Role of Autophagy in Brain Sculpture: Physiological and Pathological Implications

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Abstract The brain has the ability to change during the life rearranging itself by the elimination and the formation of new connections between neurons. This dynamic capacity is known as brain plasticity or neuroplasticity, and is associated with functional changes involving functional recovery after brain damage, learning, memory, and addiction. It is well defined that protein synthesis is required for neuroplasticity and the establishment of long-term memories, but protein degradation plays also a crucial role in neuronal physiology and pathology. Ubiquitin-proteasome system, which degrades short-lived proteins, is important in synaptic plasticity, learning and memory, as well as lysosome system, which involves endocytosis to degrade proteins, plays a role in synaptic plasticity regulating receptor trafficking. The third major degradation pathway is the autophagy which degrades long-lived cytoplasmic proteins or damaged organelles to maintain normal cell homeostasis. Recent evidence suggests the involvement of autophagy in synaptic plasticity, in addition to its crucial role in the quality control of proteins and organelles in neurons. Thus an impairment of the autophagic machinery is closely connected with the alteration of neuronal function and neuron ability to respond to damage. A clear understanding of neuronal autophagy in brain physiology and pathology could help to develop new pharmaceutical approaches for the treatment of neurological disorders. The current Chapter will focus on the key role of autophagy in the development and function of the central nervous system (CNS), and on the emerging evidence of autophagy deregulation in neurodegenerative disease and acute brain damage.

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Abbreviations

3-MA	3-methyladenine
AD	Alzheimer's Disease
Akt (PKB)	Protein kinase B
Ambra1	Autophagy/Beclin-1 Regulator 1
AMPAR	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
APP	Amyloid precursor protein
ASD	Autism spectrum disorders
Atg	Autophagy-related
ATP	Adenosine triphosphate
AVs	Autophagic vacuoles
Aβ	β-amyloid peptide
Bcl-2	B-cell lymphoma 2
Bcl-XL	B-cell lymphoma-extra large
Bif-1	BAX-interacting factor-1
Bim	B-cell lymphoma 2 interacting mediator of cell death
CCI	Controlled cortical impact injury
CIII PI3K	Class III phosphatidylinositol 3-kinase
CMA	Chaperone-mediated autophagy
CNS	Central nervous system
COMT	Catechol-o-methyltransferase
Deptor	DEP domain containing MTOR-interacting protein
ER	Endoplasmic reticulum
FAD	Familial AD
Fbxo7	F-box protein 7
FIP200	FAK Family Kinase-Interacting Protein of 200 kDa
FPI	Fluid percussion injury
$GABA_A$,	γ-aminobutyric acid
GCEE	γ-glutamylcysteinyl ethyl ester
H/I	Hypoxia/ischemia
HCb	Hemicerebellectomy
HD	Huntington's disease
HDAC6	Histone deacetylase 6
Htt	Huntingtin
i.c.v.	Intracerebroventricular
IO	Inferior olive
IRGM	Immune-related GTPase M
KO	Knockout
LAMP	Lysosome-associated membrane protein type
LC3	Microtubule-associated protein 1 light chain 3
LRRK2	Leucine-rich repeat kinase 2
LTD	Long-term depression
LTP	Long-term potentiation
MAO-B	Monoamine oxidase B
Mfn1	Mitofusin 1

MPP+	1-methyl-4-phenylpyridinium
mTOR	Mammalian target of rapamycin
mTORC1	mTOR complex 1
NBR1	Neighbor Of BRCA1 Gene 1
Ndp52	Nuclear dot protein 52 kDa
NGF	Nerve growth factor
NMDAR	N-methyl-D-aspartate receptor
NPCs	Neural progenitor cells
NSF	N-ethylmaleimide-sensitive factor
p62/SQSTM1	Sequestosome 1
PD	Parkinson's Disease
PE	Phosphatidylethanolamine
PI3K	Phosphatidylinositol 3-kinase
PI3P	Phosphatidylinositol 3-phosphate
PINK1	PTEN-induced putative kinase 1
pMCAO	Permanent middle cerebral artery occlusion
Pn	Pontine nuclei
polyQ	Polyglutamine
PP1	Protein phosphatase 1
PRAS40	Proline-rich Akt substrate of 40 kDa
PSD95	Postsynaptic density protein 95
PSEN	Presenilin
PTEN	Phosphatase and tensin homolog
Raptor	Regulatory-associated protein of mTOR
Rubicon	RUN and cysteine rich domain containing beclin 1 interacting protein
SCI	Spinal cord injury
SVZ	Subventricular zone
TBI	Traumatic brain injury
tMCAO	Transient middle cerebral artery occlusion
TrkA	Tropomyosin receptor kinase A
Tsc2	Tuberous sclerosis proteins
ULK1	UNC-51-like kinase 1
UVRAG	UV Radiation Resistance-Associated Gene
VDAC1	Voltage-dependent anion channel 1
VMP1	Vacuole Membrane Protein 1
WIPI-1	WD-repeat protein Interacting with PhosphoInositides-1

1 Introduction

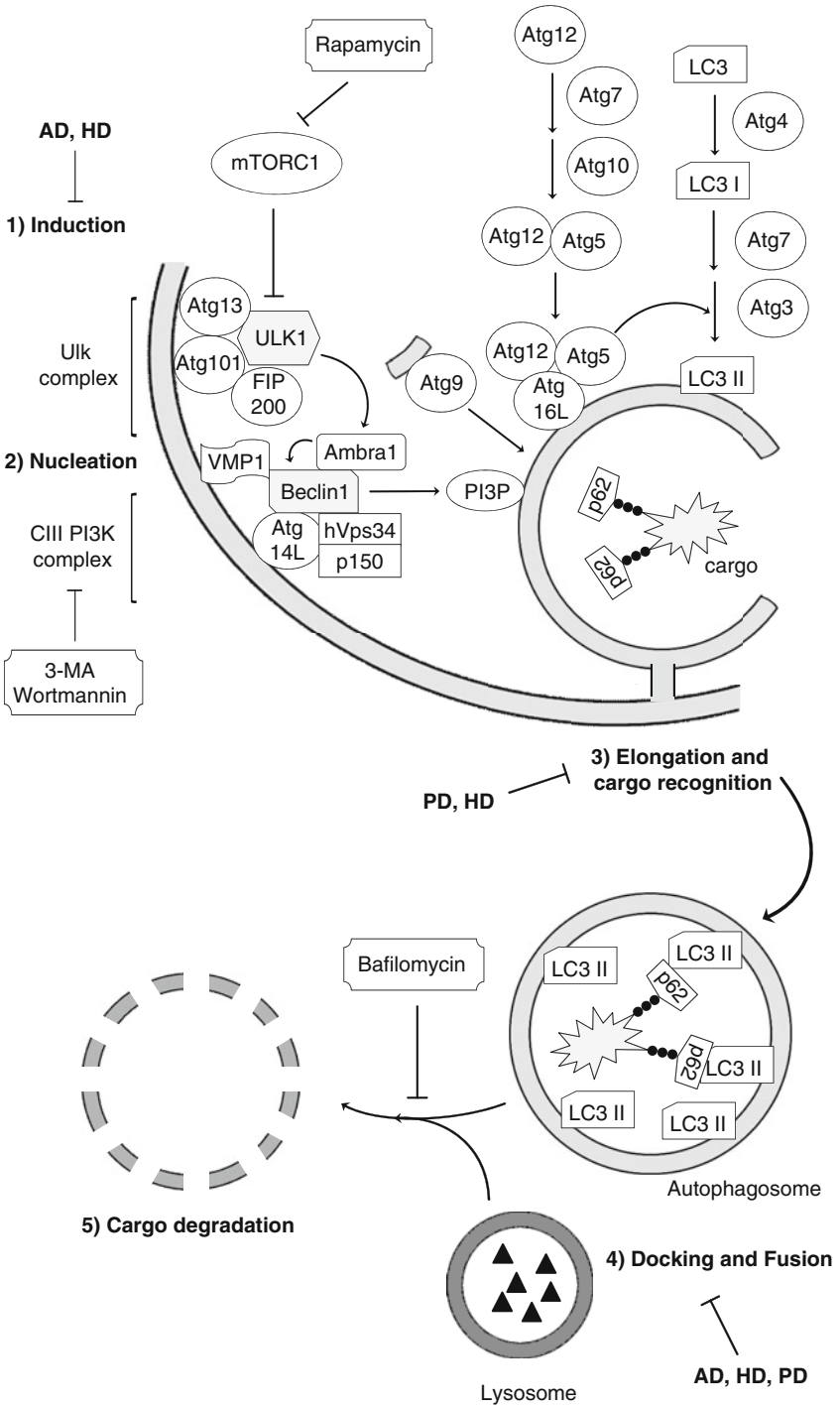
Autophagy is a catabolic mechanism that mediates degradation and recycling of cellular constituents, delivering portion of cytoplasm to the lysosomes for degradation. Autophagy is considered to be important to maintain cellular homeostasis, especially under nutrient deprivation or stress conditions, and to guarantee proteins quality control and organelles turnover. Furthermore, autophagy has been

