl-4-

phenyl-1,2,3,6-tetrahydropyridine (MPTP). MPTP treatment also increases α-synuclein and tau hyperphosphorylation in wild-type mice (Qureshi and Paudel, 2011) but not in α-synuclein-deficient mice (Duka *et al.*, 2009). This observation points to an active role for tau in the pathogenesis of synucleopathies.

A common feature of these models is the elevated activation of GSK3β kinase as a sensor of ROS production (Kozikowski *et al.*, 2006). β-sheet-enriched proteins, such as Aβ, increase intracellular ROS (Hureau and Faller, 2009). Moreover, treatment with ROS-promoting factors increases Aβ, α-synuclein and tau hyperphosphorylation (Chaves *et al.*, 2010) preceding neurodegeneration in particular settings (Ghosh *et al.*, 2012). ROS scavengers reduce cell death in experimental models of PD (Lee *et al.*, 2011; Wang *et al.*, 2015), and Aβ-mediated cell death *in vitro* (Kontogiorgis *et al.*, 2007) and *in vivo* (Ghosh *et al.*, 2012), and β-sheet forming peptides of specific prion protein sequence treatments *in vitro* (Gavin *et al.*, 2005). In addition, the increase in oxidative stress and ROS generation can trigger the generation of TNT leading to intensification of the spreading of intercellular α-synuclein (Abounit *et al.*, 2016). In conclusion, increased ROS seems to potentiate both seeding of neurotoxic PS and, in some cases, its intercellular transport, which in turn increases ROS in recipient neurons, potentially leading to a 'vicious' cycle of neurotoxicity. However, although cell death in this co-morbid scenario exemplifies the impact of two or more misbalanced processes, it is unclear whether there is a common process underlying terminal pathology leading to neurodegeneration.

5. PrP^C and tau as partners during neurodegeneration

Although tau fibrils (4RN1) are able to induce tau aggregation in cell cultures (Nonaka *et al.*, 2010), a demonstration of i) the interaction of tau as transmissible PS and PrP^C and ii) the involvement of PrP^C in tau spreading remains elusive. To date, two forms of neuronal interplay between tau and PrP^C have been described: i) direct interaction between PrP^C and tau proteins, and ii) inverse correlation of protein expression in cell lines and during neurodegeneration.

5.1. Direct interaction between PrP^C and tau

Using recombinant proteins, full length tau can bind to recombinant PrP^C *in vitro* (Wang *et al.*, 2008). In fact, the interaction of PrP^C and cytoskeletal proteins such as tubulin was described three years earlier in another study (Nieznanski *et al.*, 2005). The effects of PrP^C on tubulin dynamics include the rapid induction of tubulin oligomerization and aggregation thereby inhibiting microtubule formation (Nieznanski *et al.*, 2006; Osiecka *et al.*, 2009). In addition, increased tau levels reduce the effect of PrP^C on tubulin oligomerization; this reduction is abolished when tau is phosphorylated by both PKA and GSK3 but not after PKA phosphorylation alone (Osiecka *et al.*, 2011). Lastly, activation of the PKA-cAMP response element binding (CREB) protein reduces tau transcription (Liu *et al.*, 2015). Together, these studies point to PKA as a key intracellular kinase regulating tau (levels and phosphorylation), PrP^C and tubulin oligomerization.

The presence of mutated forms of *PRNP* correlates with increased p-tau and neurofibrillary neurodegeneration in specific familial prionopathies, particularly GSS linked to certain *PRNP* mutations. Hyperphosphorylated tau deposition is frequently observed in the brains of GSS patients carrying *PRNP* mutations *P102L* (Ishizawa *et al.*, 2002), *P105L* (Yamazaki *et al.*, 1999), *A117V* (Tranchant *et al.*, 1997), *V176G* (Simpson *et al.*, 2013), *F198S* (Ghetti *et al.*, 1994; Hsiao *et al.*, 1992), *Q217R* (Hsiao *et al.*, 1992; Woulfe *et al.*, 2005) and *Y218N* (Alzualde *et al.*, 2010) (Fig. 7). Although PrP^{C} with the *P102L* mutation shows increased tau binding (Wang *et al.*, 2008), the mechanisms of these point mutations resulting in the increase of hyperphosphorylated tau are not known. Nevertheless, the interplay between certain *PRNP* mutations and tau phosphorylation can be reproduced in differentiating neurons from induced pluripotent stem (IPs) cells reprogrammed from dermal fibroblasts carrying the *Y218N* mutation. Differentiated neurons display elevated p-tau, deficits in mitochondria transport along microtubules and increased cell death (Matamoros-Angles *et al.*, 2017).

5.2. Opposite correlation between PrP^{C} and p-tau/tau levels during neurodegeneration

Changes in 14-3-3, tau and other biomarkers have been reported in several neurodegenerative diseases including human prionopathies (CJD). For example, increased levels of tau, p-tau and α-synuclein are present in the CSF and brain parenchyma of CJD patients (Foutz *et al.*, 2017; Karch *et al.*, 2015; Lattanzio *et al.*, 2017; Llorens *et al.*, 2016a; Llorens *et al.*, 2016b; Llorens *et al.*,

2017). With respect to PrP^C, it has been shown that *PRNP* levels are reduced in CJD brains compared to controls (Llorens *et al.*, 2013a), and this decrease might be involved in the frequent seizures observed in patients (Ng *et al.*, 2014) similar to what has been reported in mice lacking the protein (Carulla *et al.*, 2011; Carulla *et al.*, 2015; Rangel *et al.*, 2009).

