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Role of Astrocytes in Neurodegeneration

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Monografia orientada pelo Professor Doutor Rui Fernando Marques da Silva, Professor Auxiliar

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Resumo

As células da glia constituem um grupo celular abundante no cérebro humano. Estas desempenham funções de suporte, de modo a auxiliar no estabelecimento de sinapses, bem como na manutenção do funcionamento e sinalização por parte dos neurónios. As células da glia são classificadas em três grupos principais: a micróglia, cujo papel está maioritariamente relacionado com o sistema imunitário; os astrócitos e, por último, os oligodendrócitos e células de Schwann, responsáveis pela formação de camadas de mielina nos axónios do sistema nervoso central e periférico, respetivamente.

Os astrócitos são as células da glia mais abundantes no sistema nervoso central. No século 19, Michael von Lehnossek introduziu o termo "astrócito", ao observar que a morfologia destas células se assemelhava ao formato de uma estrela. Os astrócitos possuem várias diferenças quanto à sua morfologia, desempenhando diversas funções essenciais para a manutenção da homeostasia cerebral. Os astrócitos executam funções de manutenção metabólica, estrutural e de suporte neuronal. Adicionalmente, as células da astroglia são essenciais para a manutenção da barreira hematoencefálica.

Relativamente à sua morfologia, os astrócitos emitem ramificações extensas a partir do corpo celular, possuindo pés terminais no final das suas ramificações, de modo a estabelecerem contacto com os capilares cerebrais. No seu citoplasma, os astrócitos possuem diversos feixes de filamentos intermédios, sendo os mesmos constituídos pela proteína acídica fibrilar glial (GFAP). A expressão de GFAP é normalmente utilizada como marcador específico na identificação de astrócitos. A proteína S100B, ligante do ião cálcio, medeia a interação entre as células da glia e os neurónios. As proteínas GFAP e S100B não são marcadores universais para todos os astrócitos.

Atualmente, sabe-se que existem diversos tipos de astrócitos com diferentes características morfológicas. No entanto, a classificação binária é bastante utilizada, dividindo os astrócitos em duas classes principais: protoplasmáticos e fibrosos. Os astrócitos protoplasmáticos encontram-se presentes na substância cinzenta cerebral, são positivos para o marcador S100B e apresentam ausência quase total de GFAP. Os seus processos são curtos e bastante complexos, estabelecendo contactos com os capilares sanguíneos e com diversas sinapses. Os astrócitos fibrosos, localizados na substância

branca cerebral, são positivos para o marcador GFAP e possuem longos processos com menor complexidade, estabelecendo contactos com os nódulos de Ranvier.

As doenças neurodegenerativas conferem aos seus doentes condições bastante debilitantes, abrangendo indivíduos de todas as faixas etárias. Estas patologias caracterizam-se por uma degeneração progressiva, acompanhada da morte dos neurónios. Consequentemente, as doenças neurodegenerativas afetam nomeadamente as capacidades motoras e cognitivas dos seus doentes, podendo mesmo originar demência. Atualmente, estas patologias representam um assunto de extrema importância a nível mundial, nomeadamente devido ao seu impacto nas áreas socioeconómicas e da saúde. Até ao momento, ainda não foi descoberta nenhuma cura definitiva para as doenças neurodegenerativas. No entanto, devido aos inúmeros estudos efetuados nesta área de investigação, os progressos são cada vez maiores.

Atualmente, a maioria das investigações efetuadas na área da neurodegeneração sugere um envolvimento dos astrócitos e da micróglia na patogénese e progressão das doenças neurodegenerativas. Os astrócitos são células imprescindíveis para um normal funcionamento cerebral. Caso haja uma disfunção astrocítica, a probabilidade do surgimento de fenómenos patogénicos que levam à morte neuronal é elevada.

A ativação dos astrócitos constitui uma característica principal nas doenças neurodegenerativas, bem como uma resposta defensiva adaptada que fornece suporte metabólico aos neurónios. Esta resposta é desencadeada por diversos fatores, nomeadamente toxinas, elementos químicos, stress oxidativo e agentes patogénicos. A formação de radicais livres, os processos inflamatórios e a neurodegeneração são fenómenos causados pela disfunção da ativação astrocítica, estando associados ao desenvolvimento deste tipo de patologias.

Os astrócitos reativos podem desempenhar dois papéis na patogénese do sistema nervoso central: providenciar neuroprotecção e reparação celular, mas podendo igualmente promover a ocorrência de fenómenos neurodegenerativos. Consequentemente, os astrócitos reativos poderão ser classificados nos tipos A1 (neurotóxicos) e A2 (neuroprotetores).

A doença de Alzheimer é uma doença neurodegenerativa e a principal causa de demência. Esta patologia constitui a terceira causa de mortalidade em Portugal. Uma das principais preocupações relacionadas com a doença de Alzheimer consiste no seu

diagnóstico tardio, uma vez que os sintomas iniciais surgem anos após as primeiras mudanças patológicas cerebrais. Consequentemente, um dos principais objetivos na comunidade médica e científica passa por uma deteção precoce, de modo a prevenir, abrandar ou mesmo parar a sua progressão.

A doença de Alzheimer é caracterizada pela acumulação de fragmentos proteicos do péptido β-amilóide (Aβ), resultando na formação de placas de Aβ no parênquima cerebral, bem como na acumulação de uma forma atípica da proteína tau no meio intracelular dos neurónios, originando novelos neurofibrilares (NFTs). A acumulação de placas de Aβ poderá desencadear a morte neuronal ao interferir na comunicação sináptica entre neurónios. No meio intracelular, os NFTs poderão desencadear o bloqueio do transporte de nutrientes e outras moléculas essenciais para o bom funcionamento neuronal e a sobrevivência destas células.

Adicionalmente, a doença de Alzheimer é caracterizada pela ocorrência de neuroinflamação e atrofia cerebral. Pensa-se que a presença de Aβ e de NFTs desencadeiam a ativação da micróglia que, por sua vez, tenta eliminar as proteínas tóxicas, bem como a disseminação de resíduos resultantes da morte neuronal. A partir de certo ponto, a micróglia deixa de conseguir exercer as suas funções, levando a um estado de inflamação crónica.

Os astrócitos contribuem para a perda de neuroprotecção e aparecimento de fenómenos patológicos na doença de Alzheimer. Inicialmente, os astrócitos possuem um papel protetor, ao captarem e eliminarem os fragmentos Aβ. No entanto, com a progressão da doença, os astrócitos perdem a capacidade de *clearance*, levando à acumulação de Aβ que, por sua vez, causa a estimulação dos astrócitos, levando à produção de mediadores pro-inflamatórios e, por último ao dano neuronal.

A doença de Parkinson é uma doença neurodegenerativa caracterizada pela perda de neurónios dopaminérgicos na *substantia nigra*. Consequentemente, os baixos níveis de dopamina levam ao surgimento de sintomas a nível do sistema locomotor, como acinesia/bradicinesia, rigidez, tremor e instabilidade postural. Para além destes sintomas, poderá ainda surgir outro tipo de sintomatologia, nomeadamente depressão e distúrbio comportamental do sono REM.

A doença de Parkinson é caracterizada pelo aparecimento dos corpos de Lewy, que consistem em agregados de proteínas que contêm nomeadamente a proteína α-

sinucleína. O mecanismo que leva ao surgimento desta patologia continua por clarificar. No entanto, considera-se que diversos fatores poderão levar à apoptose, tais como o stress oxidativo, a neuroinflamação, toxicidade ao nível da α-sinucleína, défice mitocondrial, entre outros.

Tal como referido anteriormente, os astrócitos tornam-se reativos em resposta à secreção de diversos fatores pela micróglia, adotando um fenótipo pró-inflamatório. Adicionalmente, os astrócitos podem ainda efetuar a endocitose de α-sinucleína libertada pelos neurónios. Estudos demonstraram que a acumulação de α-sinucleína nos astrócitos favorece a disfunção dos mesmos, promovendo ainda a ativação da micróglia, nomeadamente em regiões do cérebro onde existe uma diminuição significativa de neurónios dopaminérgicos, favorecendo a neurodegeneração.

A esclerose lateral amiotrófica caracteriza-se pela degeneração progressiva dos neurónios motores na medula espinal, no tronco cerebral e no córtex motor. Esta patologia leva à perda de diversos fatores relacionados com a fisiologia dos neurónios motores, bem como à perda de sinapses neuromusculares, causando a paralisia de músculos voluntários. A principal causa de morte relacionada com a esclerose lateral amiotrófica está diretamente relacionada com incapacidade respiratória.

A neuroinflamação e a ativação das células da glia são observadas quer no início da doença, quer ao longo da sua progressão. Adicionalmente, é possível observar astrócitos reativos no córtex motor e na medula espinal dos doentes. Este tipo de astrócitos perdem as suas funções homeostáticas e, consequentemente, tornam-se neurotóxicos, levando à neurodegeneração.

Na esclerose lateral amiotrófica, existem vários fenótipos de astrócitos, bem como uma diversidade regional. A patogénese desta doença resulta de uma variedade de interações celulares. Atualmente, várias investigações relativas a esta patologia abordam diversas possibilidades terapêuticas, nomeadamente quanto à regulação da função dos astrócitos e promoção da sobrevivência e regeneração dos neurónios motores.

De modo a procurar a resolução e tratamento para as doenças neurodegenerativas, é essencial que as investigações e estudos nesta área continuem a evoluir. O desenvolvimento de novas abordagens quanto à preservação dos mecanismos neuroprotetores dos astrócitos, bem como a manutenção das propriedades fisiológicas

destas células poderiam, eventualmente, levar ao abrandamento da progressão da neurodegeneração.

A presente monografia visa estudar o impacto dos astrócitos nas doenças neurodegenerativas. Inicialmente, será efetuada uma abordagem teórica sobre os astrócitos e as suas principais funções, seguindo-se uma breve revisão sobre as doenças neurodegenerativas. O foco desta monografia será a doença de Alzheimer, devido ao seu grande impacto na população mundial, sendo uma das grandes causas de mortalidade em Portugal. No entanto, será feita uma breve abordagem sobre o papel dos astrócitos na progressão da doença de Parkinson e na esclerose lateral amiotrófica. Por último, abordar-se-ão estudos recentes, de modo a analisar quais as perspetivas futuras relativamente ao tema em causa.

Palavras-chave: Astrócitos; Doenças Neurodegenerativas; Doença de Alzheimer; Doença de Parkinson; Esclerose Lateral Amiotrófica.

Abstract

Astrocytes are the most abundant glial cells in the central nervous system (CNS). These cells have a highly heterogenous morphology. Astrocytes are known to maintain structural, metabolic and guidance support for neuronal function. These cells play critical roles in diverse physiological CNS processes, acting as neuroprotective cells. They are also essential for the integrity of the blood-brain barrier (BBB).

Neurodegenerative diseases are characterized by a progressive degeneration and/or neuronal cell death, which may affect body's movement and cerebral performance, leading to dementia. Nowadays, neurodegenerative diseases are one of the biggest medical and socioeconomical issues worldwide and the causes of their emergence are still unknown. There has still not been discovered a definite cure for these diseases.

Most of the investigations suggest that microglia and astrocytes causally participate in the pathogenesis and progression of many neurodegenerative diseases. Considering the importance of astrocyte functions for CNS performance, it is reasonable to say that astrocyte dysfunction can be a primary event of a pathogenic cascade ultimately leading to neuronal loss. The activation of astrocytes is a pathological feature of neurodegenerative diseases and an adaptive defense response that provides essential metabolic support for neurons. The formation of free-radicals, inflammation, elevations of glutamate levels and neuronal degeneration that are caused by the dysfunction of astrocyte activation are closely related to the development of neurodegenerative diseases. However, reactive astrocytes play opposite roles in the diseased CNS: they promote neurodegeneration, but they can also support neuroprotection and repair.

Alzheimer's disease (AD) is the most common cause of dementia and one of the major causes of death worldwide. One of the major concerns of AD is its late diagnosis, since symptoms only start to appear many years after the first physiological changes in human brain. Therefore, AD has become a significant area of research.

The current dissertation intends to review the role of astrocytes in neurodegenerative diseases. Alzheimer's disease will be the focus of this monograph, but Parkinson's disease and amyotrophic lateral sclerosis will also be briefly discussed.

Keywords: Astrocytes; Neurodegenerative Diseases; Alzheimer's Disease; Parkinson's Disease; Amyotrophic Lateral Sclerosis.

"Everyone you meet is fighting a battle you known nothing about. Be kind. Always."

-Robin Williams, victim of Lewy body dementia

In memory of my beloved grandparents, João and Mabília, who

always inspired me to never give up on my dreams.

I will always carry you in my heart.