implicated in various cellular processes, such as development, differentiation, ageing and immunity [77, 101, 102].

Autophagy is highly conserved from yeast to mammals and it can be classified in three principal types: microautophagy, chaperone-mediated autophagy (CMA) and macroautophagy [115]. Microautophagy leads to degradation of sequestered portions of cytosol by direct invagination of lysosome membrane [99]. CMA delivers cytosolic protein containing a KFERQ-like motif to the lysosomal lumen via chaperone Hsc70 and LAMP-2A complex [66]. Macroautophagy (hereafter referred to as autophagy) is the well-characterised form of autophagy that leads to the formation of a double-membraned vacuole, the autophagosome, containing cytoplasmic material, such as macromolecules and organelles. Autophagy requires several steps: induction and nucleation of phagophore (the isolation membrane), elongation of phagophore to constitute the autophagosome, maturation of autophagosome into amphisome/autolysosome by fusion with endosome/lysosome and, finally, degradation of membrane contents (Fig. 1). Autophagy is constitutively activated at a basal level to maintain cellular homeostasis but it can also be induced by several input signals, such as nutrient deprivation, change of intracellular levels of Ca^{2+}, ATP and cAMP, hormones, protein accumulation and damaged organelles [136]. The main regulator of autophagy is mTOR (mammalian target of rapamycin) complex 1 (mTORC1), a polyprotein complex that contains mTOR, Raptor, mLST8/GßL, Deptor and PRAS40 [22]; however the autophagy is also regulated by mTOR-independent pathways even though the effectors involved in the autophagosome biogenesis are not clear [136].

More than 30 autophagy-related (Atg) proteins have been identified and characterized in yeast [60, 61] and conserved in mammals, where additional Atg proteins have been identified. However, less than half, the "core Atg proteins" [110, 166], are

Fig. 1 Schematic representation of mammalian autophagy pathway. Following inhibition of mTOR, ULK complex, composed of ULK1, Atg13, FIP200 and Atg101, is activated and starts the autophagosome nucleation. ULK1 also phosphorylates Ambra1, leading to the activation of Beclin1, a component of CIII PI3K complex (or Beclin1 complex), which consists of PI3K or hVps34, Beclin1 and p150. In this step, VMP1 recruits Beclin1 to the phagophore where the complex is required for generating a pool of phosphatidylinositol 3-phosphate (PI3P). To expand the autophagosome membrane, Atg12-Atg5-Atg16L complex and LC3 ubiquitin-like conjugation systems are required. The Atg12-Atg5-Atg16L multimeric complex is formed by subsequent steps involving Atg7 and Atg10 and is also required for the efficient function of LC3. The second system mediates the conjugation of LC3 to phosphatidylethanolamine (PE). LC3 is processed by Atg4, forming the cytosolic LC3 I. Atg7 binds LC3 I and transfers it to Atg3, which catalyses the conjunction to the lipid PE and the conversion of LC3 I in LC3 II. The complete autophagosome fuses with the lysosome and cargo molecules engulfed by autophagosomes are degraded by lysosomal hydrolases and recycled back to the cytoplasm. The specificity of cargo degradation is mediated by selective adaptor proteins, such as p62 that binds ubiquitinated residues of the target proteins or organelles. The steps known to be affected in neurodegenerative diseases and the action of drugs modulating autophagy are indicated. *AD* Alzheimer disease, *HD* Huntington disease, *PD* Parkinson disease. Regulators of autophagy are also indicated. 3-MA, 3-methyladenine


Rapamycin
Atg12
Atg7
Atg10
LC3
Atg4
LC3 I
AD, HD
mTORC1
1) Induction
Atg12
Atg5
Atg7
Atg3
Atg13
ULK1
Atg101
FIP
200
Ulk
complex
Atg12
Atg5
Atg9
Atg
16L
LC3 II
2) Nucleation
VMP1
Ambra1
Beclin1
PI3P
p62
p62
cargo
CIII PI3K
complex
Atg
14L
hVps34
p150
3-MA
Wortmannin
PD, HD
3) Elongation and
cargo recognition
Bafilomycin
LC3 II
LC3 II
p62
p62
LC3 II
LC3 II
LC3 II
Autophagosome
5) Cargo degradation
4) Docking and Fusion
AD, HD, PD
Lysosome

involved in autophagosome formation. These proteins can be subdivided in three functional groups: (1) two kinase complex, ULK complex (consisting of ULK1, Atg13, FIP200 and Atg101) and the class III phosphatidylinositol 3-kinase (CIII PI3K) complex (or Beclin1 complex, comprising PI3K or hVps34, Beclin1 and p150); (2) two ubiquitin-like protein conjugation systems, ATG16L1 complex (Atg12-Atg5-Atg16L) and LC3; (3) two transmembrane proteins, Atg9 (and associated proteins involved in its movement such as WIPI-1) and VMP1.

The activity of ULK complex is important in the induction step of autophagy and is negatively regulated by mTORC1. After autophagy induction, mTORC1 is inactivated, leading to dephosphorylation of ULK1 and Atg13. This event causes the activation of these proteins and the consequent phosphorylation of FIP200 by ULK1. Recently it has been identified a novel ULK complex interactor, Atg101, important for both the stability of Atg13 and basal phosphorylation of Atg13 and ULK1 [48, 98].

Beclin1-CIII PI3K complex is required for the isolation membrane nucleation, generating a pool of phosphatidylinositol 3-phosphate (PI3P), which acts as platform for other Atg proteins recruitment. Beclin1 interacts with several proteins, which are able to modulate the complex activity: Atg14L, UVRAG, Ambra1, and Bif-1 enhance Beclin1 complex activity; the transmembrane protein VMP1 is crucial for the nucleation step, recruiting Beclin1 (and other components of the Beclin1 complex) to the phagophore; whereas Rubicon, the anti-apoptotic proteins Bcl-2 and Bcl-XL and the pro-apoptotic protein Bim negatively regulate autophagy [136].

Following the autophagy induction, ULK1 phosphorylates Ambra1, leading to the activation of Beclin1 and the translocation of the Beclin1-CIII PI3K complex from the microtubule network to the endoplasmic reticulum (ER), which is considered the main membrane source for the biogenesis of phagophore [1, 28, 44]. The fundamental contribution to the biogenesis and elongation of the phagophore from other organelles membrane is mediated by Atg9 [169].