Decreased PrP^C-mediated signalling may also generate increased levels of tau, hyperphosphorylated tau and α-synuclein. Experiments carried out to develop models of gain and loss of PrP^C function using neuroblastoma (N2a) or human embryonic kidney (HEK293) cells have shown that cellular levels of tau and p-tau inversely correlate with *PRNP* and PrP^c expression levels *in vitro* (Schmitz *et al.*, 2014; Vergara *et al.*, 2015). The molecular mechanism responsible for the decrease in tau and p-tau levels and their associated toxicity in cell lines remains to be elucidated. Regulation of the activity of the *MAPT* promoter by PrP^C-mediated effectors such as specific miRNAs**,** including miR-34c-5p (Wu *et al.*, 2013), or histone deacetylases such as HDAC2 (Liu *et al.*, 2017), has tentatively been considered. However, an interesting hypothesis suggests that these changes may be associated with oxidative damage, as PrP^C-dependent actions on tau and p-tau reduction are only relevant under oxidative conditions (Schmitz *et al.*, 2014). On the other hand, the effects of a decrease in PrP^C are not exclusive to the *MAPT* promoter since other promoters also increase particular mRNA transcription in the absence of *PRNP* expression (Vergara *et al.*, 2015). Finally, PrP^C overexpression also reduces huntingtin aggregation and toxicity in neuronal cells (Lee *et al.*, 2007). Therefore, it can be suggested that PrP^C and its associated signalling

participate in regulating common intracellular signalling mechanisms, decreasing the expression of tau, p-tau, huntingtin and other molecules involved in neurodegeneration. Whether this intracellular signalling involves ROS generation and Ca^{2+} imbalance as main actors regulating the *MAPT* promoter warrants further study.

6. PrP^C as a binding receptor for PS

6.1. PrP^C and Aβo

Increasing evidence suggests that several membrane receptors can bind to Aβo. Among others, these receptors include PrP^C (Dohler *et al.*, 2014; Fluharty *et al.*, 2013; Freir *et al.*, 2011; Ganzinger *et al.*, 2014; Lauren *et al.*, 2009; Zou *et al.*, 2011), the α7 nicotinic acetylcholine receptor (α7nAChR) (Kar *et al.*, 1998), Fcγ receptor II-b (FcγRIIb) (Kam *et al.*, 2013), the p75 neurotrophin receptor (p75NTR) (Yaar *et al.*, 1997), the paired immunoglobulin-like receptor B (PirB) (Wang *et al.*, 2012), the PirB human orthologue receptor (LilrB2) (Kim *et al.*, 2013), the β-adrenergic receptors (β-ARs) (Wang *et al.*, 2011) and the Eph receptors (Cisse *et al.*, 2011).

PrP^C is one of the binding partners for Aβo (Dohler *et al.*, 2014; Fluharty *et al.*, 2013; Freir *et al.*, 2011; Ganzinger *et al.*, 2014; Lauren *et al.*, 2009; Zou *et al.*, 2011). Indeed, some studies have demonstrated that the N-terminal residues 23-27 as well as the ≈ 94–110 region (CC2) of the PrP^C molecule are relevant for PrP^C interactions with Aβo, as the deletion of any of these regions results in

a major loss of PrP^C-Aβo interaction. In addition to these residues, a recent study pointed to the involvement of the C-terminal domain (120-144) in Aβ fibril formation, modulating the interaction of the N-terminal regions of PrP^C with Aβ (Bove-Fenderson *et al.*, 2017). With respect to the two N-terminal PrP^C interacting domains, they also act in a coordinated manner to provide high affinity binding sites for Aβo. After binding, the Aβo-PrP^C complex activates Fyn kinase through metabotropic glutamate receptor 5 (mGluR5) (Larson *et al.*, 2012; Um and Strittmatter, 2013). mGluR5 does not interact directly with Aβo (Beraldo *et al.*, 2016; Um *et al.*, 2013); however, the ternary complex (Aβo-PrP^C-mGluR5) may act upstream of N-methyl-D-aspartate receptor subunit NR2B (NR2B) phosphorylation (Larson *et al.*, 2012). In addition, PrP^C is linked by mGluR5 to the cellular protein mediators Homer 1b/c, non-receptor tyrosine kinase of the focal adhesion kinase family (Pyk2) and calmodulin-dependent protein kinase II (CamKII) (Haas *et al.*, 2016; Haas and Strittmatter, 2016).

Binding Aβo with PrP^C can be blocked with antibodies (Freir *et al.*, 2011) and with STI1 (Caetano *et al.*, 2008; Ostapchenko *et al.*, 2013). Blockage with antibodies prevents the binding of Aβo (Freir *et al.*, 2011) but can also trigger neurotoxicity in a PrP^C-dependent way (Solforosi *et al.*, 2004; Sonati *et al.*, 2013). In contrast, blockage with STI1 prevents the toxicity mediated by Aβo in cultured neurons and brain slices (Ostapchenko *et al.*, 2013). Recently, a peptide mimicking the binding site of laminin onto PrP^C (Ln-γ1) was shown to promote the internalization of PrP^C-mGluR5 and to transiently decrease Aβo binding to neurons without affecting their neurotoxicity (Beraldo *et al.*, 2016).

Decreased Aβo binding seems to be the result of the increased internalization of the PrP^C-mGluR5 complex (Beraldo *et al.*, 2016).

In parallel, binding Aβo to PrP^C also triggers an increase in tau phosphorylation (Larson *et al.*, 2012). Furthermore, tau hyperphosphorylation and increased oxidative stress have been described as direct cellular effects of Aβo in primary cultures and organotypic slice cultures (Johansson *et al.*, 2006; Lloret *et al.*, 2011; Zempel *et al.*, 2010), 3D neuronal cultures (Choi *et al.*, 2014) and *in vivo* (Chabrier *et al.*, 2012). In fact, Aβo treatment induces activation of caspase-3 and elongator factor 2 (EF2) (a mediator of several stress responses) (Harding *et al.*, 2003) together with abnormal phosphorylation and cleavage of tau (Tanokashira *et al.*, 2017). The increase in EF2 phosphorylation in cortical neurons after Aβo treatment is mediated by Fyn (Um *et al.*, 2013). Although not described, it is reasonable to consider that PrP^C may mediate Aβo-dependent EF2 phosphorylation.