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Abbreviations

CNS	Central Nervous System
GFAP	Glial Fibrillary Acid Protein
Aldh1L1	Aldehyde Dehydrogenase-1 family member L-1
GPC4	Glypican 4
GPC6	Glypican 6
SPARCL1	Hevin
THBS1	Thrombospondin 1
THBS2	Thrombospondin 2
MEGF10	Multiple EGF-like domains 10
MERTK	MER Proto-oncogene Tyrosine Kinase
GABA	Gamma-Aminobutyric Acid
ATP	Adenosine Triphosphate
AQP4	Aquaporin 4
EAAT	Excitatory Amino-Acid Transporter
GLAST	Glutamate Aspartate Transporter 1
GLT-1	Glutamate Transporter-1
BBB	Blood-Brain Barrier
GLUT1	Glucose Transporter 1
TGF-β	Transforming Growth Factor beta
GDNF	Glial cell line-Derived Neurotrophic Factor
FGF	Fibroblast Growth Factor
IL-6	Interleukin 6
SSeCKS	Src-Suppressed C-Kinase Substrate
VEGF	Vascular Endothelial Growth Factor
ATPase	Adenosine Triphosphatase

Kir	Inward-Rectifier Potassium Channel
CD40	Cluster of Differentiation 40
DNA	Deoxyribonucleic Acid
TDP-43	Transactive DNA-binding Protein 43
NFT	Neurofibrillary Tangles
AD	Alzheimer's disease
PD	Parkinson's disease
DLB	Dementia with Lewy Bodies
ALS	Amyotrophic Lateral Sclerosis
WHO	World Health Organization
ADI	Alzheimer's disease International
COVID-19	Coronavirus disease 2019
DALY	Disability-Adjusted Life Year
Aβ	Beta-amyloid
APP	Amyloid Precursor Protein
PHF	Paired Helical Filament
CDK5	Cyclin-dependent Kinase 5
MAPK	Mitogen-activated protein kinase
PPA2	Protein Phosphatase 2
MCI	Mild Cognitive Impairment
NIA-AA	National institute on Aging and Alzheimer's Association
PET	Positron Emission Tomography
CSF	Cerebrospinal Fluid
sMRI	Structural Magnetic Resonance Imaging
FDG-PET	^{18}F-fluorodeoxyglucose positron emission tomography
ApoE4	Apolipoprotein E4

NAAQS	National Ambient Air Quality Standards
CVD	Cardiovascular Disease
IGT	Impaired Glucose Intolerance
IRS-1	Insulin Receptor Substrate 1
EOAD	Early-onset AD
PSEN-1	Presenilin-1
PSEN-2	Presenilin-2
ApoE2	Apolipoprotein E2
ApoE3	Apolipoprotein E3
LOAD	Late-onset AD
CAA	Cerebral Amyloid Angiopathy
PTPN9	Protein Tyrosine Phosphatase Non-Receptor Type 9
PCDHA4	Protocadherin Alpha-4
ROS	Reactive Oxygen Species
IL-1β	Interleukin-1β
TNF-α	Tumor Necrosis Factor α
BDNF	Brain-Derived Neurotrophic Factor
CCL2	β-chemokine 2
CCL20	β-chemokine 20
CXCL10	α-chemokine 10
CXCL12	α-chemokine 12
HB-EGF	Heparin-Binding Epidermal Growth Factor
ApoJ	Clusterin
SORL1	Sortilin-Related Receptor 1
FERM2	Fermitin Family Member 2
6E10	Aβ Recombinant Monoclonal Antibody

APP/PS1	Transgenic mouse that expresses chimeric mouse/human APP and a mutant human PSEN-1
PSD95	Postsynaptic Density Protein 95
JAK2-STAT3	Janus Kinase 2 signal transducer and activator of transcription 3
TFEB	Transcription Factor EB
BACE1	Beta-secretase 1
IFN-γ	Interferon-γ
TLR	Toll-Like Receptor
RBD	REM sleep behavior disorder
ENS	Enteric Nervous System
SNCA	Synuclein Alpha
NLY101	Glucagon-like peptide-1 receptor agonist
SOD1	Superoxide Dismutase 1
C9orf72	ofr 72 on chromosome 9
TARDBP	TAR DNA Binding Protein
FUS	Fused in Sarcoma
RNA	Ribonucleic Acid
mSOD1	mutant SOD1
Cx43	Connexin-43
HMGB1	High Mobility Group Box Protein 1
NF-kB	Nuclear Factor Kappa B
GluR2	Glutamate Receptor 2
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
iNOS	inducible Nitric Synthase
SPD1	S-Phase Delaying Protein 1
ES cells	Embryonic Stem Cells

AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazopropionic acid receptor
GluA2	Glutamate receptor 2
PPARγ	Proliferator-activated receptor γ
NMDA	N-methyl-D-aspartate
MAO-B	Monoamine Oxidase B
miRNA	MicroRNA
TF	Transcription Factors

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1 Introduction

1.1 Astrocytes

The glial cells, also known as glia (from the Greek word for "glue"), are non-neuronal cells located in the brain. In general, glial cells play supportive functions to help define synaptic contacts and maintain the signaling abilities of neurons. Based on their shape, function and location, glial cells are classified into three main types: 1) microglia, which act as immune cells; 2) astrocytes; 3) oligodendrocytes and Schwann cells, which form myelin layers around axons in the central and peripheral nervous systems, respectively (1, 2).

Astrocytes are the most abundant glial cells in the Central Nervous System (CNS). These elements possess various structures and functions and are ubiquitous in all regions of the CNS. They are known to maintain structural, metabolic, and guidance support for neuronal function. Astrocytes play critical roles in a broad range of physiological CNS processes. They are also essential for the integrity of the blood-brain barrier. However, astrocytes are important contributors to the progression of CNS injury and neurodegeneration (3-5).

In 1856, Rudolf Virchow first introduced the idea of "neuroglia", describing it as a "nerve glue", a connective tissue in which the nervous system's elements are embedded (6-8). A decade later, Otto Deiters made the first key description and visualization of an astrocyte. The German scientist was the first to draw the astrocytes as stellate cells. Years later, Jacob Henle and Friedrich Merkel observed the network of astrocytes in the brain. In 1873 Camillo Golgi, by using his silver nitrate staining technique (6-8), demonstrated the existence of glial-vascular contacts between astrocyte end-feet and brain microvasculature. In his theory he postulated that there was a link between the morphology and the function of astrocytes in the CNS.

By the end of the 19th century, Michael von Lehnossek first used the term "astrocyte" due to the stellate morphology of these cells. The origin of this word arose from a combination of the Latin word *astra*, for stars, with the suffix *cyte*, for cell (7, 9). Years later, Santiago Ramón y Cajal developed the gold chloride-sublimate staining method, known as the first specific stain for astrocytes. This technique helped him to demonstrate astrocytes' morphological heterogeneity, as illustrated in Figure 1 (6, 7).
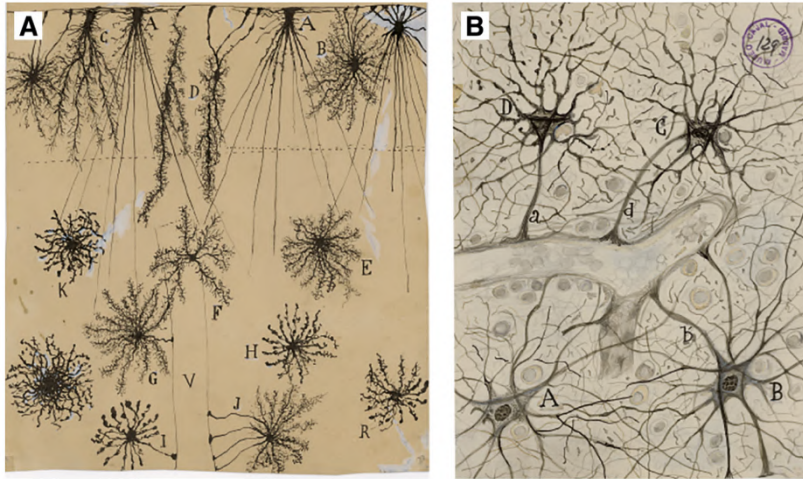
Figure 1. Images of astrocytes drawn by Santiago Ramon y Cajal (10).

A: Golgi impregnated glia from human cortex in the plexiform layer (A-D), second and third layers (E-H and K, R, respectively) and perivascular glia (I and J); V, blood vessel. B: perivascular astrocytes.

1.1.1 Morphology

Astrocytes have a highly heterogenous morphology. These cells emit many long branching extensions from the cell body, with endfeet usually formed at the extended ends of the astrocyte branches, where those that are connected to the capillary wall are called perivascular end-feet. Astrocytes exhibit many interlaced fibrils in their cytoplasm, constituting the main component of their skeleton. The structure of these fibers is called glial filament, which is an intermediate filament. Glial filaments are composed of glial fibrillary acid protein (GFAP) which, alongside vimentin, acts as a main factor for the intermediate filaments that constitute the cytoskeleton of astrocytes (11, 12).

The expression of GFAP is commonly used as a specific marker for the identification of astrocytes. This marker is expressed by astrocytes cultured *in vitro*, but *in situ* the levels of GFAP expression vary considerably (11). S100β is a calcium, copper and zinc-binding protein, which mediates the interaction between glial cells and neurons. Like GFAP, S100β is not a universal marker for all astrocytes. Recent studies have singled out a novel marker expressed by both protoplasmic and fibrous astrocytes, the aldehyde dehydrogenase-1 family member L-1 (Aldh1L1). Aldh1L1 is a 10-formyltetrahydrofolate dehydrogenase, a participant in astrocyte folate metabolism.

This protein has been identified in both immature and mature astrocytes in grey and white matter within the central nervous system (13).

Two major classes of astrocytes were first described in the 19th century by using the Golgi staining, which revealed their distinct morphological pattern: the protoplasmic and fibrous astrocytes.

Protoplasmic astrocytes are present in the grey matter. They have a rounded cell body shape and present many fine processes (approximately 50 μm long), which are extremely elaborate and complex. These processes contact blood vessels (establishing perivascular endfeet) and synapses. Protoplasmic astrocytes are S100β positive and have low or absent GFAP. As for fibrous astrocytes, they are located in the white matter and have long processes (up to 300 μm), though much less complex as compared to protoplasmic astroglia. The processes of these cells establish several perivascular endfeet. These structures also send numerous extensions that contact axons at nodes of Ranvier. Fibrous astrocytes are GFAP positive, with a star-like shape. (6, 11). Nowadays, some other types of astrocytes have been discovered in the brain (Table 1).

While the binary classification is widely used, astrocytes form a very heterogenous population containing many different subtypes. Furthermore, astrocytes even differ within the same region of the brain. For example, Müller cells and Bergmann glia are both present in the cerebellum (2).

Other subtypes of astrocytes include the radial astrocytes that are bipolar cells with an ovoid shape and elongated processes. This type of glia is a common feature of the developing brain, as these are the first cells to appear in the neural tube during the course of embryogenesis; from very early embryonic stages, radial glial cells also form a scaffold, aiding in neuronal migration. After maturation, radial glial cells differentiate, becoming stellate astrocytes, although radial astrocytes remain in the retina (Müller glia) and the cerebellum (Bergmann glia) (15).

Interlaminar astrocytes are GFAP positive cells that can be found in layer 1 of cerebral cortex and only appear after birth. The varicose projection astrocytes are also GFAP positive cells located in layers 5 and 6 which exhibit long processes with many varicosities (16). Velate astrocytes are located in the granular cell layer of the cerebellar cortex and exhibit rounded cell bodies with thin, long, veil-like processes that wrap granule cells and synapses (17). Pituicytes, located in neurohypophysis, express GFAP

and S100β. The neurohypophysis does not contain neuronal bodies, but only terminals of magnocellular neurons. These terminals are surrounded by pituicytes, which provide for osmosensitivity and regulate neuropeptides secretion through release of taurine and through dynamic modulation of terminal coverage. In contrast to other astrocytes, pituicytes are sensitive to several neurotransmitters and neurohormones (10).