The elongation of the phagophore requires two ubiquitin-like protein conjugation systems. Firstly, Atg5 and Atg12 are conjugated by the ubiquitin-activating E1-like enzyme Atg7. In particular, Atg7 activates Atg12, which is transferred to Atg10, an E2-like enzyme, and then covalently linked with Atg5 ([105, 148]). The Atg12-Atg5 heterodimer binds Atg16L forming a large multimeric complex required for elongation of phagophore [103] and for the efficient function of LC3. The second system mediates the conjugation of LC3 to phosphatidylethanolamine (PE) [41, 103]. LC3 is processed by the protease Atg4, forming the cytosolic LC3 I. Atg7 binds LC3 I and transferred it to Atg3 (E2-like), which catalyses the conjunction to the lipid PE and the conversion of LC3 I in LC3 II. During the expansion of autophagosome, LC3 II is attached both to the inner and outer membrane of this structure. However, until the fusion with the lysosome LC3 II is removed from the outer membrane, while the one in the inner membrane is degraded with the autophagosome cargo [52, 59].

Autophagy has been originally identified as a non-selective process, but recent evidence underlines the importance of selective mechanism, specially involved in the quality control of proteins and organelles. Several post-translational modifica-

tions have been implicated in the regulation of autophagy, one of which is the ubiquitination. The residues of ubiquitin facilitate the recruitment of autophagic receptors and selective adaptor proteins, such as p62 (also called SQSTM1), NBR1, HDAC6, Nix, Ndp52, which tether the targeted substrate to core of Atg proteins, such LC3 [139].

The autophagy mechanism has been analyzed in several tissues and although it occurs in all cell types and involves the same protein complexes, recent studies suggest that this mechanism is tissue-specific regulated. In particular, autophagy plays a crucial role in the brain, in which it is involved as well as in stress response, in quality control of proteins and organelles, even in specific function, such as neurodevelopment, differentiation, learning and memory.

In this Chapter we will discuss the physiological roles of autophagy in neurons and the pathological implications occurring when this process is dysregulated.

2 Neuronal Autophagy

Neurons are highly specialized cells, composed by specific compartments including neurites (axons and dendrites), synapses and soma, in which the synthesis, transport and degradation processes are finely regulated. Moreover, mature neurons are postmitotic cells, which require an efficient protein quality control system to avoid accumulation of misfolded or aggregated proteins and damaged organelles that cannot be diluted through cell division. Autophagy is of particular importance in the synaptic compartments, characterized by high-energy demand and where a fine control of proteins and organelles turnover is necessary to ensure the activity.

Compared to other organs (such as heart, liver, pancreas, kidney or muscle), in basal condition the brain shows higher level of LC3 and a low number of autophagosomes [112], which do not increase even under nutrient deprivation [106]. Several studies demonstrated that the low presence of autophagic vesicles, analyzed as a low amount of LC3 II then LC3 I, depends on the fast kinetics of vesicles formation and degradation, that reflects the high efficiency of autophagosome turnover [8, 107].

3 The Role of Autophagy in Neurodevelopment and Neurogenesis

The importance of autophagy in development has long been suggested by several studies in which autophagic structures were analyzed during embryogenesis [20, 83] and by studying mutant mice for several *Atg* genes, which have different role in the regulation of development. In fact, mice lacking *Atg3, Atg5, Atg7, Atg9* and *Atg16L1* complete the embryonic development, but die shortly after birth, suggesting that the proteins encoded by these genes are not essential for embryogenesis but have a crucial role in the regulation of perinatal starvation [64, 68, 131, 132, 145]. Instead, Beclin1, Ambra1 or FIP200 deficient mice are embryonic lethal at the

stage E7.5, E10–E14 and E13.5–E16, respectively [35, 37, 174]. The differences among *Atg* genes are not well understood. It seems possible that the role of Beclin1 and FIP200, which interact with several factors, may be related to other function; alternatively, the different lethality may depend on the step in which each factor is involved [104]. In the latter case, Atg9 constitutes an exception because it acts in early phase of autophagy process but its loss causes a less severe phenotype [146].

The short survival time after birth of *Atg* knockout (KO) mice prevents the study of the Atg proteins role, whereas the generation of conditional KO mice allowed to understand their function in specific tissues. Brain-specific deletion of *Atg5* and *Atg7* suggests that these proteins are involved in motor function and that their deficiency results in the development of progressive motor and behavioural deficits. The histological analysis of Atg5 conditional KO mice shows partial loss of Purkinje cells and neuronal inclusion bodies accumulation. Aggregates of ubiquitinated proteins, which accumulate in a time-dependent manner, are also detected starting at embryonic day E15.5. It has been proposed that basal autophagy in Purkinje neurons is necessary to ensure the correct protein turnover, avoiding aggregates formation [42]. Atg5 KO embryos also display defects in apoptotic corpse engulfment in photoreceptor and ganglion cell layers of the retina at E18.5 [123]. A recent work has also proposed that Atg5 plays a central role in developing embryonic cortex to ensure the formation of correct multiple layers architecture. By silencing Atg5 expression in cortical neural progenitor cells (NPCs), it has been observed that the loss of Atg5 function leads to unbalanced cortical NPCs differentiation and proliferation and causes the abnormal morphology of cortical neurons. Atg5 exerts its function in strictly cooperation with β-Catenin and both are required to regulate cortical NPCs differentiation and proliferation [87].

Studies performed on Atg7 conditional KO mice show that the loss of this protein leads to axonal swellings, with accumulation of aberrant membrane structures and progressive dystrophy and degeneration of the axon terminals in Purkinje cells. Interestingly, these events occur much earlier than the neuronal death, suggesting that basal autophagy is required to regulate membrane homeostasis in the axonal terminals in addition to protein quality control [65].

ULK1 protein regulates axon outgrowth in cerebellum granule neurons and it has been demonstrated that ULK1 is expressed in different neuron population during development and is particular abundant in developing cerebellar granular cells. The inactivation of ULK1 by retroviral injection of its dominant negative form demonstrates that this kinase has a pivotal role in neurite extension/parallel fiber formation [151]. Recent studies also suggest that ULK1 regulates endocytotic trafficking of growth factors, that are necessary during polarized axon elongation. However is not well understood whether ULK1 acts influencing the autophagy mechanism or has a different function through the interaction with several proteins, for example in NGF-TrkA endocytosis [152, 180].

Another example of the importance of autophagy during neurodevelopment comes from the studies conducted on Ambra1 protein. Ambra1 is strongly expressed in developing neuronal tissue starting at the embryonic day E8.5, where is detected in neuroepithelium. Subsequently, a massive expression is observable in the ventral

part of the spinal cord, in the encephalic vesicles, in the neural retina, in the limbs and in the dorsal root ganglia (E11.5); at later stages, Ambra1 is expressed in the entire developing nervous system; finally, in postnatal brain Ambra1 is particularly abundant in the cortex, hippocampus and striatum. Ambra1-deficient mice display defects in neural tube closure, as well as increased cell proliferation and cell death, that cause exencephaly and/or spina bifida phenotypes [35]. Therefore, Ambra1 appears to be involved in cell proliferation and survival during neurodevelopment, perhaps controlling the degradation of key development regulators, as demonstrated by the increase of ubiquitinated proteins in Ambra1-deficient mice, or directly regulating cell proliferation.

A central role for autophagy has recently been proposed also in adult neurogenesis. Ambra1 and Beclin1 are highly expressed in adult subventricular zone (SVZ) of the lateral ventricles, an area where new neurons are generated and then migrate through the rostral migratory stream to the olfactory bulb to become interneurons [100]. It has been demonstrated that autophagy is involved in two different mechanisms in SVZ: on the one hand it sustains the pool of stem cell, on the other hand it enhances the survival of neuronal precursors. In fact, *Beclin1* heterozygous mice show a significant decrease in cell division and an increase in the number of apoptotic cells in SVZ compared to wild-type [171].

