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Autophagy in axonal and dendritic degeneration

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Abstract

Axonal and dendritic degeneration is a common, early pathological feature of many neurodegenerative disorders and thought to be regulated by mechanisms distinct from that of the soma death. The unique structures of axons and dendrites (collectively neurites) may cause them to be particularly vulnerable to the accumulation of protein aggregates and damaged organelles. Autophagy is a known catabolic mechanism whereby cells clear protein aggregates and damaged organelles. Basal autophagy occurs continuously as a housekeeping function, and can be acutely expanded in response to various stress or injuries. Emerging evidence shows that insufficient or excessive autophagy contributes to neuritic degeneration. Here, we review the recent progress that has begun to reveal the role of autophagy in neuritic functions and degeneration.

Keywords

autophagy; axon; dendrite; neurodegenerative diseases

Introduction

Neurons comprise the soma, axon and dendrites. The latter two are collectively known as neurites, in which molecules including proteins are synthesized, delivered, and degraded for their dynamic functions in synaptic growth and activity. Upon certain stimuli, the axons and dendrites of cultured neurons exhibit features of degeneration, including terminal degradation, neuritic retraction, synaptic pathology, and marked focal beading or swelling. The term ‘dystrophic neurites’ is used to describe these processes, which is analogous to neurite dystrophy *in vivo*. In neurodegenerative disorders, axons, dendrites and synapses often degenerate prior to the loss of the cell bodies, which may contribute to pre-clinical episodes of disease process and eventual clinical symptoms [1, 2].

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Autophagy is a cell self-digesting system that is responsible for the clearance of long-lived proteins and damaged organelles by the lysosome. A number of evolutionarily conserved gene products, known as the Atg proteins, are required for autophagosome (autophagic vacuoles) synthesis, maturation, trafficking and clearance [3]. Several functionally distinct groups are responsible for autophagy execution, including the Ulk1-Atg13-FIP200 kinase complex, the class III PI3K complex containing the core proteins Vps34, p150 and Beclin 1, the lipid-binding Atg18 homologs, the multi-spanning transmembrane protein Atg9, the Atg12 conjugation system and the ubiquitin-like LC3 conjugation system [3]. Examples of upstream signaling molecules that regulate autophagic activity include the mammalian target of rapamycin (mTOR), a Ser/Thr kinase which prevents autophagy induction through Ulk1 inactivation, and AMP-activated protein kinase (AMPK), an energy-sensing kinase that promotes autophagy by inducing Ulk1 activation [4]. In fact, pharmacological induction of autophagy can be achieved using rapamycin, which inhibits mTOR. In addition, class III PI3K-Beclin 1 complex controls the nucleation process of the phagophore, a precursor of autophagosome. 3-methyladenine (3MA) or wortmannin, which inhibits class III PI3K activity, is often used to block autophagy.

Emerging evidence demonstrates a critical role of macroautophagy (hereafter referred to as “autophagy”) in the maintenance of protein and organelle homeostasis in the axons and protection of axons. Dysfunctional autophagy has been observed in experimental neuritic degeneration in both primary neuronal cultures [5–7] and *in vivo* animal models [8–13]. Insufficient autophagic clearance and perhaps excessive autophagy induction, characterized by the accumulations of autophagy-related vesicular compartments, are also observed in degenerating neurites of brain specimens from patients with several neurodegenerative diseases, including Alzheimer’s disease (AD) [14–17], Parkinson’s disease (PD) [18, 19] and Huntington’s disease (HD) [20]. A growing body of evidence shows the indispensible role of constitutive autophagy in preventing neuritic degeneration, whereas imbalanced induction of autophagy can contribute to neuritic degeneration. This review focuses on current progress that has begun to reveal autophagic processes in the axons and dendrites. The roles for autophagy in neuronal death and neurological disorders, and the potential molecular mechanism for the pathogenic function of autophagy, have been recently reviewed [21–23].