Table 1. Different types of astrocytes. Adapted (7, 11, 14, 15, 18)

Type of astrocyte	Morphology	Location	Characteristics
Protoplasmic astrocytes	Ovoid cell body with numerous thick and short primary branches that divide into secondary branchlets, tertiary leaflets and, finally, endfeet	Grey matter	The endfeet are capable of enveloping blood vessels and synapses
Fibrous astrocytes	Possess long and thin processes that remain mostly unbranched, with endfeet at the end	White matter	The endfeet envelop the nodes of Ranvier and the blood vessels
Bergmann glia	Small cell bodies, with processes that extend from the Purkinje cell layer to the pial surface. Their processes are extremely elaborated	Purkinje cell layer of the cerebellum	- Several Bergmann glial cells surround a single Purkinje neuron and their processes ensheath the Purkinje cell's dendrites - Their processes form close contacts with synapses formed by parallel fibers on Purkinje neuron dendrites

Müller glia	Extended longitudinal processes	Retina	Establishment of contacts with retinal neurons
Interlaminar astrocytes	Small and rounded cell bodies with two types of processes: tangential fibers travelling radially and "cable-like", long, vertical processes	1st layer of the cerebral cortex	• Presence of short processes that extend in all directions and participate in the pial glia limitans by forming a network of GFAP fibers • Capability of processes to propagate Ca^{2+} waves
Velate astrocytes	Possess thin veil-like processes that spread out and overlap each other	Cerebellum	Formation of a sheath surrounding granule neurons
Varicose projection astrocytes	Exhibit 1 to 5 long (up to 1 mm) processes, characterized by the presence of varicosities distributed around 10 μm apart	5th and 6th layers of the cerebral cortex	• GFAP+ cells • The main processes are straighter and less branched, than protoplasmic astrocytes • The processes terminate in the neuropil or on blood vessels
Pituicytes	Irregular in shape with many cytoplasmic processes extending in the proximity of the capillaries	Neurohypophysis	• Their processes surround neuro-secretory axons and axonal

			endings under resting conditions • Their cytoplasm contains large lipid droplets
		24	

1.1.2 Functions

Astrocytes are cells that do not present axons and do not generate action potentials. Therefore, they were initially thought to be mere "brain glue", supporting neuronal activity. However, astrocytes play critical roles, including promoting neuronal maturation, synapse formation, neuronal survival during development, regulation of angiogenesis and maintenance of a viable microenvironment for neurons.

According to their microenvironment, astrocytes may perform specialized functions. For example, fibrous astrocytes, located in the white matter, contact the nodes of Ranvier, where they may regulate spike propagation, while protoplasmic astrocytes, located in the grey matter, enwrap synaptic terminals, influencing synaptic transmission (19).

Astrocytes in synaptogenesis

Astrocytes are active participants in synaptogenesis, either in the development phase or following lesion to the CNS (2). Pfrieger and Barres developed a study with retinal ganglion cells, in which they observed that, in the absence of glia, these cells presented little synaptic activity, while synaptic activity in the presence of astrocytes was 100 times higher (20). This increased activity mediated by astrocytes is explained by the consequent increasement in the number of synapses, which is 7 times higher in retinal ganglion cells co-cultured with astrocytes than in cells cultured without astrocytes (19).

Astrocytes are critical for the formation and maturation of inhibitory and excitatory synapses via release of a range of molecules, including cholesterol, glypicans (GPC4 and GPC6), hevin (SPARCL1) and thrombospondins (especially THBS1 and THBS2), that can control both pre- and postsynaptic function. Some factors (e.g., thrombospondins) produce synapses that are immature and functionally silent.

Glypican-4 and 6 induce functional synapse maturation by increasing the number of AMPA receptors on the surface of synapses (21, 22).

Astrocytes and synaptic pruning

It has also been shown that astrocytes participate in synapse elimination and pruning, renovating the neuronal circuits through phagocytosis. Astrocyte-mediated synapse elimination is dependent on two phagocytic receptors, Multiple EGF-like domains 10 (MEGF10) and MER Proto-oncogene tyrosine kinase (MERTK) (3).

Astrocytes and synaptic control

Astrocytes participate in synaptic transmission by releasing synaptically active molecules called gliotransmitters. These molecules are released by astrocytes in response to neuronal synaptic activity that stimulates astrocytes, therefore generating waves of $[Ca^{2+}]$ and neuronal excitability. Gliotransmitter release occurs via a number of different mechanisms: calcium-dependent exocytosis, non-exocytotic release from cytosolic pools and calcium-independent channel mediated release. The molecular mechanisms that regulate the release of these neuroactive molecules (e.g., glutamate, adenosine, GABA, ATP and D-serine) is not completely understood but is thought to be mainly controlled by calcium (2, 9).

Furthermore, astrocytes release growth factors and cytokines that exert more potent and prolonged effects on the synapse. Other substances secreted by astrocytes that may contribute to synaptic function include polyunsaturated fatty acids, steroids and other neuroactive intermediates and metabolites with affinity for GABA receptors (2).

Astrocytes and the tripartite synapse

The close morphological relation between astrocytes and synapses, as well as a functional expression of relevant receptors in astroglia prompted the concept of the "tripartite synapse". According to this model, synapses are built from three important parts: the presynaptic terminal, the postsynaptic neuronal membrane, and the surrounding astrocyte (Figure 2). The release of a neurotransmitter from the presynaptic terminal will activate receptors in both the postsynaptic neuronal membrane and the astroglial membrane which, subsequently, will result in the generation of a postsynaptic potential in the neuron and a Ca^{2+} signal in the astrocyte. This signal then propagates through the astrocyte, triggering the release of gliotransmitters that will react with the pre- and postsynaptic terminal receptors, in order to modulate synaptic transmission.
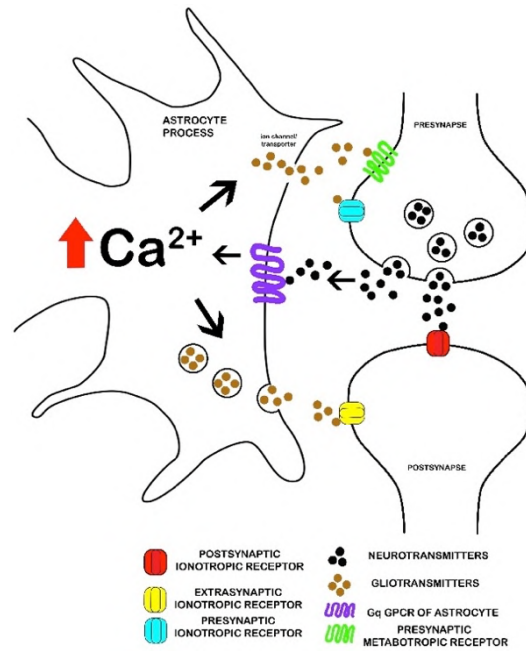
Figure 2. Representation of the Tripartite Synapse (23).

Astrocytes and the neurovascular unit

Ca^{2+} waves are not restricted to one astrocyte and can propagate via gap junctions to neighboring astrocytes (21). In fact, these gap junctions, formed by connexins 30 and 43, allow astrocytes to function as a functional syncytium, allowing the diffusion of small molecules along astrocyte's processes (24).

Astrocytic calcium waves help to control blood flow by inducing release of prostanoids, nitric oxide, arachidonic acid and other molecules that signal vasodilation or vasoconstriction depending on metabolic context, coupling blood flow to neuronal energy demand (21). Astrocytes perform this function since they have two domains: the vascular and the neuronal. This intersection of neurons, astrocytes and blood vessels is known as the "neurovascular unit" (Figure 3). Subsequently, astrocytes adjust vascular flow to the synaptic activity. Homeostasis of the neurovascular unit is essential for cognitive function, and imbalances may be linked to cognitive changes (2).
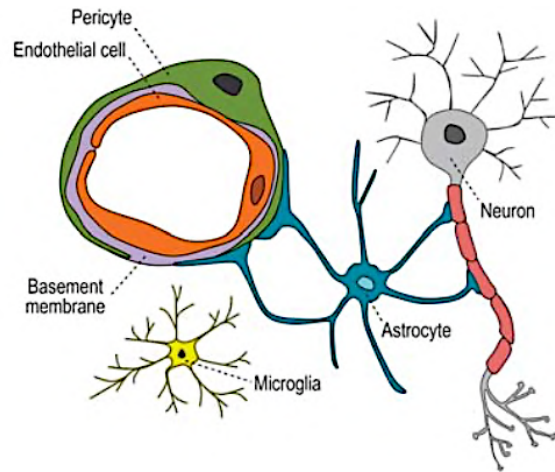
Figure 3. Diagram of the neurovascular unit (25).

Astrocyte's role in homeostasis

In addition to all the functions mentioned above, astrocytes help to maintain synaptic homeostasis of the interstitial fluid. These cells surround the synapse and maintain adequate levels of pH, ions, neurotransmitters, and fluid. Aquaporin 4 (AQP4) is a protein expressed in astrocytic projections that transports water, essential to maintain osmotic balance of the extracellular environment. These astrocytic projections also have transporters for K^+ uptake (2).

Astrocyte metabolism and neuronal support

Astrocytes are known to take up glucose and typically present a high glycolytic rate, and the metabolic needs of active neurons are at least partly met by non-oxidative glucose metabolism. The "astrocyte-neuron lactate shuttle" hypothesis proposes that astrocytes take up glucose from the blood and metabolize it to lactate, which is then taken up by neurons and used as an energy substrate. Moreover, astrocytes also store glucose in the form of glycogen, which can act as a short-term energy buffer during periods of high neuronal activity (9).

Astrocytic regulation of glutamate levels

Glutamate is a neurotransmitter that, when released in excess, acts as a powerful neurotoxin that triggers neuronal cell death in many acute and chronic brain lesions. Astrocytes have the capability to remove the bulk of glutamate from the extracellular space, by using excitatory amino acid transporters (EAAT). To date, five types of EAATs have been described in mammals, among which EAAT1 (GLAST) and EAAT2 (GLT-1) are predominantly found in astrocytes. Glutamate accumulated by astrocytes

is enzymatically converted into glutamine by glutamine-synthetase. Glutamine is not sensed by neurotransmitter receptors, is not toxic and can be safely transported to presynaptic terminals through the extracellular space; after entering the neuronal compartment, glutamine is transformed into glutamate (11, 26).

Blood-Brain Barrier induction and maintenance

The blood-brain barrier (BBB) is the specialized structure of brain microvascular endothelial cells that protects the brain from toxic substances in the blood, supplies the CNS with nutrients and filters excess and toxic molecules from the brain to the bloodstream. Astrocytes send their endfeet, which enwraps the brain vasculature. Additionally, it is thought that astrocytes regulate the induction of the BBB, i.e., tight junction formation and expression of transport systems (e.g., GLUT1). During development, astrocytes are thought to contribute to BBB tightening and up-regulation of the different transport mechanisms.

The regulations exerted by cultured astrocytes on BBB induction depend both on cell-cell contact and the diffusion of molecules. Some of the astrocytic signals that regulate different aspects of BBB properties include TGF-β, GDNF, basic FGF, IL-6 and hydrocortisone. The src-suppressed C-kinase substrate (SSeCKS) in astrocytes is responsible for the decreased expression of VEGF and increased release of the anti-permeability factor angiopotein-1. It is thought that astrocytes also maintain tight junction and microvascular permeability in the adult brain (19).

Astrocytes and K^+ buffering

During normal neuronal activity, neurotransmission leads to the buildup of K^+ into the extracellular space and, if not corrected, it may result in neuronal depolarization, hyperexcitability and seizures. Astrocytes are able to take up excess extracellular K^+, distribute these ions through the gap junction-coupled astrocytic syncytium and extrude the ions at sites of lower extracellular K^+ levels.

In order to play such role, astrocytes possess passive uptake (via channels - by inward rectifier K+ channels, Kir – and cotransporters) and active uptake (via Na^+/K^+ ATPases, leading to an increase in intracellular K^+ and water) mechanisms (11, 19).

Astrocytes and immune functions

Astrocytes can play a bridge role between CNS and immune system. Particularly, astrocytes can phagocytose cells (26), acting as antigen-presenting cells. Astrocytes can also express class II major histocompatibility complex antigens and costimulatory molecules (CD40 and B7) that are essential for antigen presentation and T-cell activation. Furthermore, astroglial cells also produce a wide array of chemokines and cytokines that act as immune mediators in cooperation with the ones produced by microglia (19).

1.2   Neurodegenerative diseases

Neurodegenerative diseases confer very debilitant conditions to the individuals suffering from it and are found among all the age groups. They are characterized by a progressive degeneration and/or neuronal cell death, which may affect body's movement and cerebral performance, leading to dementia. Nowadays, neurodegenerative diseases are one of the biggest medical and socioeconomical issues, and the causes of their emergence are still mostly unknown. Unfortunately, a cure has still not been discovered.

Human beings are born with approximately 100.000 million neurons. As the time goes by, some of these cells die, and the human organism is only capable of producing a reduced number of new neurons, which is a natural process related to ageing. However, sometimes neurons degenerate or die faster than usual, which leads to the occurrence of neurodegenerative diseases.

Neurogenerative diseases have a major impact in the patient's life (especially in what concerns professional, social and personal issues), possibly leading to a total incapability to exert any kind of quotidian activities. One of the major concerns in these patients is their late diagnosis, in which the person suffering from this medical condition is already in a stage of the disease where the treatment options are very limited and have a low efficiency.