Recent evidences support the idea that autophagy plays a fundamental role also in dendritic spine pruning during postnatal life. It has been reported an increase in spine density with reduced developmental spine pruning in layer V pyramidal neurons in postmortem temporal lobe of patients with autism spectrum disorders (ASD). These spine defects correlate with mTOR hyperactivation and autophagy impairment. In fact, Tang and colleagues, using a mTOR costitutively activated mouse model ($Tsc2^{+/-}$ mouse, with mutated Tsc2, a protein that indirectly inhibit mTOR) observed a postnatal spine pruning defects, blockage of autophagy and ASD-like behaviours. Interestingly, rapamacyn is able to revert these phenotypes in $Tsc2^{+/-}$ mice but not in $Tsc2^{+/-}$: Atg7 conditional KO double mutants, suggesting that autophagy is required for the correct remodelling of dendritic and spine architecture [147].

4 The Synaptic Role of Autophagy

Recent evidences have suggested that autophagy plays an essential role in synaptic plasticity, influencing both pre- and post-synaptic compartments by altering the efficacy of neurotransmitter release or by modifying the post-synaptic density composition (Fig. 2). There are two main forms of synaptic plasticity: long-term potentiation (LTP), where occurs a persistent increase of synaptic efficacies in response to a high-frequency stimulation, and long-term depression (LTD), characterized by a lasting decrease in synaptic effectiveness that follows a pattern of low-frequency stimulation [4, 7]. Both LTP and LTD are considered cellular correlates of learning and memory and for this reason the study of these processes is so intriguing. One of the main receptors involved in synaptic transmission is the

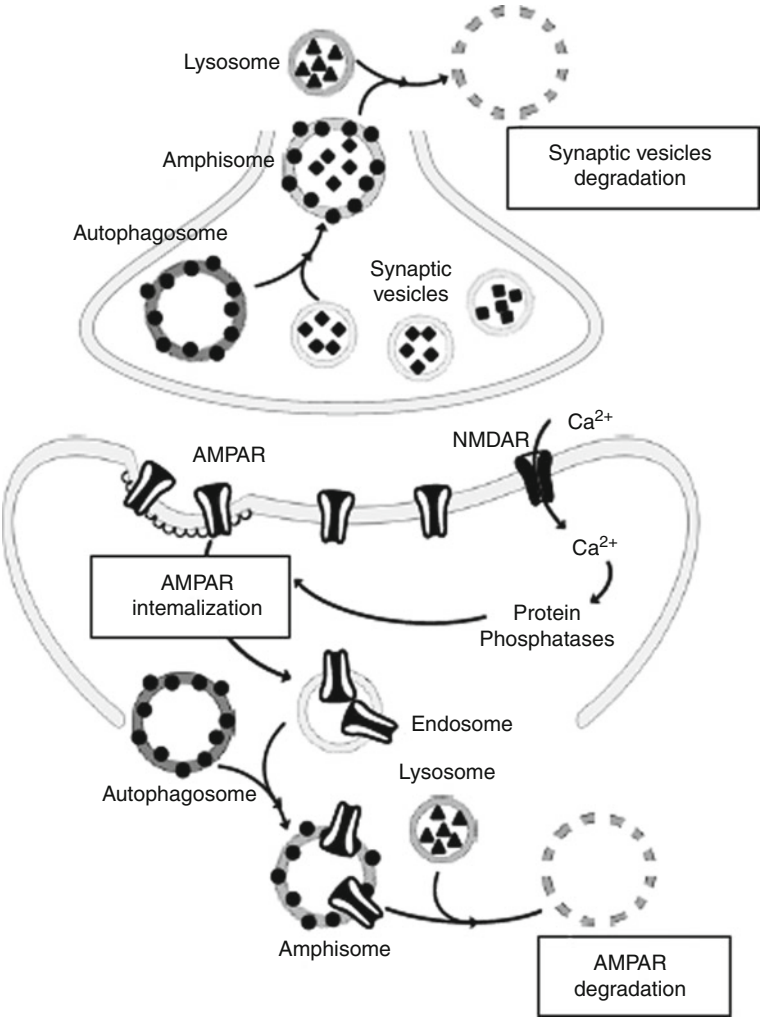


Fig. 2 Representation of autophagy role in pre- and post-synaptic compartment. In the presynaptic terminal autophagy alters the efficacy of neurotransmitter release, leading to selective degradation of neurotransmitter-containing vesicles. In the post-synaptic terminal autophagy regulates the degradation of AMPA receptor (AMPAR) after chemical induction of long-term depression (LTD). LTD leads to activation of the calcium ion-permeable NMDA receptor (NMDAR). A series of downstream intermediate signaling steps, including activation of protein phosphatases, cause AMPAR endocytosis and induction of LTD. Endosome vesicles containing AMPAR fuse with autophagosomes, forming amphisomes, that finally fuse with lysosomes leading degradation of the AMPAR

α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR), a class of ionotropic glutamate receptors that mediates the excitatory neurotransmission response. The synaptic transmission can be modulated by modifying the exposure of AMPARs in post-synaptic density [144] and by altering the trafficking of AMPARs into and out of synapses [88, 90]. Increased exposure of AMPARs causes a strengthen of synaptic transmission and generally LTP, whereas the removal of AMPARs results in LTD [141, 144]. AMPARs are heterotetrameric complexes, composed of various combination of four subunits (GluA1-4) [47]. In the adult hippocampus GluA subunits are assembled preferably in two major subtypes, GluA1-2, which represent approximately 80 % of synaptic receptors, and GluA2-4, which represent the remaining 20 % [84, 165]. Recent evidences suggest that AMPARs subunit composition and their interactors on synaptic membrane are able to regulate the trafficking and the targeting of AMPARs to synaptic sites [19, 27, 31, 43, 56, 113, 116, 117, 143, 144]. Moreover, several studies proposed that both GluA1 and GluA2 subunits have a central role in LTD [18, 73, 75, 153, 181]. Upon N-methyl-D-aspartate receptor (NMDAR)-dependent chemical LTD induction, the increase of intracellular Ca^{2+} level leads to the activation of several phosphatases, including PTEN, calcineurin and PP1, that beyond other functions are committed to dephosphorylate GluA1 at Ser 845 residue, promoting its internalization [72], and PSD95 at the Ser295, which is responsible to the recruitment of GluA1 subunit to post-synaptic surface and to the stabilization of the synaptic structure [58]. The GluA2 subunit is stabilized at the synapses by the interaction with NSF (N-ethylmaleimide-sensitive factor), an ATPase that is involved in membrane fusion events [56, 74]. During LTD, it is possible that NSF dissociates from GluA2 and that AP2 binds an overlapping region of GluA2, leading to clathrin-dependent receptor internalization [74, 91]. Internalized AMPARs can be recycled back to the synapses, pooled in the endosomes as a reserve [86, 114] or targeted to lysosome for degradation [33, 75]. Recently it has been proposed that autophagy is responsible for the regulation of neuronal activity, leading to the degradation of AMPARs contained in endocytic vesicle. In fact, after chemical LTD induction the number of autophagosome in dendritic shaft and spines of pyramidal neurons increases, together with a reduction of GluA1 subunits [140]. Degradation of AMPARs, and the consequent LTD stabilization, is partially recovered by application of Okadaic acid and dipotassium bisperoxo (5-hydroxypyridine-2-carboxyl) oxovanadate V, which are inhibitors of PP1 and PTEN, respectively. Both PP1 and PTEN regulate the phosphorylation and the activity of Akt and for this reason it has been suggested that chemical LTD leads to autophagy activation by inhibition of PI3K-Akt-mTOR pathway. Moreover, the inhibition of PI3K by wortmannin and the silencing of Atg7 by lentiviral shRNA infection determine a complete block of LTD-induced autophagy or a partial recovery of GluA1 levels, respectively. It is possible that the increase of the number of autophagosomes, observed in dendritic shafts and spines during LTD, increases the probability of fusion between autophagosomes and endosomes containing GluA subunits to form amphisomes, determining lysosomal degradation of cargo and preventing the receptors recycling in the post-synaptic site. Another interesting possibility is that after LTD induction autophagy acts as a selective degradation mechanism. It has been

reported that $GABA_A$ (γ-aminobutyric acid) receptors, but not acetylcholine receptors, are internalized and degraded selectively by autophagy in *C. elegans* neurons [128]. Moreover p62 protein, which is a selective adaptor protein between cargo and autophagosomes, has an important role in LTP and spatial memory [124] and interacts directly with AMPAR subunits influencing their trafficking to synaptic membrane [51]. It is probable that autophagosomes merge with endosomes containing AMPARs by a p62-mediated mechanism.