Biogenesis and fate of autophagosomes in neurites

Biogenesis of autophagosomes

Although there has been limited investigation of autophagosome (see Glossary) synthesis in neurons, accumulation of autophagosome-like vacuoles has frequently been observed in dystrophic axon terminals [24, 25] and axonal swellings [8, 14, 17, 26, 27] of affected neurons in diverse injury models. Time-lapse confocal imaging of cultured cerebellar granule neurons shows that vesicles labeled with green fluorescent protein-tagged microtubule-associated protein light chain 3 (GFP-LC3) travel along neurites in a retrograde direction [28], raising the possibility that autophagosomes may arise in the distal tips of axons and transport their engulfed cargo toward the soma through retrograde transport [29] (Figure 1a). The cargo may include soluble or aggregated proteins, mitochondria and possibly even nerve growth factor (NGF) [29, 30]. Indeed, a recent live-imaging study demonstrates that autophagosome initiation is a constitutive and spatially restricted process in the distal ends of *in vitro* cultured dorsal root ganglion (DRG) neurons [30]. In addition, *de novo* formation of autophagosomes is also detected in cell bodies of primary cortical neurons [31]. However, it remains unclear whether autophagosome biogenesis occurs along the axons. The origin of the membrane also remains to be established but limited evidence may suggest that autophagosomes in the axons are originated by budding from existing vesicles [31].

Maturation of autophagosomes

Autophagosome maturation requires fusion of the autophagosomes with late endosomes and/or lysosome, which provide an acidic environment and digestive function to the interior of the autophagosomes [32]. In the past, two major approaches have been applied to monitor the maturation of autophagosomes, both of which are effective for analyzing autophagosome maturation in living neurites. First neurons have been labeled with an autophagosome marker (e.g. LC3 [33]) and a lysosome/endosome marker, such as the late endosome/lysosome marker lysosomalassociated membrane protein 1 (LAMP1) [30, 31], Rab7 [31], LysoTracker [30, 34] and lysosome-associated membrane glycoprotein 1 (Lgp120) [34], followed by analysis of co-localization or comigration. Alternatively, dual-color fluorescent LC3 reporters have been used; these fluoresce in different colors depending on the pH. Examples of these are: mRFP-GFP tandem fluorescent-tagged LC3 (tfLC3) [35], mCherry-EGFP-LC3 [36] and a recently reported mTagRFP-mWasabi-LC3 [37]. GFP does not fluoresce under acidic and degradative conditions whereas mRFP or mCherry do. mCherry-EGFP-LC3-labeled DRG neurons show a reducing gradient of autophagosomes positive for both mCherry and EGFP between distal and proximal axon regions, suggesting that autophagosomes are transported to proximal axons and become fully acidified [30]. Thus, following synthesis, autophagosomes exit from the distal pool, become acidified, and initiate retrograde transport along the axons [28, 30, 31] (Figure 1a).

Autophagosomes mature to autolysosomes after fusion with late endosomes and lysosomes during their axonal transport [31], and an impairment of autophagosome maturation could alter their transport properties and favor autophagosome accumulation (Figure 1b). Once fully acidified in the proximal part of the axons, autophagosomes may switch to bidirectional motility resembling that of lysosomes [30, 34] and initiate the proteolytic clearance of the autophagy substrates [31] (Figure 1a). In contrast to a number of studies of the biogenesis and maturation of autophagosomes in axons, no report has described the similar process in dendrites.

Dynamics of autophagosomes and autolysosomes in neurites

Microtubule-dependent transport

Microtubules are involved in autophagosome formation and appear to facilitate targeting and fusion of autophagosomes to lysosomes [38], although some of these conclusions have been questioned and the precise mechanism remains unclear [39]. MAP1B plays an essential role in regulating microtubule stability in the axons. Thus LC3-MAP1B interaction may regulate autophagosomes in an axon-specific, microtubule-dependent manner [8, 28] (Figure 1a). The administration of nocodazole, a microtubule disruption agent, clearly reduced the activity of autolysosomes in PC12 neurites under starvation conditions [34]. In accordance with the results, a recent report shows that microtubule cytoskeleton and molecular motor defects are the early pathogenic events in the PS1/APP mouse amyloid model, which may lead to transport abnormalities, local accumulation of autophagosomes and promote neuritic dystrophy [40]. It is well known that axons of vertebrate neurons display uniformly plus-enddistal microtubules, whereas their dendrites display non-uniformly oriented microtubules [41]. Although axons and dendrites differ in the arrangement of microtubules, it remains unclear whether there is any difference in the autophagosome dynamics between the two different compartments.