It is estimated that 153.000 Portuguese people and 35,6 million worldwide citizens suffer from some type of dementia. The current expectation is for these numbers to double by the year of 2030 and to triple by 2050. Annually, it is estimated that the number of new diagnoses of dementia is approximately 7,7 million (27).

Neurodegenerative diseases are characterized by progressive loss of vulnerable populations of neurons. They can be classified according to primary clinical features

(e.g., dementia, parkinsonism), anatomic distribution of neurodegeneration (e.g., extrapyramidal disorders, frontotemporal degenerations), or main molecular abnormalities. Although neurodegenerative diseases are commonly defined by specific protein accumulations and anatomical vulnerability, they share many fundamental processes associated with progressive neuronal dysfunction and death, such as proteotoxic stress and its attendant abnormalities in ubiquitin – proteasomal and autophagosomal/lysosomal systems, oxidative stress, programmed cell death and neuroinflammation. Moreover, it is essential to take in account that protein abnormalities can be present before the onset of clinical features.

The most common neurodegenerative disorders are amyloidosis, tauopathies, α-synucleinopathies and transactivation response DNA binding protein 43 (TDP-43) proteinopathies. To make a specific neuropathologic diagnosis, some of the major histopathological features to consider are abnormal protein conformations in these disorders and their cellular and neuroanatomical distribution. Examples of protein accumulations within the neurons include tau in neurofibrillary tangles (NFTs) or Pick bodies, α-synuclein in Lewy bodies and TDP-43 in neuronal cytoplasmic and neuronal intranuclear inclusions.

Due to the analysis of cross-sectional *post-mortem* evaluations of a number of human brains with a range of clinical and pathological severity, it is known that many neurodegenerative diseases have a stereotypic progression that can be described by stages. For example, staging schemes have been developed for Alzheimer's disease (AD), Parkinson's disease (PD), dementia with Lewy Bodies (DLB) and amyotrophic lateral sclerosis (ALS) (28).

1.2.1 Alzheimer's disease

Alzheimer's disease (AD) is a neurodegenerative disease and the most common cause of dementia. AD causes emotional, physical, social and financial distress for patients and their relatives (29). This pathology is known to be one of the major causes of death and, consequently, it has become a significant area of research. Even though the research that has been carried along the decades has revealed a great deal about Alzheimer's disease, there are still many unknown and important characteristics, especially in what concerns the biological changes that cause AD, its progression, and how this disease can be prevented or even treated (30).

Even though long-term memory is preserved, patients affected by this neurodegenerative disease face difficulties with short-term memories. Moreover, with the progression of the disease, patients start having difficulties in their daily routines (e.g., spatial disorientation and language disabilities), which can be accompanied by depression, irritability and delusions (29).

One of the major concerns of this type of dementia is its late diagnosis, since the early symptoms only start to appear years after the first changes in the brain. Subsequently, researchers believe that an early detection will be the major factor to prevent, slow and even stop the disease.

1.2.1.1 Dementia

Dementia is classified as a clinical syndrome characterized by a cluster of symptoms and signs manifested by difficulties in memory, disturbances in language, psychological and psychiatric changes, and impairment in common daily activities (e.g., paying bills or making a meal) (31). Its early recognition is not easy, due to the insidious and variable onset of the syndrome, which emerges through the personality of the patient, sometimes not showing a clear demarcation until a late state of the disease. Diagnosing dementia requires a history evaluation for cognitive decline and impairment in daily activities, with corroboration from a close relative. Additionally, a mental status examination shall be performed to analyze impairments in memory, language, attention, spatial orientation, executive function and mood. Physical examination may help to identify its etiology (31, 32).

Different causes of dementia are associated with distinct symptom patterns and brain abnormalities. The causes are of major importance, because different types of dementia can have different courses, with different patterns of symptoms, and can respond differently to treatment (Table 2).

Table 2. The main causes of dementia and their respective characteristics. Adapted (30-32)
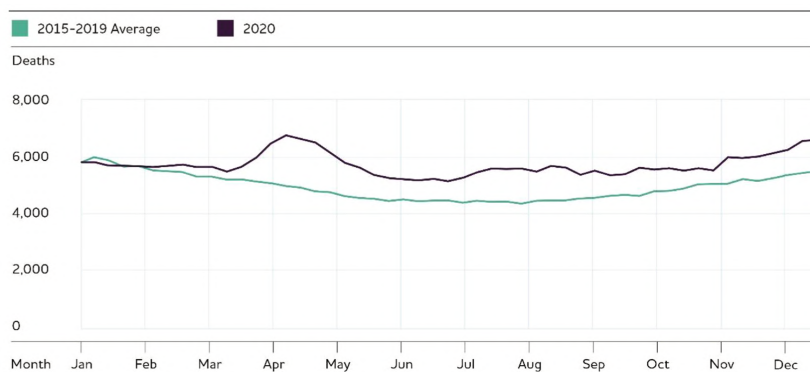
Causes			
	Alzheimer's Disease	The most common cause of dementia	Progressive accumulation of the protein fragment β-amyloid (plaques) and twisted strands of the protein tau (tangles) inside neurons
	Vascular Dementia	Common in older individuals, especially in patients with pathological evidence of vascular damage (infarcts)	Occurs mostly from blood vessel blockage or damage, leading to infarcts
	Lewy Body Dementia	Initial symptoms: sleep disturbances, hallucinations, slowness and gait imbalance	Abnormal aggregations of the protein α-synuclein in neurons. The onset of the disease is marked by cognitive impairment
	Mixed Dementia	About half of older patients with dementia present more than just one cause of dementia	Characterized by the abnormalities of more than just one cause of dementia (most commonly, AD combined with vascular dementia)
	Frontotemporal Dementia	Initial symptoms: marked changes in personality and/or difficulty with producing or comprehending language	Frontal lobe and temporal lobes are especially affected areas of the brain. Upper layers of the cortex typically become soft and spongy and have protein inclusions (tau proteins or the transactive response DNA-binding protein)

1.2.1.2 Epidemiology

According to World Heath Organization (WHO), over 55 million people live with dementia worldwide. In 2019, WHO ranked Alzheimer's Disease and other forms of dementia as the 7th leading cause of death, in which 65% of these deaths are women (33, 34). Alzheimer's Disease International (ADI) estimates that globally 75% of

people with dementia are not diagnosed; this value can be as high as 90% in some low- and middle-income countries, where stigma and lack of awareness of dementia remain major barriers to diagnosis (35). AD is a leading cause of disability and morbidity in older adults. Before the patient dies, he/she lives through years of morbidity as the disease progresses (30).

As most countries enforced lockdown measures to avoid the spread of COVID-19 during 2020-2021, movement restriction cut off much access to healthcare services for people with dementia symptoms. In the United States of America, as shown in Figure 4 the number of deaths due to AD and other dementias was more accentuated, in comparison to those that occurred between 2015 and 2019 (30, 35)

Figure 4. Deaths caused by AD and other dementias in the United States of America in 2020, compared with 2015-2019 (30).

In Portugal, Alzheimer's disease is the 3rd major cause of death among the citizens (Figure 5) and also one of the main causes of disability-adjusted life years (DALYs) (36).
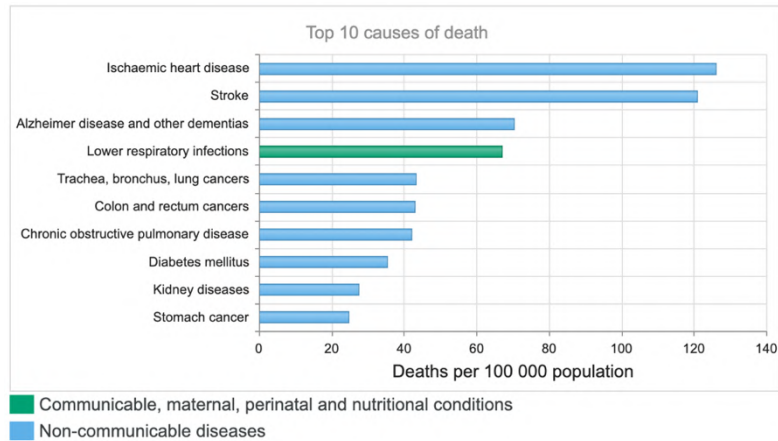
Figure 5. Top 10 causes of death in Portugal (2019) for both genders in all the age groups (36).

1.2.1.3 Pathophysiology

In healthy human brains, neurons establish connections with each other through their branching extensions. These connections, called synapses, allow the transmission of information in tiny bursts of chemicals that are released by one neuron and detected by another one. The human brain contains about 100 billion synapses and these neuron-to-neuron connections allow signals to travel rapidly through the brain, and the information carried along creates the cellular basis of memories, sensations, thoughts, emotions and movements (30).

Two of the major brain changes associated with AD are the accumulation of the protein fragment β-amyloid (Aβ) into clumps (known as β-amyloid plaques or senile plaques) outside neurons and the accumulation of an untypical form of the protein tau (known as neurofibrillary tangles) inside neurons. Plaques and smaller accumulations of β-amyloid may contribute to the damage and death of neurons (neurodegeneration) by interfering with neuron-to-neuron communication at synapses. Inside neurons, neurofibrillary tangles can block the transport of nutrients and other molecules that are important for normal function and neuron survival (30).

The senile plaques, as previously mentioned, are extracellular deposits of β-amyloid protein with different morphological forms (neuritic, diffuse, dense-cored, or classic). These plaques aggregate within the isocortex and are found in all six cortical layers. Proteolytic enzymes (β-secretase and γ-secretase) are responsible for the biosynthesis of β-amyloid deposits from the membrane amyloid-β precursor protein (APP). These enzymes cleave APP into several amino acid fragments (43, 45, 46, 48,

49 and 51), which reach the final forms Aβ40 and Aβ42. Aβ42 seems to be deposited initially and may have a role in initiating the events that ultimately lead to amyloid deposition. Since there are several types of Aβ monomers (e.g., large and insoluble amyloid fibrils), β-amyloid plays a key role in neurotoxicity and neuronal function. Consequently, accumulation of denser plaques can cause stimulation of astrocytes and microglia, damage to axons, dendrites and loss of synapses (37, 38).

Neurofibrillary tangles (NFTs) are unusual filaments of the hyperphosphorylated tau protein that, in some stages, can be twisted around each other, forming a paired helical filament (PHF) that accumulates in axons and dendrites, which can then lead to a loss of cytoskeletal microtubules and tubulin-associated proteins. The progression of AD can reflect NFTs morphological stages, including 1) pre-tangle phase, where phosphorylated tau proteins are accumulated in the somatodendritic compartment without the formation of PHF; 2) mature NFTs, characterized by filament aggregation of tau protein without the displacement of the nucleus to the periphery part of the soma; 3) the extracellular tangles, which result from a neuronal loss due to the large amounts of filamentous tau protein with partial resistance to proteolysis. According to studies carried in *post-mortem* specimens, NFTs were shown to be densely associated with the areas of the brain that were most affected by the disease (37, 38).

In AD, hyperphosphorylation of tau protein is produced by glycogen-synthase-kinase 3β, cyclin-dependent kinase 5 (CDK5), mitogen-activated protein kinase (MAPK) and many others. Additionally, decreased phosphatases (which dephosphorylate tau) have been found in AD *post-mortem* specimens. Protein phosphatase 2 (PPA2) has been shown to increase tau hyperphosphorylation and has been demonstrated to be reduced in AD (37).

Other brain changes related to AD include inflammation and atrophy (decreased brain volume). It is believed that the presence of Aβ and tau proteins activate the microglial cells. Microglia try to clean the toxic proteins as well as widespread debris from dead/dying cells. When the microglia cannot keep up with all of these needs, chronic inflammation may set in. Atrophy occurs due to cell loss. The normal function of the human brain is further compromised in AD by decrease in brain's capability to metabolize glucose, its main fuel (30).

1.2.1.4 The stages of Alzheimer's disease

Alzheimer's disease is a progressive neurodegenerative disease that can be divided into three different stages: pre-clinical, mild cognitive impairment (MCI) and dementia. The length of each phase is influenced by age, genetics, gender and other factors.

Pre-clinical phase

This stage can last for several years, where the patients are asymptomatic. Identifying the biomarkers can help diagnosing AD in this stage (39). The National Institute on Aging and Alzheimer's Association (NIA-AA) conceptualized pre-clinical AD in three stages (Figure 6). The individuals in stage 1 have biomarker evidence of Aβ, which can be demonstrated by positron emission tomography (PET) amyloid imaging or cerebrospinal fluid (CSF) Aβ levels. However, there are no suggestions of neurodegeneration or subtle cognitive/behavioral symptoms. In stage 2, there is also evidence for amyloid positivity and, in addition, neuronal injury markers as evidenced by brain atrophy on structural magnetic resonance imaging (sMRI), hypometabolism on ^{18}F-fluorodeoxyglucose positron emission tomography (FDG-PET), or elevated levels of CSF tau. In stage 3 of pre-clinical AD, the patients have biomarker evidence of amyloid accumulation, early neurodegeneration, as well as subtle evidence of cognitive decline (40, 41).