Other evidences suggest that autophagy has also a pre-synaptic role regulating neurotransmission [46]. Conditional Atg7 KO mice, in which autophagy deficiency is restricted to dopaminergic neurons, show that inhibition of autophagy in pre-synaptic terminal alters pre-synaptic structure and neurotransmission. In particular, the absence of autophagy results in increased size of axon terminal, increased evoked dopamine release and more rapid pre-synaptic reuptake of neurotransmitter. The pre-synaptic role of autophagy is mTOR-regulated. In fact, acute rapamycin treatment induces a transient augment in the number of autolysosomes in synaptic terminals and at the same time reduces the number of neurotransmitter vesicles. This effect is absent in Atg7 conditional KO mice, indicating that the induction of autophagosome formation by rapamycin requires Atg7. It has been suggested that this mechanism is not specific to dopaminergic neurons. Indeed, rapamycin induces autophagosome-like structures also in other neurons, such as glutamatergic, GABAergic and cholinergic [46].

5 Role of Autophagy in the CNS Diseases

Considering the complexity of autophagic pathway and its importance in neuronal function is not surprisingly that neurons are particularly sensitive to autophagy defects with several pathological implications. Acute and chronic neurodegenerative diseases – including stroke, brain trauma, spinal cord injury, Alzheimer's disease, Parkinson's disease, Huntington's disease – are characterized by mitochondria dysfunction and extensive neuronal cell death. Moreover, the characterization of the autophagic molecular machinery has been followed by abundant studies supporting the key role of autophagy alterations in several human disorders including the above cited brain diseases (Fig. 1).

6 Acute Brain Damage

6.1 Ischemic Stroke

Stroke is one of the main causes of mortality and long-term disability worldwide and the majority of cases are ischemic. Ischemic stroke occurs as a result of a transient or permanent reduction in cerebral blood flow, with consequent lack of oxygen and nutrients in ischemic areas leading to neuronal death. Cerebral ischemia results

in severe intracellular energy stress leading to cell death by a combination of necrotic cell death in infarct core and apoptotic cell death in the ischemic penumbra [32]. In this scenario, the cellular defences against the exhaustion of energy and metabolic stress contribute to neuronal survival. Under stress condition, in neuronal cells exists a subtle balance between life and death and the ultimate fate depends on the cross-talk between different cellular pathways. Since autophagy is induced by cellular stress to enhance cell survival, and many of these stresses (including nutrient deprivation and oxidative stress) occur during cerebral ischemia, in the last years numerous studies have been interested in disentangling the role of autophagy in brain ischemia. Although increased autophagy has been reported in different types of cerebral ischemia (including focal, global and hypoxic/ischemic injury), its role in neuronal death remains controversial. In fact, several works demonstrate that autophagy acts as a protective response after ischemic strokes, whereas other studies show that autophagy inhibition can reduce ischemic damage.

It has been shown that hypoxia/ischemia (H/I) induces Beclin1 expression in the hippocampus and cerebral cortex of neonatal rats (postnatal day 7), and that *in vivo* pharmacological activation of autophagy by rapamycin treatment reduces necrotic cell death and brain injury. Moreover, the inhibition of autophagy by 3-methyladenine (3-MA) accelerates the progression towards necrotic cell death [13]. In addition, both hypoxic preconditioning and prophylactic treatment with simvastatin, which have neuroprotective effect when administered before the onset of H/I in neonatal rats, increase Beclin1 expression after H/I and reduce brain damage, suggesting a protective role of autophagy in the neurodegenerative process that follows neonatal hypoxic-ischemic insult.

Conversely, Koike and Colleagues described that autophagy is strongly induced in the hippocampus following H/I in neonatal mice (postnatal day 7), and they suggested its involvement in neuronal death. In fact Atg7-deficiency protects hippocampal pyramidal neurons from cell death and reduces damaged areas after H/I [63]. In agreement with this work, other studies suggest that autophagy can be implicated in ischemia-induced neuronal death. It has been demonstrated that autophagy activity is increased after transient focal cerebral ischemia in neonatal rats (postnatal day 12) mainly in the border of the lesion, and that post-ischemic intracerebroventricular (i.c.v.) injections of autophagy inhibitor 3-MA reduces the lesion volume. The activation of autophagy in the ischemic penumbra, where delayed cell death occurs, and the neuroprotective effect of post-ischemic autophagy inhibition suggest a detrimental effect of autophagy on neuronal survival [121]. In agreement with these studies, permanent focal ischemia induced in the cortex of adult rats by permanent middle cerebral artery occlusion (pMCAO) causes an increase of autophagosomes, autolysosomes and LC3 conversion (from LC3-I to LC3-II) in cortical neurons. A single i.c.v. injection of bafilomycin or 3-MA after the onset of ischemia reduces the infarct volume [164].

In support of protective role of autophagy, it has been demonstrated that IRGM (immune-related GTPase M, IRGM1 in mouse), a protein that can regulate the survival of immune cells through autophagy, is upregulated in the ischemic side in pMCAO mouse model, concomitantly with a strong autophagic response [45].

Notably, autophagy activation following pMCAO is almost completely lost in IRGM1 KO mice with an increase of infarct volume. Moreover, IRGM1-mediated activation of autophagy in the early phase of ischemia (within 24 h after injury) protects neurons from necrotic cell death in the core of lesion but promotes apoptosis in the penumbra [45].

Furthermore, in adult rats transient middle cerebral artery occlusion (tMCAO) activates autophagy, and in particular mitophagy (a process involved in selective removal of mitochondria by autophagy) [79]. In tMCAO rapamycin attenuates infarct volumes and neurological deficits after cerebral ischemia and 3-MA blocks this protective effect. The observation that rapamycin enhances mitophagy and improves mitochondrial function suggests that the selective removal of damaged mitochondria by autophagy can play an important protective role in ischemic brain injury [79].

Zhang and Colleagues [177] used both pMCAO and tMCAO mouse models of ischemia to understand the role of autophagy in the ischemic and reperfusion phases. Interestingly, they observed that in the pMCAO model, the pre-treatment with 3-MA reduces the ischemia-induced infarct, whereas i.c.v. injection of 3-MA at the onset of reperfusion (tMCAO mice) significantly aggravates brain injury, suggesting that autophagy plays detrimental and protective roles in the ischemia and reperfusion phases, respectively.

Although autophagy is generally considered a cell survival mechanism, massive autophagy can also be associated with cell death through excessive self-digestion and degradation of cellular components. Depending on the context and amount, autophagy may then either protect cell from death or induce cell death. In conclusion, the net effect of ischemic injury-induced autophagy remains controversial and might depend on brain region and maturity, on severity of injury, and on timing of therapeutic interventions. In fact, autophagy could have dissimilar effects depending on stroke model and on the different phases of ischemia (early or late), as well as playing different roles in the core of lesion and in the penumbra area.