6.2. PrP^C and α-synuclein

As observed for Aβo, several membrane-associated proteins have been described as binding receptors for α-synuclein. This protein binds directly to Na^+/K^+-ATPase subunit α3 (Shrivastava *et al.*, 2015), glucose-regulated protein 78 (GRP78) (Bellani *et al.*, 2014), lymphocyte-activation gene 3 (LAG3) (Mao *et al.*, 2016), neurexin (Mao *et al.*, 2016; Shrivastava *et al.*, 2015) and amyloid β precursor-like protein 1 (APLP1) (Mao *et al.*, 2016). α-synuclein may bind to HSPGs, as happens with other PS (Holmes *et al.*, 2013; Shrivastava *et al.*,

2015). Recently, PrP^C has been discovered to be a binding partner of α-synuclein (Aulic *et al.*, 2017; Ferreira *et al.*, 2017; Urrea *et al.*, 2017). Although the majority of these proteins are implicated in the binding and/or uptake of α-synuclein protofibrils, details of the process are lacking and, for most of them, further participation in the spreading of α-synuclein and interneuronal transport has not been investigated. Nonetheless, the binding and participation of LAG3 and PrP^C in α-synuclein spreading has been analysed *in vivo*: the absence of LAG3 or PrP^C decreases but does not fully impair α-synuclein spreading after PS injection *in vivo* (Aulic *et al.*, 2017; Mao *et al.*, 2016; Urrea *et al.*, 2017). In addition, PrP^C overexpression enhances α-synuclein spreading and pathogenic p-α-synuclein generation (Aulic *et al.*, 2017; Urrea *et al.*, 2017). At the cellular level, GPI-linked PrP^C overexpression enhances α-synuclein binding in primary cultured neurons and in cell lines (HEK293). However, recombinant α-synuclein binding to PrP^C decreases in the absence of the second charged cluster domain (CC2). α-synuclein shares this binding motif of PrP^C with Aβo (Ferreira *et al.*, 2017; Urrea *et al.*, 2017) but not with other β-rich misfolded peptides or infectious scrapie prions (Rambold *et al.*, 2008). Detailed observation of HEK293 *Prnp*-transfected cells after treatment with recombinant α-synuclein demonstrates that fibres bind to plasma membrane of transfected cells in PrP^C-rich domains as well as in apparent PrP^C-free membrane regions (Fig. 8). This result supports a novel line of biophysical studies reporting the direct interaction of α-synuclein with membrane lipids (Chaudhary *et al.*, 2017).

Considering PrP^C as a receptor for α-synuclein, it should be noted that Aβ and α-synuclein share the CC2 domain of PrP^C as a 'binding' domain. Thus, the search for molecules that block this interaction represents a strategy parallel to the current strategies aimed at targeting the seeding or aggregation process of several PS using (-)-epigallocatechin-3-gallate (EGCG) or Z-Phe-Ala-diazomethylketone (PADK) (Andrich and Bieschke, 2015; Guay *et al.*, 2010).

7. Physiological relevance of PrP^C-PS interaction: a 'twister' puzzle to decipher

The PrP^C central region contains a central hydrophobic domain (HD or HR, aa 110/113-133) and a second charged cluster domain (aa 94-110), both involved in binding with different oligomeric species (i.e., scrapie prions and Aβo/α-synuclein, respectively). Nevertheless, the physiological consequence of these interactions is not yet clear. The pioneering description of the Aβo/PrP^C interaction has led researchers to explore the physiological relevance of the interaction, and some descriptions (albeit controversial) have emerged in recent years. In addition, the latest report of the PrP^C/α-synuclein interaction (Ferreira *et al.*, 2017) also points to common intracellular signalling pathways between Aβo/PrP^C and α-synuclein/PrP^C.

Two groups of results have been published focusing on PS/PrP^C interaction: i) studies describing the role of PrP^C in amyloid-mediated neurotoxicity, and ii) reports analysing the functions of PrP^C in amyloid-mediated neurotransmission/synapse plasticity changes. With respect to cell death, the

participation of PrP^C in the neurotoxic effects triggered by aggregated Aβo and α-synuclein seems to be mandatory; PrP^C is mainly located in lipid rafts, and the integrity of lipid rafts is required to trigger Aβo-mediated cell death (Malchiodi-Albedi *et al.*, 2010). However, the data available do not fully explain other observations when considering only amyloid/PrP^C interaction. For example, the absence of PrP^C does not overcome cell death mediated by Aβo (Balducci *et al.*, 2010; Forloni and Balducci, 2011) or by other β-rich peptides (Brown *et al.*, 1998; Fioriti *et al.*, 2005; Gavin *et al.*, 2005; Vilches *et al.*, 2013). Indeed, Brown *et al.* suggested that prion mimicking peptides are nontoxic to *Prnp*$^{0/0}$ cells not because of an inability to interact with these cells but because of the loss of some aspect of a PrP^C expression-dependent phenotype (Brown *et al.*, 1998). Thus, the direct action of amyloids, for example by modification of membrane dynamics or the generation of ion channels (Arispe *et al.*, 1993), cannot be overlooked in the analysis of the PrP^C-mediated effects by misfolded aggregates.

Furthermore, the interaction of Aβo/PrP^C (mainly at 94-110 residues) and α-synuclein/PrP^C (93-109) leads to Fyn activation (Ferreira *et al.*, 2017; Larson *et al.*, 2012; Um *et al.*, 2012) through mGluR5 (Ferreira *et al.*, 2017; Haas *et al.*, 2016). PrP^C-dependent Fyn activation has also been reported after clustering PrP^C with specific antibodies (e.g., SAF61, recognizing residues 142-160 of PrP^C) (Gavin *et al.*, 2005; Mouillet-Richard *et al.*, 2000; Schneider *et al.*, 2003). This is in contrast with other antibodies recognizing PrP^C epitopes 93-105 (≈ CC2 domain), 143-153 aa, 95-105 aa (6D11 antibody) or 4H11 antibody (≈ HD / HR domain) (Klohn *et al.*, 2012; Um *et al.*, 2012). In fact, SAF61-mediated

dimerization activates Ser/Thr kinase extracellular-regulated kinase 1 and 2 (ERK1/2), and NADPH oxidase leads to cell death *in vitro* and *in vivo* (Solforosi *et al.*, 2004). In this respect, the 113-133 aa of PrP^C (HD/HR domain) is required for its dimerization (Rambold *et al.*, 2008).

As indicated by Um *et al.*, binding PrP^C with 6D11 (inside CC2) does not induce Fyn activation but rather blocks Aβo binding and neurotoxicity (Um *et al.*, 2012). This is contrast to Ferreira *et al.*, who indicated that 6D11 is able to block Fyn activation and NMDAR2B phosphorylation after α-synuclein oligomer incubation (Ferreira *et al.*, 2017). These are two conflicting results using different amyloids and the same receptor. Although additional studies are needed, it may be speculated that the interaction of Aβo or α-synuclein with PrP^C does not compromise the HD/HR domain, thereby leaving PrP^C free for putative dimerization triggering Fyn activation. An additional unsolved issue is whether Aβo or α-synuclein/PrP^C /Fyn activation leads to cell death or not, and, more relevantly, whether it can be assumed that this antibody-mediated PrP^C dimerization is a physiological signalling mechanism of PrP^C or rather is merely an 'aberrant' effect.