Mild Cognitive Impairment (MCI)

Mild cognitive impairment is defined as a transitional stage between normal aging and dementia, that is, the symptomatic pre-dementia stage, which does not fulfill the criterion for dementia diagnosis (42, 43). The prevalence of MCI in adults older than 60 years is approximately 6.7% to 25.2% and the annual rate of progression to dementia is approximately 5% to 17%. Some established biomarkers associated with the progression from MCI to AD are a positive amyloid PET scan, apolipoprotein E4 (ApoE4) genotype, abnormal CSF tau levels and a positive PET scan due to tau deposition into the lateral temporal lobe structures (44). NIA-AA established clinical criteria for MCI due to Alzheimer's disease: 1) concern about a change in cognition reported by the patient, an informant or a skilled clinician; 2) impairment of one or more cognitive functions (e.g., memory, problem solving) that is greater than expected for the patient's age and education; 3) preserved ability to function independently in daily life, though some complex tasks may be more difficult than before; 4) no dementia (45).
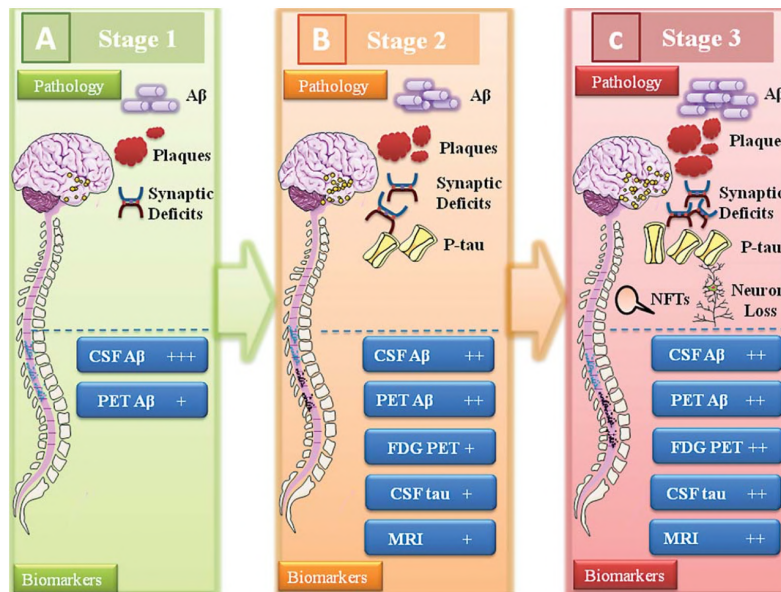
Figure 6. Schematic representation of pre-clinical phase in Alzheimer's disease (40).

Dementia

Dementia due to AD is characterized by noticeable memory/thinking/behavior symptoms that impair a person's ability to function in daily life, combined with biomarker evidence of Alzheimer's-related brain changes. As time goes by and the disease progresses, the patient will experience multiple types of symptoms that change with time, reflecting the degree of damage to nerve cells in different parts of the brain (30).

In mild stage Alzheimer's dementia, patients are likely to require assistance with some activities of their daily routines to maximize their independence and safety. In the moderate stage of AD dementia, which is considered to be the longest stage, patients may have difficulties communicating and performing routine tasks (e.g., bathing, dressing). During this stage, the individuals are likely to have changes in their personality, including suspiciousness and agitation (30). Lastly, in the sever stage of Alzheimer's dementia, patients are extremely likely to require a full-time care service. During this phase, the disease spreads through the cortex area with a severe accumulation of neuritic plaques and neurofibrillary tangles, resulting in a progressive functional and cognitive impairment where the patients cannot recognize their relative ones. In this stage, the individuals may become bedridden, due to the damage caused in the areas of the brain involved in movement. Consequently, these patients are more vulnerable to conditions (e.g., infections, sepsis, blood clots). If the areas of the brain

that control swallowing are affected, the patients will be in extreme danger of swallowing food into the trachea, leading to the deposition of food particles in the lungs, causing aspiration pneumonia (one of the major causes of deaths in AD patients) (30, 38).

1.2.1.5 Risk factors

It is believed that Alzheimer's disease results from multiple factors, in similarity to other medical conditions.

Age and family history

Increasing age is one of the greatest risk factors for Alzheimer's disease. It is rare for younger individuals to develop AD. People with at least 65 years old are more susceptible to develop this neurodegenerative disease. However, it is important to know that this type of dementia is not a normal part of aging and that the increasing age is not sufficient to cause Alzheimer's disease (46, 47).

A family history of AD is not necessary for an individual to develop this neurodegenerative disease. However, patients with a first-degree relative (e.g., parent or sibling) with Alzheimer's are more susceptible to develop the disease. Also, individuals with more than one first-degree relative with AD have an even higher risk to develop the disease (39).

Environmental factors

Environmental risk factors may induce oxidative stress and inflammation and increase the risk for developing AD. The air pollution has recently been associated with Alzheimer's. In the United States of America, the National Ambient Air Quality Standards (NAAQS) has defined six air pollutants as a threat to human health, including ozone, nitrogen oxides, carbon monoxide, particulate matter, sulfur dioxide and lead. In individuals exposed to air pollutants there is a link between oxidative stress, neuroinflammation and neurodegeneration, with the presence of hyperphosphorylated tau and Aβ plaques in the frontal cortex. Therefore, the air pollutants can lead to an increase in Aβ42 formation, accumulation and impaired cognitive function (38, 48).

Nutrition and cardiovascular disease factors

Malnutrition is another risk factor for AD. Deficiency in nutrients such as folate, vitamin B12 and vitamin D may cause a decrease in cognitive function (38).

Brain health is also affected by the health of the heart and blood vessels. A healthy heart ensures that enough blood is pumped to the brain, while healthy blood vessels enable the oxygen- and nutrient-rich blood to reach the brain so it can function normally (30). The coronary heart disease's hypothesis indicates that atherosclerosis, peripheral artery disease, hypo-perfusion and emboli are all related to an increased risk of AD. Hypertension is associated with thickening of vessel walls and narrowing of the lumen, which reduce the cerebral blood flow and, in chronic cases, it may lead to cerebral edema, contributing as risk factors for AD and cardiovascular disease (CVD). However, cardiovascular disease is considered to be a modifiable risk factor for AD, which means that individuals can focus on obtaining a pathway to prevent and delay the disease (49).

Obesity and diabetes

Obesity is widely associated with an increased risk of hypertension, stroke and diabetes. These conditions may be factors that increase the risk of cognitive decline, thereby playing an indirect role in the development of AD. Overweight patients experience white matter atrophy in their basal ganglia and corona radiata (50, 51). The obesity and other factors can lead to impaired glucose tolerance (IGT) or diabetes, which is characterized by hyperglycemia that affects peripheral tissues and blood vessels. Chronic hyperglycemia can induce cognitive impairment as a consequence of increasing Aβ accumulation, oxidative stress, mitochondrial dysfunction and neuroinflammation. Obesity is marked by increasing pro-inflammatory cytokine secretion (e.g., tumor necrosis factor-α, interleukin-1β, interleukin-6) from adipose tissue (50), which stimulates macrophages and lymphocytes, eventually leading to local and systemic inflammation. This inflammation promotes insulin resistance and, consequently, hyperglycemia. Brain inflammation causes an increase in microglia and results in reduced synaptic plasticity and impaired neurogenesis. Microglia can affect insulin receptor substrate 1 (IRS-1) and block intracellular insulin signaling, resulting in Aβ accumulation and a reduction in tau protein's degradation, both associated with AD (38).

Genetics

Studies of twins showed that the risk of AD is 60-80% dependent on heritable factors (46). Genetic factors have been investigated, for many years, as a cure of AD, and were found to play a key role in the development of the disease. Most of the cases

of early-onset AD (EOAD) are inherited in an autosomal dominant pattern and mutations in dominant genes (amyloid precursor protein (APP), presenilin-1 (PSEN-1) and presenilin-2 (PSEN-2)) are associated with AD (38).

ApoE lipoprotein is highly expressed in the liver, in astrocytes and in some microglia, serving as a receptor-mediated endocytosis ligand for lipoprotein particles like cholesterol, which is essential for myelin production and normal brain function. The ApoE gene located on chromosome 19 has three isoforms, ApoE2, ApoE3 and ApoE4 (38). The ApoE4 allele has been found to be by far the strongest genetic risk factor for late-onset AD (LOAD) and EOAD. ApoE4 is present in roughly 50-60% of patients with AD compared to 20-25% in healthy elderly adults without the history of familial AD. All the individuals inherit a copy of some form of ApoE from each parent (52). Having one copy of ApoE4 is associated with a three-fold increased risk of developing AD and, if two copies are present, there is an eight-fold increased risk (53). ApoE4 plays a major role in Aβ deposition as a senile plaque and causes cerebral amyloid angiopathy (CAA), which is known to be a marker for AD. It is also associated with vascular damage in the brain (54).

APP is encoded by the APP gene on chromosome 21 and is predominantly cut by the protease α-secretase, producing nonpathogenic soluble fragments. Additionally, the extracellular part of APP can be cleaved by the β-secretase, resulting in a soluble fragment. After this extracellular cleavage, the remaining part of APP is then cleaved by the γ-secretase. After this process, Aβ peptide is generated and released into the cytoplasm. Aβ40 and Aβ42 are particularly prone to aggregation into toxic oligomers, leading to the formation of amyloid plaques. Mutations in APP lead to overproduction of pathological Aβ fragments and consequently to amyloid pathology with increased plaque formation (55).

Presenilin-1 (PSEN-1) and presenilin-2 (PSEN-2) are both essential proteins of the catalytic core of the γ-secretase complex, which catalyzes the cleavage of membrane proteins, including APP. PSEN-1 and PSEN-2 genes are also the autosomal dominant form of EOAD located on chromosomes 14 and 1, respectively. Mutations in PSEN-1 are the most common cause of familial EOAD and are characterized by the earliest onset ages (on average 8.4 and 14.2 years earlier compared to APP and PSEN-2 mutations, respectively) (56). Mutations in the PSEN-1 gene cause a change in γ-

secretase activity, increasing the ratio of Aβ42/ Aβ40 by decreasing Aβ40 levels. Contrarily, PSEN-2 mutations are extremely rare, playing a minor role in Aβ production. Some of the PSEN-2 mutations cause a significant increase in γ-secretase activity with an elevation in the Aβ42 and Aβ42/ Aβ40 ratio level (e.g., N141I, T122P, M239V and M239I) (38, 56).

A research team led by Chunshui Yu and Mulin Jun Li (57) has discovered two new genes potentially involved in AD. The investigators identified genes expressed at higher or lower levels in the hippocampus of individuals with AD compared to healthy brains. They identified 24 Alzheimer's-related genes that appear to have an effect through the hippocampus, using previous genomic and hippocampus gene expression data. Most of the genes were already known to be correlated with the disease, but two were unknown, PTPN9 and PCDHA4. The research team then validated their findings by comparing gene expression for the two dozen genes with images of the individuals' brains. In AD, damage and loss of neurons causes the hippocampus to shrink, which can be measured through medical imaging. The investigators established that expression of two of the genes is related to the size of the hippocampus and a diagnosis of the neurodegenerative disease (57).

2 Objectives

The current dissertation aims to explore the impact of astrocytes in neurodegenerative diseases, specially bringing an overview of their functions in healthy brain conditions, their morphology and a brief review of their discovery and description since the 19th century.

This monograph specially focuses on Alzheimer's disease, since it is one of the major causes of death worldwide, occupying a third place in Portugal's top 10 causes of mortality. Moreover, this dissertation also briefly approaches Parkinson's disease and amyotrophic lateral sclerosis, two of the most common neurodegenerative disorders.

The role that astrocytes play in those diseases is still a not well known field. Yet, this topic of research has increased in popularity over the years among the scientific community. Nowadays, astrocytes are known to be promising therapeutic targets in many neurodegenerative diseases. Therefore, scientists all over the world are exploring new therapeutical possibilities for neurodegeneration using these cells.

3 Materials and methods

Many browsers for Scientific publications were used throughout this dissertation, especially PubMed (pubmed.ncbi.nlm.nih.gov) managed by the National Library of Medicine (NLM), ScienceDirect (www.sciencedirect.com) provided by Elsevier, ResearchGate (www.reserachgate.net), and Google Scholar (scholar.google.com).