6.2 Traumatic Brain Injury

Traumatic brain injury (TBI) refers to brain damage caused by an external mechanical force, which can lead to permanent or temporary impairment of cognitive, physical and psychosocial functions [89]. TBI is the leading cause of mortality and disability in the young aged population under 45 years and represents one of the major causes of hospitalization nowadays: ten million hospitalizations and/or deaths annually are attributable to TBI worldwide [70]. TBI is a complex disease process causing structural damage and motor and cognitive dysfunction produced by both primary and secondary mechanisms. Physical trauma results in the primary early mechanical damage of brain tissue (including hematomas, contusions, ischemia, axonal injury and diffuse swelling). Primary injury can initiate secondary

brain damage (from minutes to months after the trauma) including alteration in neurotransmitter release, calcium-mediated damage, mitochondrial dysfunction, oxidative stress, leading to cell death, tissue damage and atrophy [89, 168]. Neuronal death in TBI is widely attributed to the apoptotic process but several studies have also shown an increase of autophagy after TBI.

In light of the heterogeneity of the clinical aspects of TBI, different animal models (principally rodents) have been developed. Among these, fluid percussion injury (FPI), controlled cortical impact injury (CCI), weight drop-impact acceleration injury, and blast injury are widely used [17, 29, 30, 76, 80, 93].

The use of FPI and CCI rat models has allowed to observe that autophagy is persistently activated after TBI [82, 178]. Autophagosomes accumulate early after TBI (1–4 h) and activation of autophagy persists for days (15–32 days). Since oxygen radicals are involved in the pathogenesis of TBI and autophagy can be induced by mitochondrial oxidative stress, Lai and colleagues analyzed the effects of the antioxidant γ-glutamylcysteinyl ethyl ester (GCEE) on autophagy and neurologic outcome in CCI mouse model of TBI [69]. GCEE treatment decreases the oxidative stress in CCI mice and, more interesting, reduces autophagy levels after TBI, with an improvement of cognitive performance in Morris water maze test and a partial reduction of histological damage. These data suggest that oxidative stress is involved in the neuropathology of TBI and can influence autophagy activity after acute brain injury.

In addition to oxidative stress, glutamate excitotoxicity also plays an important role in TBI. Hyperactivation of NMDARs is associated with TBI-induced neuronal death and excitotoxicity has been linked to autopahgy. Interestingly, it has been demonstrated that NMDAR subunit GluN2A (and its signalling intermediates PSD-95, Homer and Shank) interacts with Beclin1 in membrane rafts of rat cerebral cortex neurons. FPI-induced TBI, in addition to increase the levels of GluN2A, causes a rapid redistribution of Beclin1 out of membrane rafts and activates autophagy pathway [6]. These data suggest that the release of Beclin1 (or PSD95/Shank/Homer/Beclin1) from the GluN2B multi-protein signalling complex in response to TBI-induced excessive stimulation of GluN2A could be a key event involved in the activation of neuronal autophagy.

Although several studies demonstrated that autophagy is activated in different TBI models, its role as a protective or detrimental process remains unclear. In order to investigate the function of autophagy after TBI, Luo and Colleagues tested the effects of autophagy inhibitors in weight-drop mouse model. They observed that the administration of autophagy inhibitors (3-MA and bafilomycin; single i.c.v. injection before TBI) blocks TBI-induced autophagy, attenuates TBI-induced cell death and brain lesions, and improves TBI-induced motor and learning deficits [85].

In conclusion, TBI causes pathophysiological responses that lead to oxidative stress, glutamate excitotoxicity, cell death, motor and cognitive outcome deficits. In this context, autophagy has been identified as part of the responses leading to cell injury after TBI and different compounds tested as neuroprotective in TBI have been shown to reduce apoptotic neuronal death as well as autophagic activity [25, 81, 161, 158, 176].

6.3 Spinal Cord Injury

Spinal cord injury (SCI) is a high-cost neurological disability that can leave the individual with severe life-lasting impairment affecting all organ systems, with strong impact on the patient, the family, health care service, and society. SCI refers to any injury, complete or incomplete, to the spinal cord causing different types of motor, sensory and sphincter dysfunction, as well as dystonia [10]. Extreme sports, high-speed transport, and traumatic accidents in general are linked with a particularly high incidence of SCI, which has an annual incidence of 50 individuals per million population with prevalence in young adults [120, 162].

SCI can be studied in mice and rats models, by means the hemisection of spinal cord at different vertebral levels. It has been observed that SCI causes a fast autophagy activation at the lesion site. Specifically, the upregulation of autophagic markers starts 4 h after the lesion, with a peak at 3 days, and lasts for 21 days [49, 53]. The observed persistent increase of key proteins involved in autophagic pathway in degenerating axons [127] suggests that autophagy might be involved in axonal degeneration following traumatic injury.

However, the role of autophagy in SCI needs to be further investigated in order to understand whether this process contributes to neuronal death or represents a neuroprotective response.

6.4 Remote Damage

Acute brain injury is characterized by two events: (1) early primary damage that directly causes cell death and degeneration, and (2) late secondary damage that induces delayed neurodegeneration through other mechanisms that are not limited to the lesion site but can involve remote areas. Remote neurodegeneration is a multifactorial phenomenon that develops days or months after acute damage and strongly affects the clinical outcome in many CNS disorders [156]. Axotomized neurons undergo a series of morphological changes before dying and the severity of remote cell death is associated to several factors, such as the type and extent of the primary lesion, the distance between axonal trauma and the soma, the type of connectivity, and the intrinsic vulnerability of the involved circuits [34].

Remote damage can be studied in animal models by means axotomy and target deprivation in order to analyze the morphological, biochemical, and ultrastructural changes that occur days to months after injury in different brain circuits. The hemicerebellectomy (HCb) is a widely used model to study the mechanisms of remote cell death. HCb consists in the ablation of half of the cerebellum, which leads, because of the crossed input–output cerebellar organization, to the damage of all neuronal axons of the contralateral inferior olive (IO) and pontine nuclei (Pn) and to deprivation of nearly all cerebellar input of the contralateral cerebral cortex [108, 156]. Recently, we analyzed autophagy function and kinetics during apoptotic cell

death in HCb-induced remote damage [155]. We demonstrated that acute brain lesions activate autophagy in axotomized neurons and that this event is subsequent to cytochrome c release from the mitochondria. Importantly, we showed that autophagy stimulation by the mTOR inhibitor rapamycin reduces neuronal death and improves functional recovery after HCb. By contrast, autophagy-impaired *Beclin1* heterozygous mice undergo a greater degeneration of axotomized neurons. These data suggest that, in remote damage induced by HCb, activation of autophagy in axotomized neurons acts as a reactive response that protects neurons by engulfing damaged mitochondria and neutralizing pro-apoptotic factors that can cause cell death [155]. In agreement with this hypothesis we recently observed that HCb alters mitochondrial dynamics (fusion/fission) balance in axotomized neurons and that the neuroprotective effect of rapamycin seems to be the result of a dual role: on one hand the stimulation of autophagy leads to damaged mitochondria removal and on the other hand the enhancement of mitochondria fission allows their elimination by mitophagy [14].

Conversely, in remote damage induced by corticovascular focal lesion autophagy activation seems to have detrimental effects. Focal cerebral infarction can be induced by distal MCAO and can cause secondary degeneration of thalamus and delay functional recovery. After the lesion, autophagy is activated in the ipsilateral thalamus and its inhibition (by using *Beclin1* KO and 3-MA treatment) results in a decrease of neuronal loss, gliosis and apoptosis [167]. These data suggest that the inhibition of autophagy can attenuate the secondary thalamic damage after focal cerebral infarction.

Although it is not possible to draw clear and general conclusions on the role of autophagy in remote damage, because of the different responses depending on the type of primary lesion, several lines of evidence implicate autophagy as a pathophysiological mechanism of remote damage and suggest that drugs targeting this process could be useful to reduce remote neurodegeneration.