Concerning neurotransmission and synapse plasticity, several studies, mainly from the Strittmatter lab, have reported that Aβo/PrP^C/mGluR5 complexes are responsible for facilitating long-term depression (LTD) and dendritic spine plasticity in cultured neurons. These effects are mediated by the phosphorylation of N-methyl-D-aspartate (NMDA) receptor subunits (Salazar and Strittmatter, 2017). Similar effects have recently been shown for α-

synuclein and PrP^C (Ferreira *et al.*, 2017). In addition, Haas *et al.* recently described additional effectors of Aβ-PrP^C binding including Homer, EF2, CamKII and Pyk2 (Haas *et al.*, 2016), some of which have relevant roles in synapse plasticity and neurodegeneration. Thus, although their participation in the neurodegenerative effects of Aβo is still controversial (Wulf *et al.*, 2017), it seems that the implication of PrP^C in synaptic plasticity after Aβo or α-synuclein binding is well established.

With respect to Aβo, two separate signalling cascades, one dependent on extracellular Ca^{2+} and Fyn kinase activation and the other on the release of Ca^{2+} from intracellular stores, have been proposed after Aβo/PrP^C/mGluR5 interactions (Haas *et al.*, 2016). With respect to α-synuclein/PrP^C/mGluR5, a Ca^{2+}-dependent mechanism has been postulated (Ferreira *et al.*, 2017). mGluR5 expression is increased in PD (Price *et al.*, 2010) and it enhances the neurotoxic effects in PD models (Battaglia *et al.*, 2004). mGluR5 blockers protect against cell death in these models (Flor *et al.*, 2002). Taking these coincidences into account, the current interventions in AD (Salazar *et al.*, 2017) may also be relevant in PD and related synucleopathies.

8. Concluding remarks

Emerging evidence points to interneuronal transport of insoluble misfolded proteins *in vitro* and *in vivo*. A full description of the cellular mechanisms playing roles in this spreading is missing for most PS. Indeed, our knowledge of the process is only partial, and several questions remain unsolved. Most studies

have used recombinant proteins to construct misfolded aggregates that do not fully mimic the biochemical properties of PS-derived from affected human brains. In addition, the relative concentration of the PS in these experiments is very high when compared to diseased human brains. Moreover, most spreading experiments are performed by inoculating the PS (recombinant or brain-derived) (for example tau) in mice overexpressing the native (*MAPT*) or mutated form (P301S-*MAPT*) to potentiate the aggregation or the seeding properties of the PS. Whether all the mechanisms of spreading described are physiologically relevant or not must be critically reviewed. Differential seeding properties and specific strains must be considered and the components accompanying PS derived from humans should be fully dissected to learn about the primary microenvironment of the human PS used for seeding. Furthermore, the putative role of glial cells, mainly astrocytes, in these processes is largely unknown.

Researchers are interested in determining specific receptors for particular PS, and several interactions with particular proteins have been reported. However, evidence of the physiological implications of this binding/interaction is only just being obtained. For example, a common region of PrP^C has been described for PrP^C, Aβ and α-synuclein interactions, thus opening new avenues for drug discovery. Eventually, these observations will help to better delineate the functions of PrP^C in both healthy subjects and brain disease.

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Figure legends

Figure 1: AD. Neurofibrillary tangles (long arrows) and senile plaques (short arrows) as seen with silver staining (A). Senile plaques composed of Aβ (m0872 antibody) (B) are surrounded by neuronal processes and dystrophic neurites containing hyper-phosphorylated tau (C, D). Neuropil threads and neurofibrillary tangles immunostained with anti-phosphorylated tau antibodies (clone AT8) (E, F). Neurofibrillary tangles are composed of PHF (G) which are gold-immunolabelled with anti-p-tau antibodies (H). Dystrophic neurites (arrows) surrounding Aβ cores (asterisk) contain, in addition to PHFs, abnormal mitochondria and cellular debris in lysosomes (I). Western blotting of sarkosyl-insoluble fractions in two AD cases shows the typical pattern of tau with upper bands of 68, 64 and 60kD, and several truncated forms at the carboxy terminal (phosphorylation) site at amino acid Ser422. A-F, paraffin sections. Scale bars: A = 40µm; B = 60µm; C and D = 50µm; E and F = 25µm; G-I: electron microscopy; G = 1µm; H = 0.2µm; I = 2µm.

Figure 2: DLB. LB and LN staining. Typical LB in a pigmented neuron of the substantia nigra pars compacta stained with hematoxylin and eosin (HE) (A). LB (B-H) and LN (I-L) containing abnormal α-synuclein in nucleus basalis of Meynert (B), spinal cord (C), reticular formation (D), hypothalamus (E), dorsal thalamus (F), amygdala (G), cerebral cortex (H), reticular formation (I), substantia nigra (J), CA3 region of the hippocampus (K) and amygdala (L). Paraffin sections. Scale bars: A = 20µm; B-K, bar in K = 6µm; L = 60µm.

Figure 3: PrP^{res} (M7216 antibody) deposits in CJD: synaptic pattern in sCJD (MM1 type) (A); perivacuolar deposits delineating confluent spongiform change in sCJD (MM2 type) cortical (B); perineuronal deposits in sCJD (VV2 type); kuru plaques (arrows) as seen with HE (D) and PrP immunohistochemistry (E) in sCJD (MV2 type); and globular PrP deposits in atypical sCJD (F). Variant CJD (vCJD) having typical florid plaques stained with HE (G) containing a core of PrP^{res} (H); cluster plaques and granular deposits in the cerebellum in vCJD (I). Paraffin sections. Scale bar = 20µm.