The criteria for the article selection went through a filtering of research according to more recent publications, namely between 2016 and 2021. However, for basic theoretical concepts, the selected articles were published prior to that period. The research was mostly done in English and specific keywords were used: glia; astrocytes; astrocytes functions; astrocytes morphology; astrocytes history; neurodegeneration; neurodegenerative diseases; astrocytes in neurodegenerative diseases; reactive astrocytes; astrocytes dysfunction; Alzheimer's disease; dementia; Parkinson's disease; amyotrophic lateral sclerosis.

In addition to the mentioned platforms of research, other sources were used during the elaboration of the monograph, including World Health Organization (WHO), Fundação Calouste Gulbenkian, Alzheimer's Association, British Medical Journal, Journal of Alzheimer's disease, American Journal of Alzheimer's disease & Other Dementias, and the Journal of Neuroscience Research. All these platforms were essential to allow this dissertation to be developed based on trustworthy and current references.

The information research process and analysis took place between January 2021 and October 2021, and the redaction of the current dissertation happened between May 2021 and November 2021.

4 The involvement of astrocytes in neurodegenerative diseases

Neurodegenerative diseases are characterized by the development of progressive clinical symptoms such as decline in cognitive, behavioral or motor functions, primarily attributed to the loss of vulnerable neuronal populations in specific CNS regions of affected individuals (58). Neurodegenerative disorders are often fatal, untreatable neurological conditions greatly impairing the quality of life of those that are affected and their caregivers (59). Classically, the onset and progression of neurodegenerative diseases were attributed to cell autonomous damage mechanisms, with dysfunction and loss of susceptible neuronal populations being sufficient to drive pathology. Nowadays, it is well accepted that non-cell autonomous mechanisms are essential contributors to neurodegeneration. Most of the investigations suggest that microglia and astrocytes casually participate in the pathogenesis and progression of many neurodegenerative disorders (58).

Growing evidence suggests an active role of glial cells in the CNS functioning. Among glia, astrocytes emerge as a critical and highly heterogenous population that perform a wide array of functions, as previously mentioned in chapter 1.1.2. Considering the importance of astrocyte functions for CNS performance, it is reasonable to say that astrocyte dysfunction can be a primary event of a pathogenic cascade ultimately leading to neuronal loss. Moreover, astrocyte dysfunction can also play a main role in the pathogenesis of several chronic neurodegenerative diseases (59).

The activation of astrocytes is a main pathological feature of neurodegenerative diseases and an adaptive defense response that provides essential metabolic support for neurons. The formation of free radicals, inflammation, elevations of the excitatory neurotransmitter glutamate and neuronal degeneration that are caused by the dysfunction of astrocyte activation are closely related to the development of neurodegenerative diseases. The activation of astrocytes in neurodegenerative diseases is a complex and multifaceted process, in which astrocyte-related gene and protein expression, as well as astrocytes' morphological structure and physiological function undergo progressive changes (60).

Astrocyte reactivity (Figure 7) is triggered by chemicals, toxins, oxidative stress or pathogens. After the stimulation caused by these agents, astrocytes secrete pro-inflammatory molecules, such as reactive oxygen species (ROS), IL-6, interleukin-1β (IL-1β) and tumor necrosis factor α (TNF-α). On the other hand, astrocytes also release protective factors, such as vascular endothelial growth factor (VEGF), brain-derived neurotrophic factor (BDNF) and nerve grow factor (61). Consequently, reactive astrocytes elicit the release of chemokines, such as β-chemokine 2 (CCL2), β-chemokine 20 (CCL20), α-chemokine 10 (CXCL10) and α-chemokine 12 (CXCL12), as well as cell adhesion molecules, including vascular cell adhesion molecule 1, neural cell adhesion molecule 1 and intercellular adhesion molecule 1. Furthermore, reactive astrocytes can also secrete vasoactive molecules (e.g., VEGF, heparin-binding epidermal growth factor (HB-EGF) (62). Even though these cells can present gain or loss of function at the site of insult, it is still not clear whether this is beneficial or damaging to their surroundings (63).
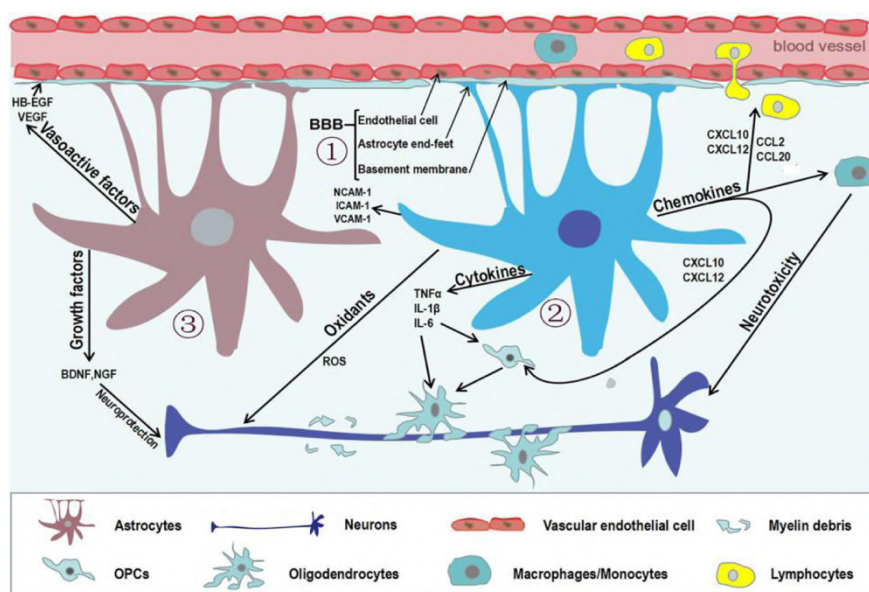
Figure 7. Reactive astrocytes in neurodegenerative diseases.

Astrocytes can be activated by multiple factors. 1) The BBB maintains homeostasis of the CNS. 2) Reactive astrocytes lose their endfeet that surround capillaries, resulting in damage to the BBB. Meanwhile, reactive astrocytes release oxidants, cytokines, chemokines and cell adhesion molecules, leading to damage of oligodendrocyte precursor cells, oligodendrocytes and neurons. 3) Activated astrocytes secrete vasoactive factors and affect normal endothelial cells by regulating junction-related proteins, preserving the integrity of the BBB. Reactive astrocytes can also secrete growth factors, promoting remyelination and neuronal regeneration (62).

As mentioned above, reactive astrocytes play opposite roles in the diseased CNS: they promote neuropathology and neurodegeneration, yet they can also support neuroprotection and repair. Under the influence of microglial or neuronal signals, reactive astrocytes convert either into the pro-inflammatory, neurotoxic A1 phenotype or the anti-inflammatory, neuroprotective A2 phenotype. In neurodegenerative diseases, A1 astrocytes are often increased in number, just like in the aging brain (58).
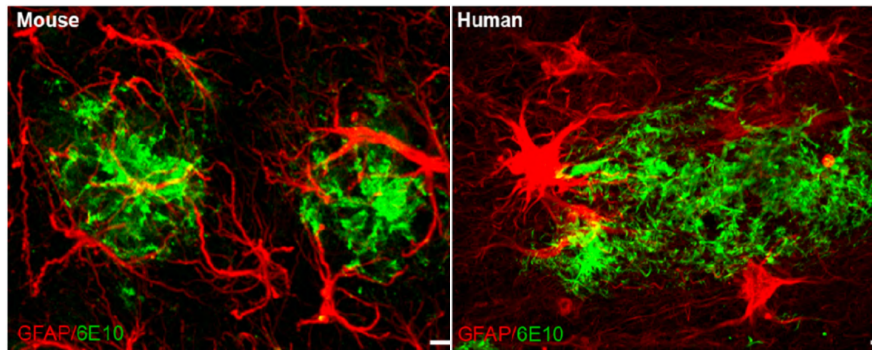
4.1 Astrocytes in Alzheimer's disease

During the last few years, the study of the role of astrocytes in AD has grown considerably. Astrocytes in this pathology contribute to the loss of neuroprotection and to the gaining of pathological characteristics. Initially, astrocytes have a protective role uptaking and degrading Aβ. However, the progression of the disease leads to reduced astrocyte clearance of Aβ and their consequent accumulation, which will stimulate astroglia to produce pro-inflammatory mediators, including chemokines, pro-inflammatory cytokines and ROS, resulting in neuronal damage (64).

Genetic data show that most of the total risk for developing AD is associated with genes mainly expressed in glial cells. Among these, Clusterin (ApoJ), Sortilin-related receptor 1 (SORL1), Fermitin family member 2 (FERM2) and ApoE (the major risk factor for AD) are mainly expressed by astrocytes, suggesting a crucial role of astrocytes in the pathogenesis of Alzheimer's disease. Astroglia undergo several morphological, molecular and functional changes in this neurodegenerative disease (65).

Morphological studies in *post-mortem* AD patient brains demonstrated close interaction between astrocytes and Aβ deposition (Figure 8). Astrocytes, when associated with senile plaques, become reactive with morphological hypertrophy manifested by thicker processes and increased expression of the intermediate filament proteins GFAP, vimentin, nestin and synemin (66). It has been suggested that by limiting the extent of astrocyte projections, the roles that astrocytes play to support neuronal survival and synapse function become limited (67).

Figure 8. Astrocyte morphologies in Alzheimer's disease brains. Adapted (66). Close interaction of both mouse and human astrocytes with Aβ plaques. GFAP-positive mouse or human astrocytes (red) around Aβ plaques (6E10, green) in the cortex of an APP/PS1 mouse and in the entorhinal cortex of an AD patient brain. 6E10: recombinant monoclonal antibody to Aβ that binds to amino acid residues 1-16 (66, 67)

Hypertrophic, reactive astrocytes are found close to amyloid plaques. They maintain their normal territory and do not overlap with neighboring astrocytes but produce calcium waves. However, reactive astrocytes contribute to the neuroinflammatory processes in AD. Reactive astrocytes in the vicinity of Aβ plaques display aberrant calcium dynamics, which can promote the release of detrimental factors, alter neuron-glia communication and impair synaptic transmission and plasticity (65, 66).

Studies in mouse models suggest that the early morphological changes in astrocytes occur in a regionally specific manner. At pre-plaques phases, atrophy is noted first in the entorhinal cortex, then in the prefrontal cortex and later in the hippocampus. At later stages of the disease, when Aβ plaques emerge, the response of astrocytes is different. Then, hypertrophy is observed in astrocytes surrounding amyloid plaques and is prevalent in the hippocampus, as opposed to the entorhinal or the pre-frontal cortex, where Aβ does not induce reactivity (68).

While Aβ affects astrocyte morphology, it also alters their function. Thus, exposure of cultured cortical astrocytes to Aβ decreases the secretion of the extracellular matrix protein thrombospondins, THBS1, from astrocytes. The reduction of THBS1 secretion is associated with a significant decrease in synaptophysin and PSD95 synaptic proteins in hippocampal neuronal cultures exposed to conditioned media from the Aβ-treated astrocytes. Ceyzeriat et al. (69) have also shown, in studies performed in mice, that

astrocytes from late stage significantly upregulate GFAP. However, inhibition of their reactivity by modulation of the JAK2-STAT3 pathway reduced not only GFAP upregulation, but also the number of amyloid deposits in the hippocampus, synaptic deficits and caused improvements in spatial learning (63, 69). This data shows that astroglial cells are influenced by the environment to change their function, but these changes can also affect their environment.

Pro-inflammatory reactive astrocyte phenotypes have been linked to synaptic degeneration and glutamate dysregulation. Knockout of astrocytic glutamate transporters EAAT1 (glutamate/aspartate transporter, GLAST) and EAAT2 (glutamate transporter-1, GLT-1) caused excitotoxicity and synaptic hyperexcitability in an AD model (70).

In the AD mouse model, calcineurin (Ca^{2+}/calmodulin-dependent phosphatase), a factor of the activated T-4 cells signaling pathway, has been found to connect between astrocyte activation and hyperexcitability during AD. This way, astrocytes alter their function and morphology during AD and may have different functions, as the disease progresses (70).

Astrocytes also play another role in the development of AD, related to the secretion of relevant agents. Apolipoprotein E (ApoE) carries lipids to neurons. The secretion of ApoE from astrocytes is positively controlled by Axl, a tyrosine kinase receptor. This mechanism supports the development of AD, while the inhibition of the receptor results in the inhibition of ApoE release and, thus, in a slowing down of the AD process (71).

Aβ may also affect calcium regulation in astrocytes. As much as 32 genes from the transcriptome of astrocytes microdissected from AD patients have been associated with Ca^{2+} signaling, suggesting a possible contribution of altered astrocyte Ca^{2+} dynamics to AD events. These events are hypothesized to disrupt neuronal signal transmission and contribute to the progression of neuronal disease (72).