7 Neurodegenerative Diseases

7.1 Alzheimer's Disease

Alzheimer's Disease (AD) is a progressive and irreversible age-related neurodegenerative disorder leading to cognitive, memory and behavioural impairments and represents the most cause of dementia worldwide. The main histopathological features of AD brains are the presence of extracellular senile plaques principally formed by the β-amyloid peptide (Aβ), intracellular neurofibrillary tangles constituted by hyperphosphorylated aggregates of the microtubule-associated protein tau, altered neuronal connectivity and massive neuronal loss principally in the hippocampus and cerebral cortex [15]. AD is primarily a sporadic pathology, with age as main risk factor; however, autosomal dominant familial forms are known (familial AD,

FAD). The first mutation causing FAD has been recognized in the amyloid precursor protein (APP) encoding-gene on chromosome 21 [40, 149]. Subsequently AD-related mutations in the presenilin 1 (*PSEN1*, on chromosome 14) and presenilin 2 (*PSEN2*, on chromosome 1) genes have also been identified [78, 142]. Pathogenic mutations in these three genes account for the majority of familial cases of early-onset AD and several mutations in the *APP*, *PSEN1* and *PSEN2* genes have been described worldwide. To note, the mutations causing early-onset autosomal dominat AD affect the metabolism and the stability of Aβ peptide, a proteolytic product of APP processing, that plays a crucial role in AD pathogenesis [138].

Aβ is generated in endo-lysosomal pathway and is present in autophagosomes and in lysosomes. The evidence that autophagic vacuoles (AVs) contain APP and are enriched in β- and γ-secretase activity (responsible for amyloidogenic APP processing with production of Aβ) implicates that AVs are active compartments for Aβ generation [172, 173] and a major source of intracellular Aβ in AD brain [172]. The evidence that autophagy is induced in AD comes out from the study of vulnerable neuronal populations before the extracellular deposition of Aβ in PS1/APP transgenic mouse model of AD. Moreover, dystrophic neurites strongly accumulate autophagosomes and other immature AVs [172], involving an impairment in the normal maturation of AVs to lysosomes and suggesting that a change in the autophagy rate or abnormal AVs accumulation in affected AD neurons can contribute to Aβ deposition. However, more recently it has been demonstrated that autophagy stimulation by rapamycin does not alter APP metabolism and Aβ secretion, suggesting that autophagy is not directly involved in APP metabolism [9]. Rather, it seems likely that the accumulation of APP C-terminal fragments (observable when lysosomal flux is impaired) is caused by a defect of endosome-derived APP or APP C-terminal fragments clearance rather than to an increase of newly generated autophagosomes. In addition to Aβ pathology involvement, autophagy-lysosomal system can also degrade both soluble tau and tau aggregates and inhibition of this mechanism leads to enhanced tau aggregation and cytotoxicity [160]. The involvement of autophagy in the pathogenesis of the two hallmarks of AD brain, amyloid plaques and neurofibrillary tangles, is confirmed by the observation that the pharmacological restoring of mTOR signalling with rapamycin rescues cognitive deficits and ameliorates Aβ and tau pathology by increasing autophagy in the 3xTg-AD mouse model of AD [12].

A role of autophagy in AD pathogenesis is also suggested by the evidence that Beclin1 levels are decreased in affected brain regions of AD patients in early stage of the disease [119]. However, in two different lines of APP transgenic mice (J20 and T41 mouse models of AD) the levels of Beclin1 are not reduced at old age, while Beclin1 deficiency (*Beclin1* heterozygous mice) promotes extracellular and intraneuronal Aβ deposition in APP transgenic mice. These data suggest that the overproduction of mutant APP and the development of amyloid pathology is not sufficient to reduce Beclin1 expression in mice and that the reduction of Beclin1 observed in AD brains likely occurs upstream of APP pathology [119]. The enhancement of autophagic protein turnover rate and lysosomal cathepsin activities in TgCRND8 mouse model (by genetic deletion of cystatin B, an inhibitor of lyso-

somal cysteine proteases) rescues autophagic-lysosomal pathology and the abnormal accumulation of Aβ, ubiquitinated proteins and other autophagic substrates. The improvement of lysosomal function in this model reduces intraneuronal levels of Aβ as well as extracellular amyloid deposition, and prevents learning and memory deficits [170].

In addition to its role in the clearance of Aβ and tau in AD pathology, autophagy is also involved in the removal of damaged organelles. Mitochondrial abnormalities correlate with dystrophic neurites, dendritic branches loss and dendritic spines pathological alteration present in AD brains [2]. Several line of evidence demonstrate that Aβ damages mitochondria and the reduction of lysosomal degradative efficiency can limit mitochondrial recycling. The impairment of autophagy in aging cells and the positive correlation between Aβ content and mitochondrial damage suggest that mitochondrial turnover could progressively decline with age, with consequent increase of oxidative damage, accumulation of dysfunctional mitochondria and finally cell death [16]. The literature about AD and mitophagy is not very abundant but several evidences, including the impairment of mitochondrial fission/fusion events which are involved in mitochondrial elimination by autophagy, imply that an inefficient lysosomal system may compromise the elimination of damaged mitochondria in AD [134, 159].

7.2 Parkinson's Disease

Parkinson's Disease (PD) is the second most common neurodegenerative disorder characterized by bradykinesia, resting tremor, rigidity, and postural instability. The movement deficits principally result from the massive and selective degeneration of nigrostriatal dopaminergic neurons with consequent striatal dopamine deficiency. Another pathological feature of PD brains is the abnormal accumulation of fibrillar α-synuclein protein leading to the formation of intracellular insoluble inclusions (Lewy bodies) and degenerating ubiquitin-positive neuronal processes (Lewy neurites) in surviving neurons [36, 109]. Currently there is no convincing therapy to block or slowdown neuronal loss but only symptomatic treatments are available. Indeed, dopaminergic deficit can be temporarily compensated with deep brain stimulation and by treatment with dopamine agonists, dopamine precursor L-dopa, monoamine oxidase B (MAO-B) and catechol-o-methyltransferase (COMT) inhibitors [21]. PD incidence markedly increases with age although a rare young-onset PD occurring before age 40 exists. PD is principally a sporadic disease (90 % of cases) but several PD-related genes have been identified in a subset of familial forms of the disorder. Genetic transmission can be autosomal dominant – mutations in the genes encoding for α-synuclein and LRRK2 (leucine-rich repeat kinase) – or autosomal recessive – mutations in the genes encoding for PINK1 (PTEN-induced putative kinase 1), parkin and DJ-1 [109].

Aging, genetic factors, and environmental exposure to pesticides and heavy metals are implicated in PD pathogenesis, and mitochondrial dysfunction and oxi-

dative stress have long been associated to PD. Mitochondrial quality control ensures the functionality of the mitochondria during cell life and the deterioration of cellular mechanisms involved in mitochondria turnover has been hypothesized to underlie the pathogenesis of several neurodegenerative diseases, in particular PD [150]. Notably, among the genes related to familial forms of PD, there are two genes encoding for proteins involved in mitochondrial quality control. PINK1 is a serine/threonine kinase normally present at very low levels in healthy polarized mitochondria; however, when mitochondria are depolarized, full-length PINK1 accumulates rapidly at damaged organelles, and recruits parkin from the cytosol to the mitochondria. Parkin is an E3 ubiquitin ligase that catalyzes the polyubiquitination of several substrates, including the mitochondrial proteins Mfn1 (mitofusin 1) and VDAC1 (voltage-dependent anion channel 1), and triggers mitochondrial engulfment by autophagosomes and subsequent degradation through mitophagy [38, 39, 96, 111]. Interestingly, another protein linked to recessive juvenile parkinsonism, Fbxo7, is involved in mitochondrial maintenance regulating PINK1/parkin-mediated mitophagy. Fbxo7, in fact, participates in parkin recruitment to damaged mitochondria and Mfn1 ubiquitination [11, 179]. Rare cases of autosomal recessive PD are caused by loss-of-function mutations in the gene encoding for DJ-1, an ubiquitous redox-responsive cytoprotective protein. In addition to antioxidant effects, DJ-1 also regulates autophagy and contributes to the maintenance of mitochondrial function. In fact, the loss of DJ-1, leads to mitochondrial membrane potential reduction, mitochondrial fragmentation, and autophagic markers accumulation [50, 97]. Collectively, these observations implicate the failure of damaged mitochondria removal through mitophagy as a contributing factor in PD pathogenesis.