Figure 4: *In vitro* studies of PS propagation in microfluidic devices. A: Schematic representation of the basic microfluidic device used in numerous studies. In this model, two reservoirs, green (left) and red (right), are separated by a large number of microgrooves (grey) (≈ 100 of 5-10µm (square section) x 450-1000µm length). B: Neurons cultured in the microfluidically isolated compartment (left) project axons toward the right compartment where different cell types (neurons, glial cells and others) can be cultured. Calcein™ staining. C-D: Examples of α-synuclein intercellular transport in microfluidic devices. p-α-synuclein staining (AB5336P antibody; green) in LN-like (arrow in C) and LB-like (arrows in D) aggregates in neurons growing in the right reservoir (asterisks in A) after treatment with α-synuclein fibrils in the left reservoir (# in A). In order to avoid passive fluidic transport between reservoirs (left → right), the recipient reservoir (right) is cultured with a higher volume of medium than the donor reservoir (left). DAPI nuclear staining. E: High power photomicrographs of layer V neocortical neurons with relevant p-α-synuclein (Ser129 antibody)

accumulation (arrows) after α-synuclein inoculation in postcommissural striatum of wild-type mice. Scale bars: B = 250μm; C = 50μm pertains to D; E = 50μm.

Figure 5: DLB showing LB/LN stained with anti-α-synuclein antibody (ab5038) (A) and Aβ-containing plaques (m0872 antibody) (B, C). Paraffin sections. Scale bars: A = 15μm; B = 40μm; C = 20μm; B and C immunofluorescence and confocal microscopy.

Figure 6: Atypical MSA with cortical involvement showing intraneuronal α-synuclein (ab5038 antibody) deposits (A) together with neurofibrillary tangles (AT8-positive) and rare senile plaques (m0872–positive) (B). Double-labelling immunofluorescence and confocal microscopy show occasional co-localisation of α-synuclein (green) and hyper-phosphorylated tau (AT8) (red) in the same neuron (C, D). Paraffin sections. Scale bars: A = 40μm; B = 20μm; C = 40μm; D = 80μm.

Figure 7: GSS (P120L) 129V/V mutation in the *PRNP* gene. Neocortex: abnormal granular and globular focal deposits stained with HE (A, arrows) are composed of multicentric PrP^{res} (M7216-positive) plaques (B, C). Hyper-phosphorylated tau (clone AT8) is seen in clusters of abnormal neurites (D, E) and in neurofibrillary tangles (F). Double-labelling immunofluorescence and confocal microscopy shows clusters of neurites containing hyper-phosphorylated tau (red) in contact with multicentric PrP^{res} plaques (green) (G). Western blotting shows the typical GSS pattern of PrP^{res} (3F4 antibody)

including bands of very low molecular weight (< 20 kD) (H). Paraffin sections. Scale bars, A and B, bar in B = 60μm; C-F, bar in F = 50μm; G = 50μm.

Figure 8: Double immunofluorescence photomicrographs illustrating HEK293 cells overexpressing PrP^{C} (green, SAF61 antibody) after incubation with α-synuclein recombinant protofibrils (red, AB5336P antibody). Arrows in A point to relevant co-localisation of the two proteins in particular membrane regions. B-C: High magnification of the central region of the cell (dashed box in A) showing regions of clear co-localisation (asterisks) intermingled with areas without co-localisation. This suggests the presence of different interactions between α-synuclein fibrils and components of plasma membrane. Scale bars; A = 25μm; B = 10μm pertains to C.