Astrocytes can also exert neuroprotective effects in different stages of AD. Both astrogliosis and microgliosis in response to Aβ increase glial secretion of transforming TGF-β. TGF-β protects neurons from Aβ toxicity and enhances Aβ clearance by microglia. Furthermore, astrocytes surrounding Aβ plaques demonstrate phagocytic activity and are able to phagocytose neuritic dystrophies (66).

In Figure 9, the astrocyte-mediated mechanisms in AD are illustrated.
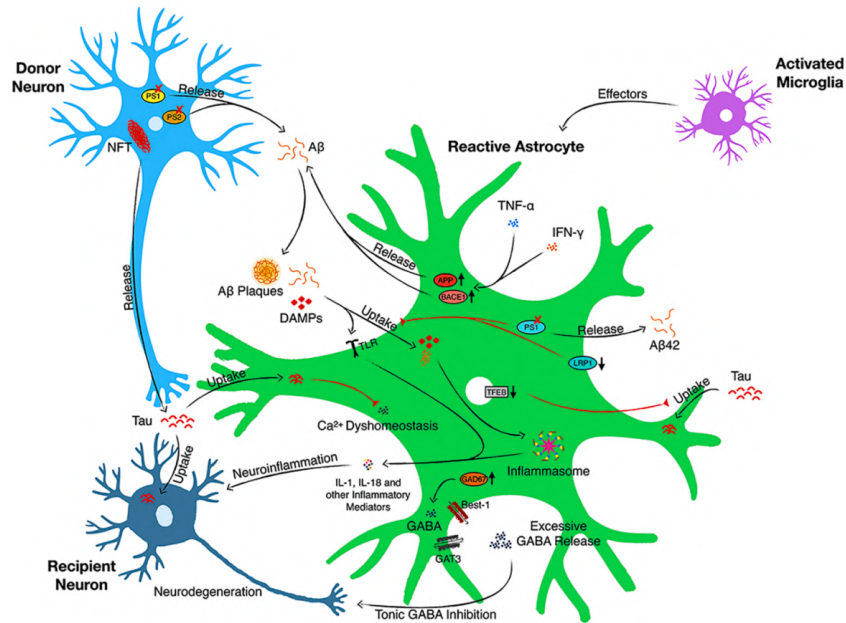
Figure 9. Astrocyte-mediated mechanisms in Alzheimer's disease.

Formation of intraneuronal NFT by phosphorylated tau and deposition of Aβ plaques in the extracellular space are hallmarks of AD, as previously mentioned. Mutations in PS1 (PSEN-1) and PS2 (PSEN-2) are associated with increased release of Aβ from neurons. The synaptic transfer of tau from donor neuron to recipient neuron leads to the propagation of tau pathology. Tau can also be taken by astrocytes. In normal situations, this mechanism is benefic, since the pathology is limited and modulated by transcription factor EB (TFEB), which mediates the transcription of genes involved in the autophagic degradation of tau. In AD, a decrease in TFEB impairs autophagy, causing accumulation of tau in astrocytes, leading to dysfunction. Astrocytes can take up and clear Aβ released by neurons, but they can also produce Aβ, since they express APP and β-site (BACE1) (their expression is increased by TNF-α and IFN-γ). The release of Aβ by astrocytes exacerbates the Aβ plaque burden. Astrocytes also induce neuroinflammation through the junction of toll-like receptors (TLRs) and the inflammasome complex. These processes will lead to the impairment of astrocyte functions (e.g., Ca^{2+} signaling, glutamate uptake and GABA release), resulting in synaptic dysfunction and neuronal death (73).

4.2 Astrocytes in other neurodegenerative diseases

4.2.1 Parkinson's disease

Parkinson's disease (PD) is a progressive neurodegenerative disease mainly characterized by the loss of dopaminergic neurons in the substantia nigra. Consequently, dopamine (DA) deficiency in the striatum causes motor symptoms, such as akinesia/bradykinesia, rigidity, tremor and postural instability. These patients also exhibit non-motor symptoms, such as hyposmia, autonomic disturbance, depression

and REM sleep behavior disorder (RBD), which precedes motor symptoms (74, 75). Pathologically, neurodegeneration is accompanied by the development of Lewy bodies, which are protein aggregates that primarily contain the protein α-synuclein (76). Currently, it is speculated that Parkinson's disease pathology propagates from the enteric nervous system (ENS) to the CNS via the vagal nerve. The mechanism of the disease remains to be clarified, yet diverse factors are thought to drive apoptosis (e.g., oxidative stress, neuroinflammation, α-synuclein toxicity, mitochondrial impairment and neuronal vulnerability). Furthermore, it is thought that non-neuronal cells contribute to the progression of PD pathology (Figure 10) (74).

α-synuclein is a soluble protein localized at presynaptic terminals. The causes of α-synuclein aggregate formation include impairment of autophagy, oxidative stress and post-translational modification of the protein. Mutations in the gene encoding α-synuclein, SNCA, have been linked with familial variants of PD. Mutations in SNCA also render the protein more prone to misfolding, leading to the formation of toxic aggregates in neurons (73).

It has been shown that astrocytes become reactive in response to activated microglial secreted signals and adopt a pro-inflammatory phenotype. This phenotype has been shown to exist in *post-mortem* brain tissue of patients with PD. Furthermore, astrocytes can also adopt a pro-inflammatory phenotype by endocytosis of α-synuclein released by neurons. Moreover, α-synuclein accumulation in human astrocytes *in vitro* resulted in severe cellular stress. As a response to this status, astrocytes sent nanotubes and enabled the transfer of intracellular α-synuclein inclusions to nearby cells, indicating that astrocytes are critically important in PD pathogenesis (77). Yun et al. demonstrated that NLY101, a glucagon-like peptide-1 receptor agonist, is neuroprotective in the performed α-synuclein preformed fibril mouse model of sporadic PD (78). This model is based on the discovery that α-synuclein protofibrils, injected in the brain, can act as seeds for the formation of α-synuclein aggregates that are toxic to the affected neurons. A particular interesting feature of this model is the generation of Lewy-like α-synuclein fibrillar inclusions with characteristics that closely resemble those seen in human PD (79). NLY101 acts to prevent astrocyte stimulation by activated microglia, blocking their conversion to an inflammatory phenotype (78).
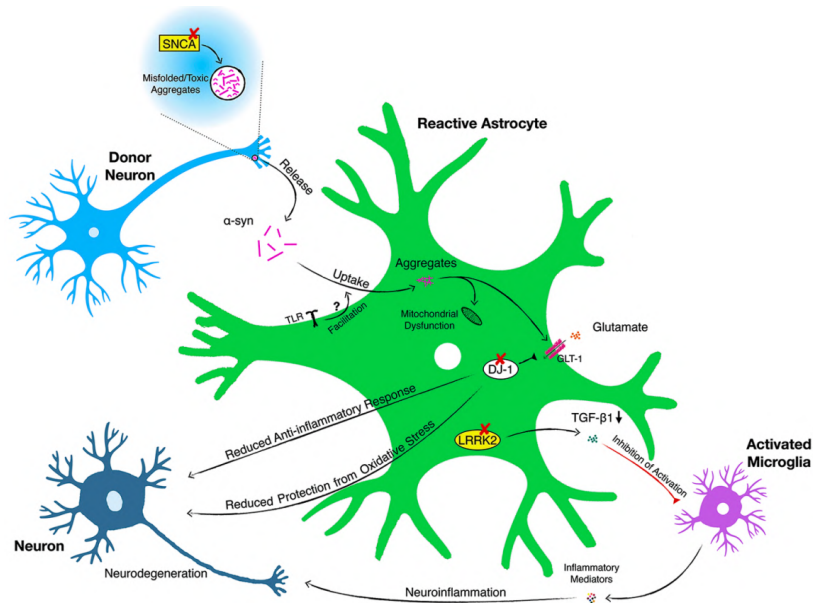
Figure 10. Astrocyte-mediated mechanisms in Parkinson's disease.

α-synuclein released by neurons is taken up by astrocytes. Accumulation of α-synuclein in astrocytes interferes with their basal function and causes mitochondrial dysfunction. Mutations in DJ-1 reduce astrocyte-mediated neuronal protection against oxidative stress. These abnormalities are also related to perturbations in glutamate uptake, leading to excitotoxicity (73).

4.2.2 Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS) is characterized by the progressive degeneration of motor neurons in the spinal cord, brainstem and motor cortex (80). Multiple aspects of motor neuron cellular physiology are perturbed and the fellow synapses are lost, causing the progressive paralysis of voluntary muscles. Death ultimately results from respiratory failure. Approximately 10% of the ALS cases appear with a familial history of the disease and are most frequently linked to a dominant mutation. The rest of the cases are sporadic and may result from yet unidentified environmental exposure or genetic mutations. The first ALS-linked gene identified encodes for the Cu^{2+}/Zn^{2+} ion-binding superoxide dismutase 1 (SOD1) protein. Another mutation associated with ALS is the expansion of a hexanucleotide repeat sequence in C9orf72 (orf 72 on chromosome 9). Other mutated genes associated with ALS include transactive response DNA binding protein (TARDBP), which encodes TAR DNA-binding protein 43 (TDP-43) and fused in sarcoma (FUS) (81). Both TDP-43 and FUS are RNA/DNA binding proteins, which regulate gene expression (58).

The fundamental pathological basis for ALS remains to be determined, just like the specific insult that targets only specific classes of motor neurons for death. Additionally, it is known that glial pathology is observed in all cases of familial and sporadic ALS and that nonneuronal cells in the spinal cord can affect the viability of motor neurons (82).

Neuroinflammation and glial activation are observed at the onset of the disease and along its progression. Reactive astrocytes are observed in the cortex and spinal cord of ALS patients. In mutant SOD1 (mSOD1 (G93A)) mice, the most used animal model to study this neurodegenerative disease, glial cell proliferation and activation are both found in motor and non-motor areas, starting at the pre-symptomatic stage (83). In ALS, reactive astrocytes lose their homeostatic functions, becoming neurotoxic and, consequently, will trigger motor neuron degeneration (84).

Moreover, both upper and lower motor neurons are affected in ALS and astrocytes reveal regional diverse phenotypes. Figure 11 illustrates this statement using experimental animal models.
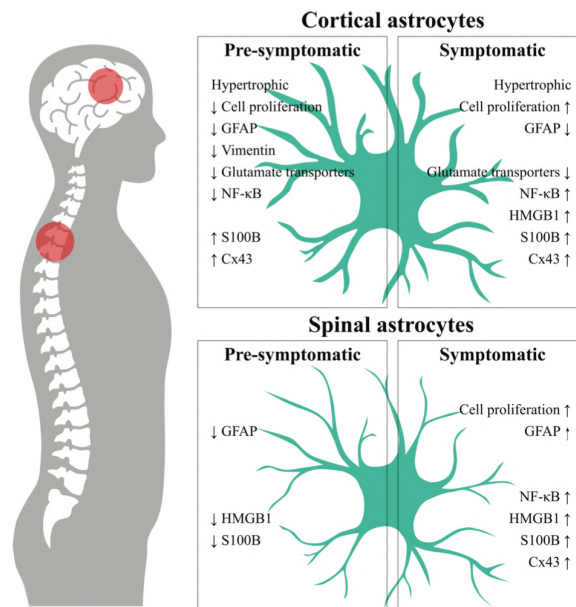
Figure 11. Astrocyte's phenotypes and regional diversity in ALS (84).

Cx43: connexin-43; GFAP: glial fibrillary acidic protein; HMGB1: high mobility group box protein 1; NF-kB: nuclear factor kappa B; S100β: S100 calcium-binding protein β. Cortical astrocytes are less proliferative in the pre-symptomatic stage, where they show a decreased expression of S100β and Cx43. In the symptomatic stage of the disease, cortical astrocytes are more proliferative, showing an increased expression of NF-kB, HMGB1, S100β and Cx43, together with a reduction of glutamate transporters and GFAP. Spinal astrocytes in the pre-symptomatic stage exhibit a decreased expression

of GFAP, S100β and HMGB1, while in the symptomatic stage astrocytes present a proliferative profile with increased expression of NF-kB, S100β, HMGB1 and Cx43.

In ALS, increased expression of HMGB1 in reactive astrocytes can lead to the activation of toll-like receptor/receptor for advanced glycation end-products (TLR/RAGE) signaling pathways, contributing to the evolution of the disease. S100β is a calcium-binding protein that is highly expressed in astrocytes and can either have beneficial or harmful effects, depending on its concentration. In ALS, S100β levels are increased in the cerebrospinal fluid, which correlates with a worse prognosis of the disease. By inhibiting S100β expression, a downregulation in the expression of GFAP and cytokines occurs, indicating its correlation to a proinflammatory phenotype in mSOD1 astrocytes. Consequently, the release of cytokines causes an activation of the NF-kB signaling cascade, a regulator of reactive astrogliosis and inflammation. Lastly, Cx43 is responsible for the communication among astrocytes. However, the overexpression of Cx43 in the astrocytes illustrated in Figure 9 is associated to astrocyte-mediated neurotoxicity (84).