Aberrant accumulation of α-synuclein, the major protein in Lewy bodies, is associated with PD pathogenesis and mutations in α-synuclein cause early-onset PD. In inducible α-synuclein-overexpressing cell line it has been demonstrated that α-synuclein can be degraded either by ubiquitin/proteasome system and autophagy pathway. In this *in vitro* model, in fact, α-synuclein is presents in structures with the morphological features of autophagic vesicles and stimulation of autophagy by rapamycin increases its clearance [163]. Afterwards, it has been demonstrated that wild-type α-synuclein is internalized and degraded in lysosomes by CMA, and that mutant forms of this protein (both A30P and A53T mutant) bound specific receptors on lysosomes membrane stronger than wild-type but are poorly internalized [24]. Thus, wild-type α-synuclein seems to be efficiently degraded via CMA, whereas the degradation of mutant α-synuclein is impaired. The blockade of CMA then results in a compensatory activation of macroautophagy, which fails to maintain normal rates of protein degradation [24]. Several studies support an involvement of proteasomal, lysosomal and autophagic pathways in PD, although there is no general consensus about the main pathway responsible for α-synuclein degradation. However, it seems that all the three proteolytic pathways are involved in α-synuclein clearance [5, 24, 163]. Numerous studies have suggested a link between LRRK2 (leucine-rich repeat kinase 2), an important genetic contributor to PD, and aberrant autophagy. LRRK2 is associated with late-onset PD displaying variable pathology depending

on the type of mutation [92, 95, 175]. Mutated LRRK2 has been shown to induce or inhibit autophagy depending on specific mutation or cell type [130, 133].

In conclusion, mounting evidence implicates dysfunctional autophagy and mitophagy in PD pathogenesis. Many gene mutations causing familial PD have been identified and many of these alter autophagy. In addition, PD toxins (as MPP+, rotenone, 6-hydroxydopamine, and paraquat) also deregulate autophagy [26], highlighting the importance of this process in PD pathogenesis.

7.3 Hungtinton's Disease

Huntington's disease (HD) is an autosomal dominant inherited neurodegenerative disorder characterized by abnormal repetitive movements called chorea, progressive dementia and psychiatric manifestations. The main pathological alteration in HD brains is the selective neuron loss occurring in the striatum and cortex. The onset of symptoms is typically in the middle-age but the disorder can manifest at any time. HD causes death 15–20 years after the onset of neurological manifestations, and although there are treatments that can help to control choreiform movements, successful therapy to block disease progression does not exist [157]. The mutant protein in HD results from an abnormal expansion of the trinucleotide sequence CAG in the first exon of *huntingtin* gene, resulting in an excessive extension of variable length of the polyglutamine (polyQ) tract at the N-terminus of huntingtin (Htt) protein with a toxic gain of function. The normal allele contains a repeated sequence of 10–35 CAG triplets. The expansion of this CAG repeat over 35 is linked with the development of disease, and the length of polyQ tract is inversely correlated with the age-onset of HD [67, 129]. The production and accumulation of misfolded Htt cause the formation of inclusion bodies in HD brain leading to the selective loss of striatal GABAergic neurons.

Different mechanisms have been suggested to contribute to the pathogenesis of HD, including excitotoxic injury, oxidative stress, mitochondrial dysfunction, and apoptosis. As autophagy is primarily responsible for maintaining normal cellular protein homeostasis in the CNS and HD is a neurodegenerative proteinopathy (as other neurodegenerative disease such as AD and PD), it is not surprising that autophagy has attracted the interest of scholars in the field. Several lines of research have reported autophagy dysfunction in HD and have shown that autophagy modulation could represent a potential therapeutic intervention.

Increased number of AVs has been reported in human HD samples and in different experimental models. Initially it has been observed that Htt abnormally accumulates in punctate cytoplasmic structures resembling endosomal-lysosomal organelles in HD brains [135] and that wild-type and mutant Htt associate with endosomes in human-derived primary fibroblasts [154]. Then, in clonal mouse striatal cell line transiently transfected with human Htt it has been demonstrated that both wild-type and mutant Htt accumulate in the cytoplasm forming vacuoles [57], which incorporate the lysosomal enzyme cathepsin D in proportion to polyQ length and have the

ultrastructural features of autophagosome [55]. Primary striatal neurons from transgenic HD mice (R6/2 mouse model expressing exon one of the human Htt gene carrying a CAG repeat expansion) exposed to neurotoxic concentration of dopamine not only present increased cell death compared to wild-type neurons, but also exhibit lysosome-associated responses with the induction of autophagic granules and electron-dense lysosomes [118].

Mutant Htt can sequester and inactivate mTOR, thus promoting autophagy in HD brain [126] and accumulation of the autophagic markers p62 and LC3 in the striatum of transgenic mouse models of HD [71]. Therefore, several studies suggest that dysfunctional autophagy can be involved in HD pathogenesis. However, the increase of autophagosomes in cellular and mouse model of HD is not accompanied by an increase of autophagic substrates degradation although autophagic flux does not appear to be affected [94]. In fact, in HD cells AVs form with normal or enhanced rates and are effectively eliminated by lysosomes but there is a defect in autophagic cargo recognition with the formation of "empty" autophagosome. As a result, despite an increase in the initiation of autophagy and the formation of AVs, aggregated proteins (including Htt) and damaged mitochondria are not degraded and accumulate in the cytoplasm contributing to toxicity [94].

Many cell types respond to autophagy blockade by upregulating CMA [54] and CMA results increased in cellular and mouse models of HD [62]. Htt has two putative KFERQ-like CMA-targeting motifs; the pentapeptide motif KFERQ is recognized by cytosolic chaperone Hsc70 that targets the substrates to lysosomes, where they are bound by the lysosome-associated membrane protein type 2A (LAMP-2A), which mediates substrate translocation into the lysosomal lumen [23]. Notably, CMA-dependent degradation of Htt fragments is less efficient for polyQ-expanded Htt fragments, perhaps because polyQ expansion in mutant Htt delays the transport across the lysosomal membrane, with consequent accumulation into the cytosol [62, 122]. CMA targets selectively N-terminal fragments of Htt (while full-length polyQ-Htt is primarily targeted by autophagy), and specific targeting of N-terminal polyQ-Htt fragment to the CMA pathway strongly reduces the formation of cytosolic inclusion and improves HD phenotypes in R6/2 mice [3].

As rapamycin treatment reduces aggregate formation and cell death in cells expressing mutant Htt [125], small molecules which enhance autophagy are able to reduce toxicity in HD models [137], and several mTOR-independent autophagy inducers increase the clearance of mutant Htt and reduce its toxicity [136], the manipulation of autophagy and CMA could represent a promising candidate for therapy development.

8 Conclusions

In this Chapter we have described autophagic mechanism and its fundamental role in neuronal function. We have highlighted that autophagy plays a key role in the physiology of the central nervous system, not only as quality control mechanism but

also for brain development and synaptic function. The emerging evidences that autophagy defects are involved in common neurodegenerative diseases and that acute brain injuries are characterized by strong autophagic responses encourage to unravel the role of autophagy in brain diseases and open the possibility of future therapeutic approaches.

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