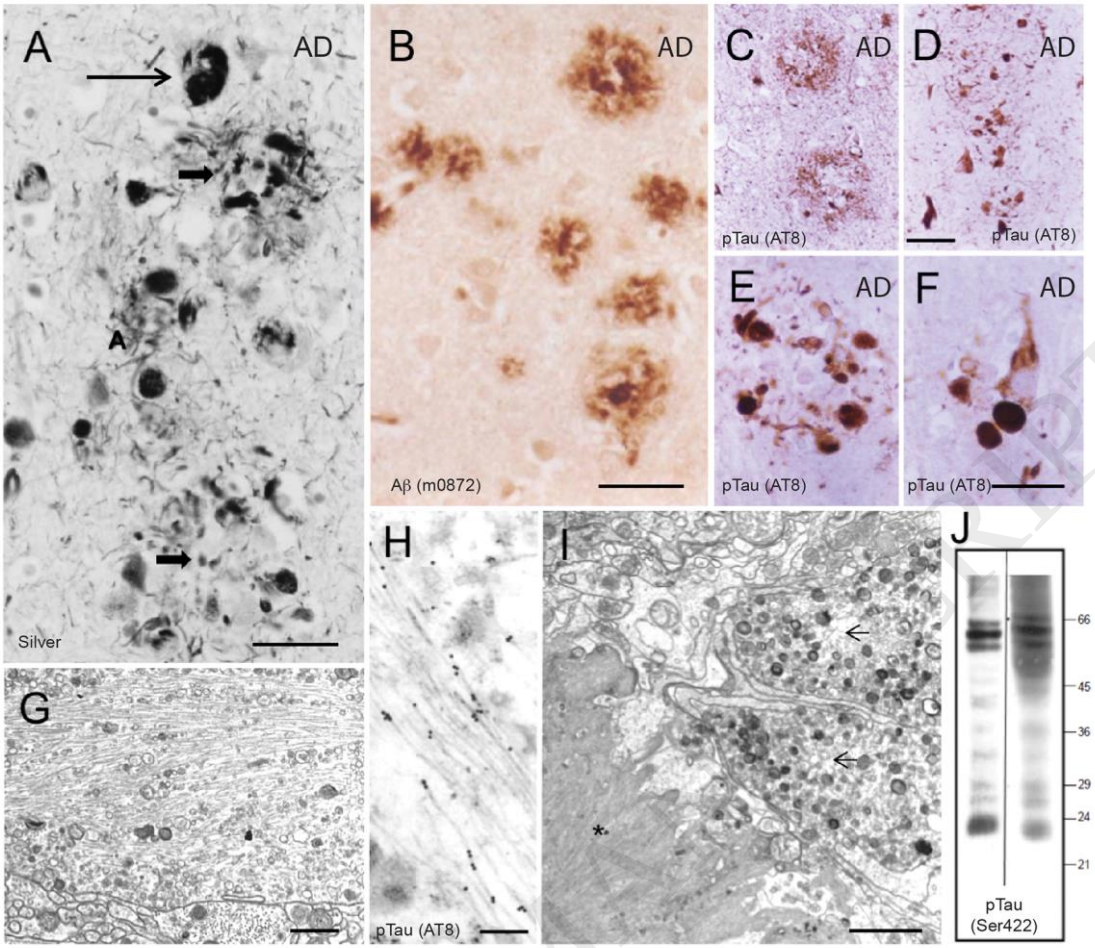

FIGURE 1

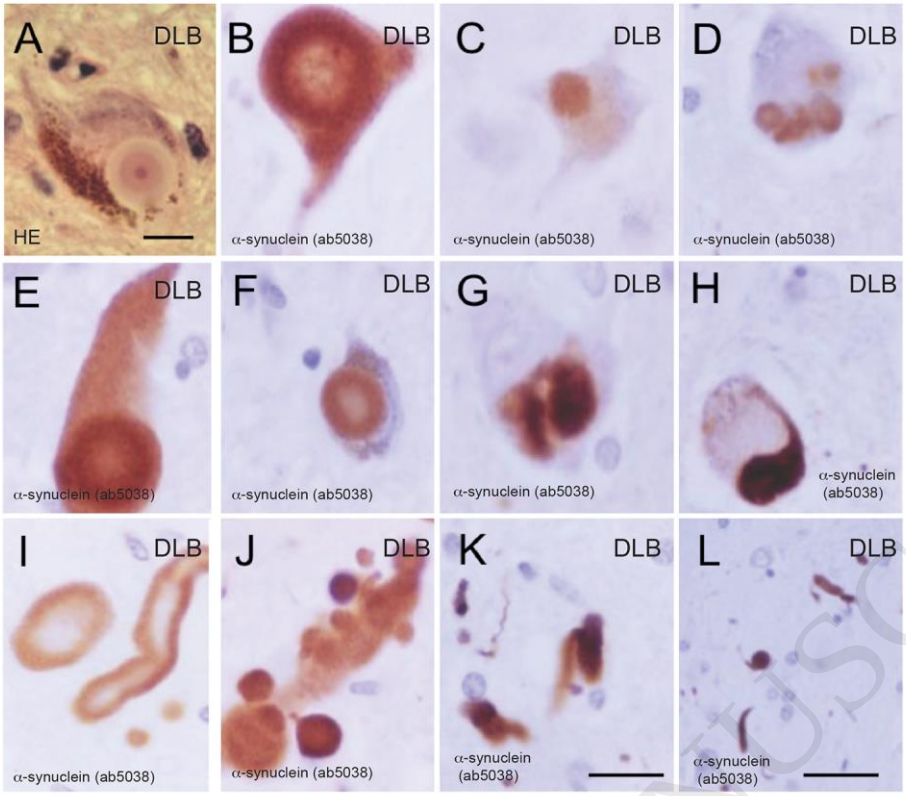

FIGURE 2

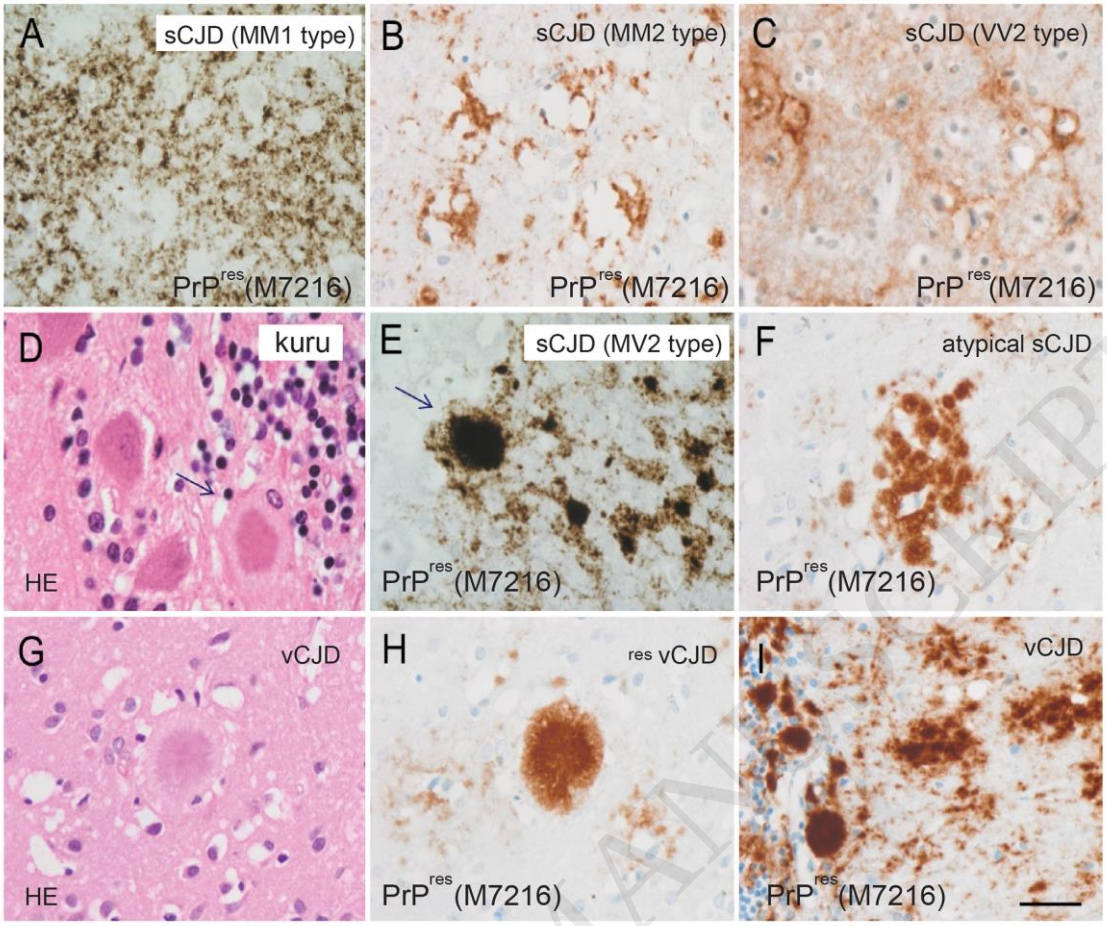