Glutamate transport dysfunction in astrocytes is the main cause of higher extracellular levels of glutamate. An increase in glutamate was found in cerebrospinal fluid in ALS patients. The inhibition of glutamate uptake can induce the selective degeneration of motor neurons (85). Thus, impaired glutamate transport in astrocytes leads to the accumulation of excitotoxic levels of extracellular glutamate (81).

The influx of Ca^{2+} caused by the overstimulation of motor neurons leads to cell death as Ca^{2+} is involved in many apoptotic pathways. The permeability of glutamate receptors is determined by GluR subunits (86). Astrocytes regulate the expression of glutamate receptor 2 (GluR2 or GluA2) subunits and the susceptibility of motor neurons to excitotoxicity by regulating the influx of calcium through AMPA receptors in these cells (85). The calcium permeability of AMPA receptors is largely determined by the presence of the GluR2 subunit. GluR2 lacking glutamate receptors are highly permeable to Ca^{2+}. It has been shown that a lack of GluR2 increased motor neuron degeneration in mice, while increasing GluR2 levels in motor neurons prolonged survival of mice (86). The presence of GluR2 renders AMPA receptors impermeable to calcium and astrocytes are able to modulate GluR2 expression in motor neurons.

ALS is associated with the expression of mSOD1 in astrocytes and inhibition of the capability of GluR2 to regulate Ca^{2+} ion penetration (60). This event causes a decreased GluR2 expression in co-cultured motor neurons and increased vulnerability to AMPA receptor-mediated glutamate excitotoxicity (81).

Moreover, mitochondrial dysfunction is known as a key mechanism of motor neuron degeneration in ALS. Additionally, abnormalities in astrocytes mitochondria cause high levels of reactive oxygen species. Likewise, high levels of NADPH oxidase, inducible nitric synthase (iNOS) and ROS production, which have been observed in human astrocytes expressing mutant s-phase delaying protein 1 (SPD1), cause noncellular autonomous toxicity in motor neurons derived from human embryonic stem cells (ES cells) (86).

The pathogenesis of amyotrophic lateral sclerosis is a result of a variety of cellular interactions (Figure 12). Currently, some of the new research topics that are under investigation for the treatment of ALS are the regulation of astrocyte function, improvements in the regeneration environment of motor neurons and promotion of the survival and regeneration of motor neurons.
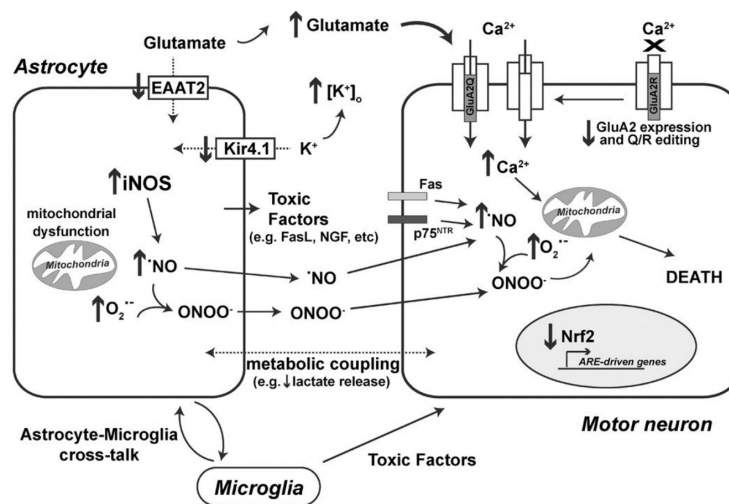
Figure 12. Schematic representation of potential pathways involved in astrocyte-mediated motor neuron death in ALS (81).

Astrocytes in ALS demonstrate a reduced capability for metabolic support to motor neurons and to effectively regulate extracellular levels of ions and neurotransmitters. Impaired glutamate transport in astrocytes causes accumulation of excitotoxic levels of extracellular glutamate. Excitotoxicity is facilitated by increased permeability of AMPA receptors to calcium as a consequence of decreased expression and pre-mRNA editing of GluA2 AMPA receptor subunits in motor neurons. Moreover, the potassium buffering capacity of astrocytes is affected by the reduced expression of astrocytic

inwardly rectifying potassium channel, Kir4.1. The rise in extracellular potassium levels contributes to neuronal hyperexcitability, decreasing the uptake of glutamate. Reactive astrocytes display increased production of $^{\cdot}$NO due to iNOS upregulation. Moreover, mitochondrial dysfunction leads to increased production of $O_2{}^{\cdot-}$ and the formation of $ONOO^-$, leading to oxidative and nitrative stress. $^{\cdot}$NO and $ONOO^-$ can diffuse across cellular membranes to directly affect mitochondrial function and induce oxidative stress in motor neurons. Increased levels of $^{\cdot}$NO also sensitize motor neurons to the apoptotic signaling mediated by FasL/Fas and NGF/p75^{NTR}.

5 Future perspectives

Neurodegenerative diseases are often fatal, untreatable disorders that cause a major impact in patient's quality of life and their caregivers. Epidemiologists estimate that neurodegenerative diseases incidence is likely to triplicate in the next 30 years, along with the progressive aging of the population.

While scientific approaches in the past have been neuron-centric, according to which insults underlying the neurodegenerative process occur only inside neurons, recent studies have shifted the paradigm towards new directions. In the last few years, the importance of glial cells to maintain CNS homeostasis in health conditions has greatly increased, along with the knowledge and awareness of their essential role in pathological conditions, leading to a more gliocentric view. As a consequence, astrocytes emerge as promising therapeutic targets in many neurodegenerative diseases.

Recent studies suggest that neurotrophic viruses, including Zika and West Nile viruses, but also SARS-CoV-2, cause damage to the CNS by interfering with astrocyte functions. Viral infections, including SARS-CoV-2, and other CNS insults are known to trigger reactive astrogliosis. Depending on the insult, astrocytes can shift to a destructive pro-inflammatory phenotype, promoting CNS damage. Reactive astrocytes can also become facultative antigen presenting cells and attract immune cells to the lesion site, further contributing to immune cell infiltration and thus neuroinflammation. These recent findings provide a first argument challenging the neuron-centric dogma. The second element of confutation of the neuron-centric hypothesis is represented by the evidence that astrocyte dysfunction can also play a key role in the pathogenesis of several chronic neurodegenerative diseases, as reviewed in chapter 4 (87, 88).

It was hypothesized that cell replacement therapy may act as a valuable therapeutic approach to tackle neurodegenerative conditions. In order to successfully implement this approach, preclinical studies were performed and demonstrated that not only healthy astrocytes can be implanted into the diseased CNS, but they can resist the neuroinflammatory environment in which they become embedded. Alternatively, aberrant pathways can be precisely targeted by using advanced delivery systems, such as functionalized nanoparticles, homing peptides or viruses, in order to convey drugs or nucleic acids into the astrocytes to correct the altered signaling cascades. Moreover, the optimal formulation of these drugs should allow non-invasive routes of

administration and should not show any toxicity. Besides, the therapeutic agent must be capable to cross the BBB, in order to access the CNS parenchyma to reach an extracellular target or to be internalized by astrocytes (88).

Phase I/IIa clinical trials were performed to investigate the safety and efficacy of different transplantation strategies. ALS patients received an intrathecal administration of human stem cell-derived astrocytes (NCT03482050), while patients affected by retinitis pigmentosa were subject to a dose of glial cells committed to the astrocyte lineage (NCT04284293). Esposito et al. also explored the possibility of transplanting astrocyte-like enteric glial cells in a rat model of AD, showing their capacity to rescue behavioral impairments (89).

Various approaches involving astrocytes have been reported recently for the treatment of AD (90). Curcumin, a natural phenol obtained from plants, has been proposed to be benefic in AD, reducing Aβ formation and decreasing neurotoxicity in the brain. In a recent study using APP/PS1 transgenic mice and primary rat mixed neuronal/glial cultures, it was reported that curcumin improves spatial memory, stimulates cholinergic neuronal function and, through PPARγ, reduces the activation of the inflammatory process in microglia and astrocytes (91).

The astrocyte carries most of the extracellular glutamate. Therefore, damage to astrocytes affects their capability to perceive or respond to an increase in glutamate levels, leading to destruction of the microenvironment near neurons, causing an over-stimulation of NMDA receptors, responsible for changes in cognitive functions in frontal cortex. Studies have shown that the damage to astrocytes induced by Aβ is responsible for the reduced expression of GLT1 in AD. Therefore, drugs that target astrocytic glutamate transporters to ameliorate their expression are possible target pharmacological approaches to increase glutamate uptake, either by increasing GLT1 promoter activation or by activating GLT1 translation. Among the compounds able to stimulate GLT1 expression, there are β-lactam antibiotics, as well as cephalosporin antibiotics (64).

Recent studies have correlated GABAergic neurotransmission with pathological changes of AD. Damaged astrocytes produce a copious amount of GABA that is released to inhibit excitatory neurotransmission in the dentate gyrus. Additionally, monoamine oxidase-B (MAO-B) has been reported to be altered in reactive astroglia

and the enzyme was found upregulated in *post-mortem* brains of AD patients. In an animal model of AD, it has been shown that administration of GABA receptor antagonists improves long-term memory in the hippocampus (92).

Experimental therapies for PD are focused on preventing dopaminergic neuron loss using pharmacological compounds or transplantation of new dopaminergic neurons. The transplantation of astrocytes derived from glial-restricted precursor cells exposed to bone morphological protein (GDA^{BMP}) into injured spinal cord have promoted the survival of multiple neuron populations. It was demonstrated that delayed transplantation of rat or human GDA^{BMP} cells into an experimental model of PD rescued parvalbumin-positive GABAergic interneurons and restored synaptophysin expression, which is essential for synaptic function. The ability of these cells to target multiple problems within the PD model indicate the potential of using astrocytes as a vehicle for restoration of the CNS (93).

Astrocyte reprogramming has recently received much attention as an avenue for increasing functional dopaminergic neurons in the mouse PD brain. By targeting a microRNA (miRNA) loop, astrocytes in the mouse brain could be reprogrammed into functional dopaminergic neurons using specific transcription factors (TF) and miRNAs. This astrocyte reprogramming in the mouse model of PD has successfully added new dopaminergic neurons to substantia nigra and increased dopaminergic levels associated with axonal projections into the striatum (94).

6 Conclusion

Astrocytes are the most abundant glial cells in CNS. The first description of astrocytes dates back to the 19th century, when they were initially thought to be mere supportive elements to neurons. Astroglial cells possess various structures and functions. They are known to maintain structural, metabolic and guidance support for neuronal function. Astrocytes play key roles in a broad range of physiological CNS processes, including maintenance and integrity of the blood-brain barrier. Therefore, astroglial cells provide a valuable microenvironment for neurons.

Neurodegenerative diseases are characterized by a progressive degeneration and/or neuronal cell death, which may affect patient's movement and cerebral performance, leading to dementia. Nowadays, neurodegenerative diseases are one of the biggest medical and socioeconomical concerning issues worldwide and the causes of their emergence are still unknown. Unfortunately, most, if not all neurodegenerative diseases still do not have a cure.

Alzheimer's disease is the most common cause of dementia and one of the major causes of death in many countries, including Portugal. One of the major concerns of AD is its late diagnosis, since symptoms only start to appear many years after the first physiological changes in human brain. Therefore, AD has become a significant area of research.

Nowadays, due to many years of investigation, knowledge of astrocytes physiology and their contribution to pathogenesis of many neurodegenerative diseases has increased exponentially. Considering the importance of astrocyte functions for CNS performance, it is reasonable to say that astrocyte dysfunction can be a primary event of a pathogenic cascade ultimately leading to neuronal loss. The activation of astrocytes is a main pathological feature of neurodegenerative diseases and an adaptive defense response that provides essential metabolic support for neurons. Yet, reactive astrocytes play opposite roles in the diseased CNS: they promote neurodegeneration, but they can also support neuroprotection and repair.

Furthermore, the emergence of sequencing techniques has provided molecular evidence with a great deal of heterogeneity of astrocytes. Understanding the characteristics and properties of these cells provides important therapeutic opportunities

and it is becoming increasingly evident that it is essential to modulate the activities of distinct subpopulations of astrocytes at different stages of the disease, in order to achieve control over neurodegeneration.

Future investigations are needed to address essential issues related to astrocytes in neurodegenerative diseases. Development of new approaches to preserve and engage astrocyte-derived neuroprotective mechanisms could slow down disease progression. The design of strategies to maintain the physiological astrocyte function could also delay disease progression. The findings discussed in this dissertation support the concept that astrocytes are promising therapeutic targets in neurodegenerative diseases.

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