FIGURE 3

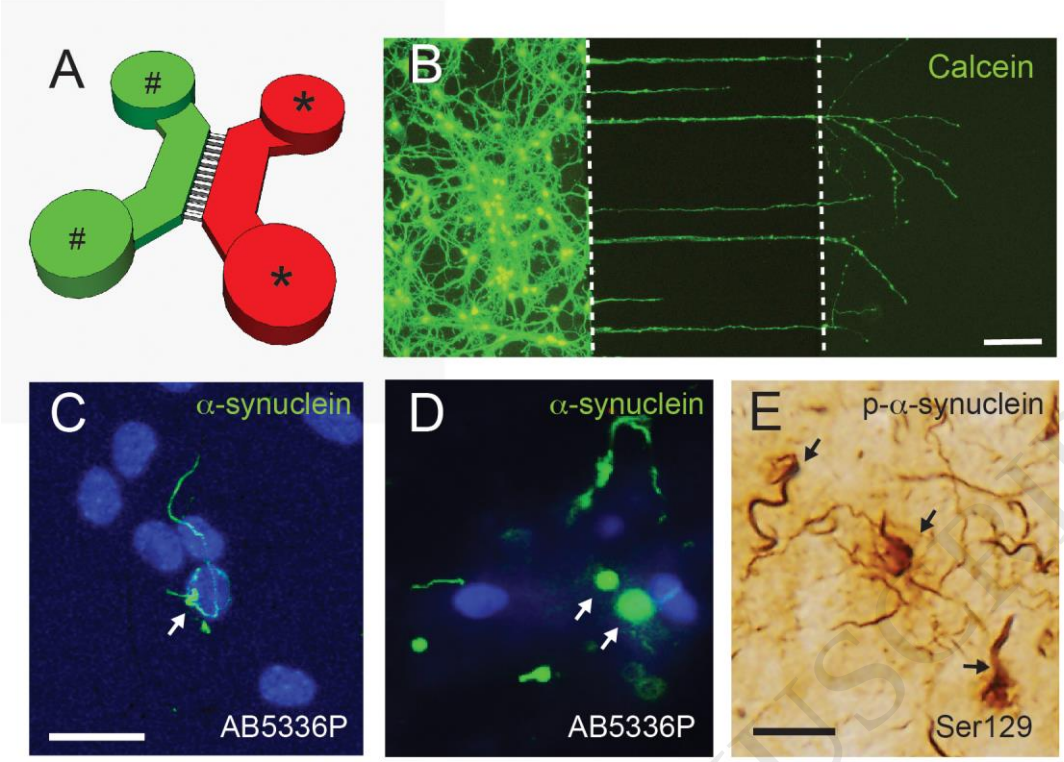

FIGURE 4

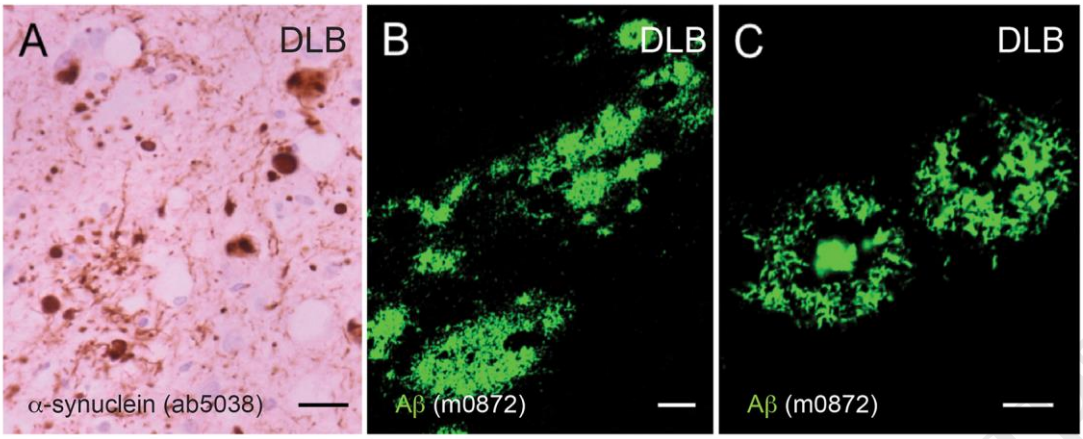

FIGURE 5

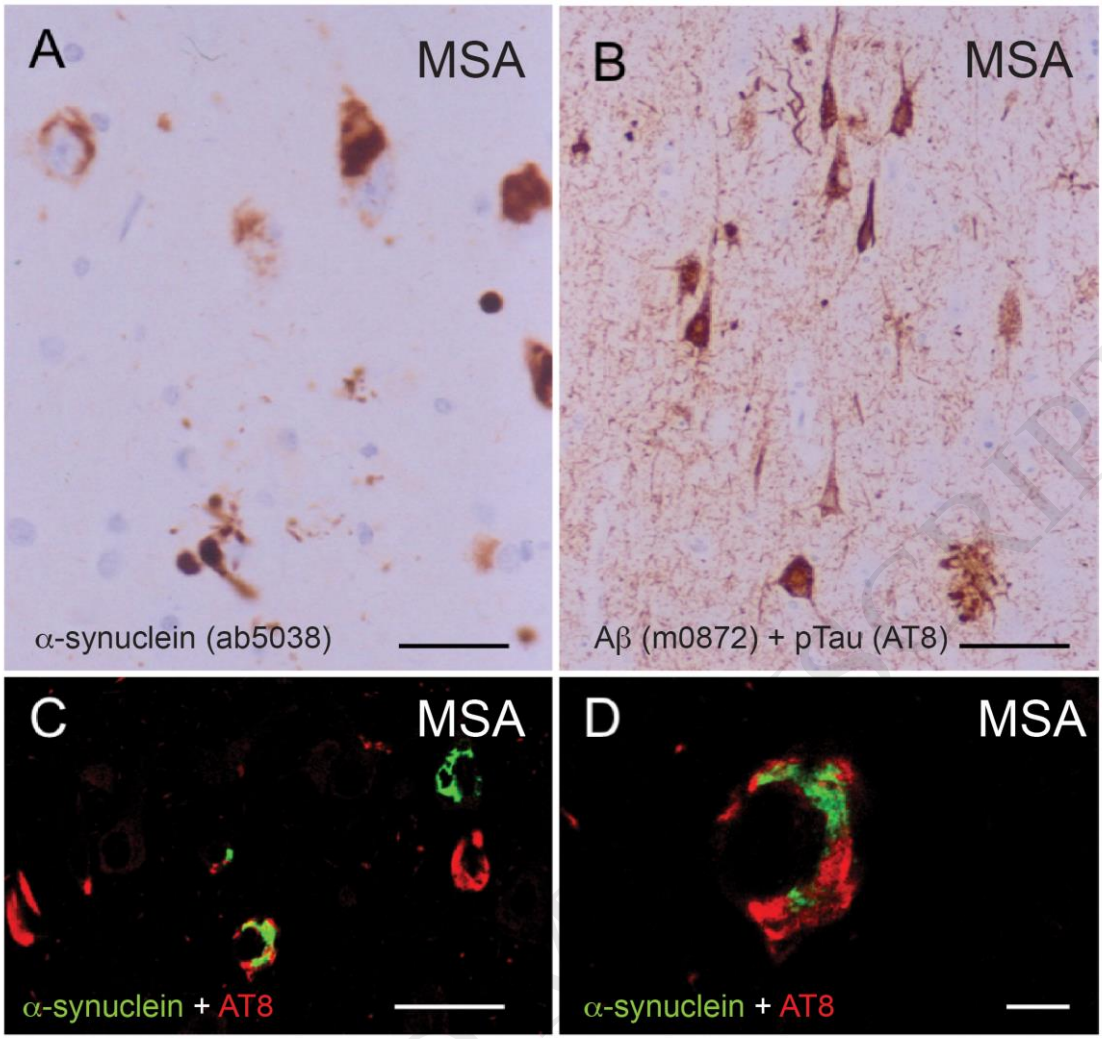

FIGURE 6

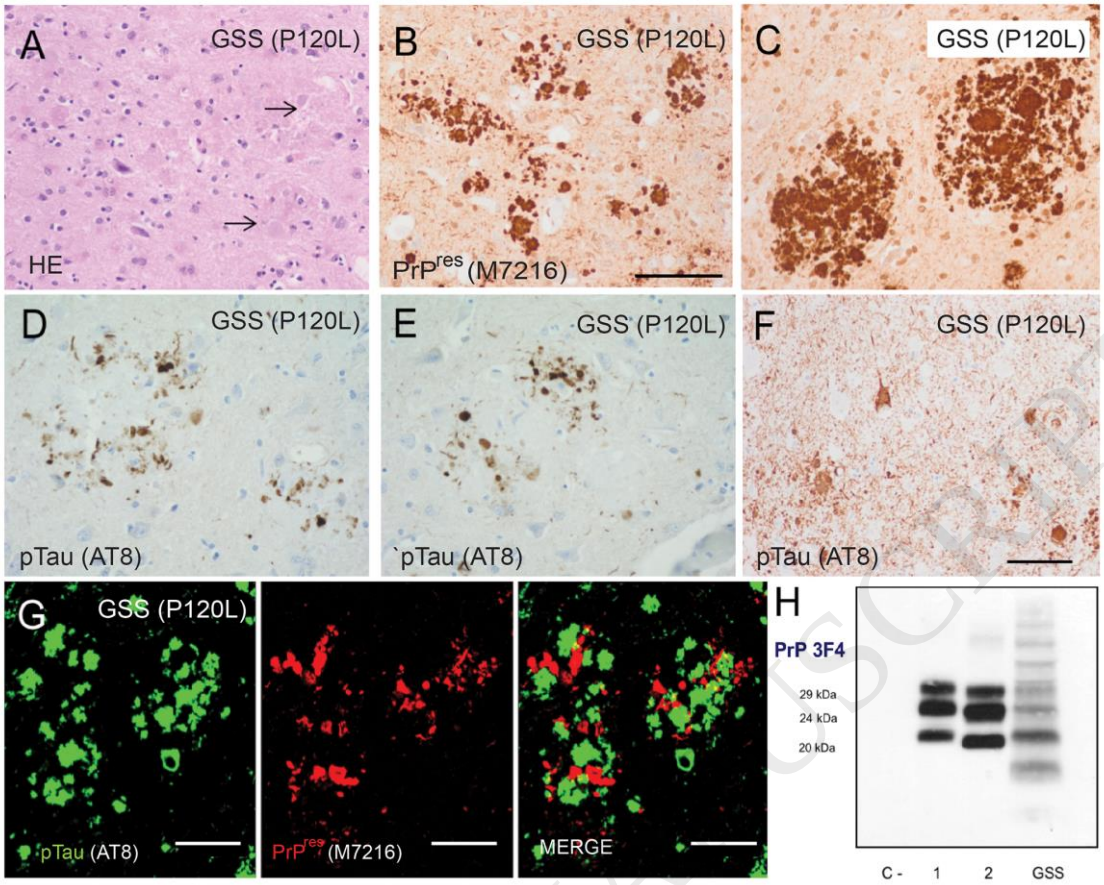

FIGURE 7

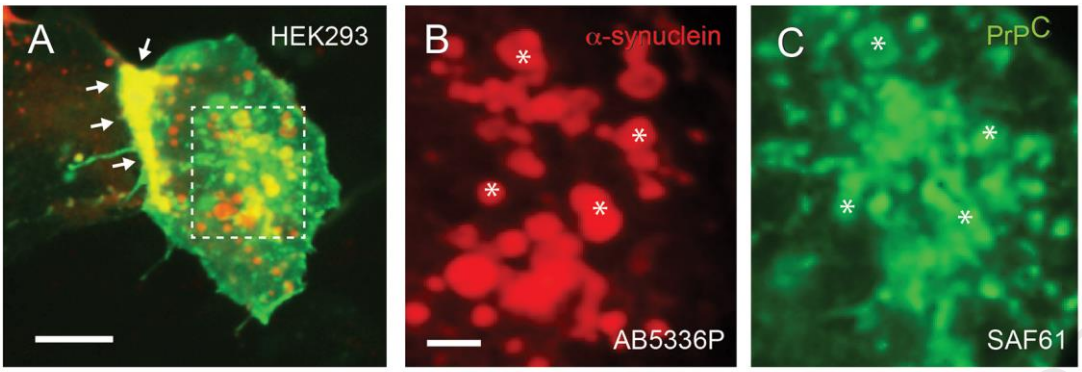

FIGURE 8