Dynein-dependent retrograde transport

Approximately all moving autophagosomes transport retrogradely along the axons [28, 31]. Increased retrograde transport of autophagic vacuoles has been previously observed when axonal elongation is blocked [42]. In cultured neurons, autophagosomes transport along the

axons with a retrograde velocity ranged from 0.25 to 0.45 μm/s during the observation period [30, 31, 43]. The retrograde transport kinetics of autophagosomes in the axons differs from that of endocytic vesicles, which show faster retrograde velocity (0.2 to 1.2 μm/s) than autophagosomes [44]. Dynein is the major minus-end motor that transports various cargos on microtubules (Figure 1a, b). Expression of a fluorescently tagged subunit of dynein in GFP-LC3 labeled neurons reveals the co-migration of dynein with GFP-LC3 puncta in the axons [30]. Such retrograde movement can be largely arrested by dynein motor inhibitor EHNA [43] or by expression of the dominant-negative dynein inhibitor CC1, which blocks the dynein-dynactin interaction [30]. Likewise, EHNA dramatically reduced the percentage of retrogradely transporting autolysosomes in PC12 neurites [34]. The study of dynein mutants links motor neuron degeneration to the defects in retrograde transport [45]. Disruption of the dynein-dynactin interaction inhibits axonal transport in motor neurons and causes late onset degeneration [46], implicating dysfunctional dynein/dynactin complex in autophagy dysregulation in neurodegenerative disease, such as amyotrophic lateral sclerosis (ALS) [47].

Kinesin-dependent anterograde transport

Kinesin-1, a plus-end directed motor on microtubules, is essential for anterograde neuritic transport (Figure 1a, b). Reduction of kinesin-1 promotes Aβ generation and its intraneuronal accumulation as well as the formation of axonal swelling in a mouse amyloid model [48]. Treatment of kinesin inhibitor AMP-PNP or knock-down of kinesin light chains KLC1 or KLC2 suppresses anterograde transport of autolysosomes in PC12 neurites [34], suggesting that the kinesin motors regulate the anterograde movement of autolysosomes in neurites. Furthermore, kinesin-1 and kinesin-2 motors are copurified with isolated autophagosomes [30]. However, these results cannot exclude the possibility that the copurification of autophagosomes and kinesin motors is due to the engulfed fraction of motor proteins in autophagosomes.

Autophagy dysfunction is a common pathological event in diverse neuritic degeneration

Terminal degradation

Growth cone collapse and degeneration of the terminal regions of long axons are recognized as the initial events in “dying back” degeneration, which is the most common pathology seen in peripheral nerve diseases caused by a wide variety of toxic, metabolic, and infectious insults [2, 49]. Our previous study shows that 3-MA prevents terminal neurites of sympathetic neurons from degeneration induced by zinc depletion [50]. Moreover, local protein degradation and synthesis are required for growth cone initiation and axonal regeneration following injury [51]. Increased number of autophagic vacuoles has been found in the axon initial segment of axotomised neurons [52], implicating autophagy in terminal regeneration through local protein degradation (Figure 2a). Nevertheless, the role of autophagy in terminal regeneration following injury remains speculative. The direct evidence that the autophagy-mediated degradation contributes to the onset of the terminal regeneration has yet to be shown. It is worth mentioning that axon terminal dystrophy of Purkinje cells deficient in *atg7* proceeds with little sign of dendritic atrophy, suggesting that axon terminals are much more vulnerable to autophagy impairment than the dendrites in this particular neuron type [25].

Neuritic retraction

Neuritic retraction, or shortening, is a progressive reduction in axonal and dendritic length. It represents a common hallmark of neurodegenerative diseases. Although little is known

about the mechanism of neuritic retraction, several extrinsic and intrinsic cues are suggested to actively signal axonal and dendritic retraction [49, 53]. Dendritic retraction and shrinkage in Purkinje cell degeneration (*pcd*) mice [9] or axonal dystrophy in *Lurcher* mice [8] each precedes Purkinje cell loss. The dendritic and axonal degeneration of Purkinje cells in both models is associated with the increased activity of autophagy pathway [8, 9] (Figure 2a). In *Lurcher* mice, the induction of autophagy is an early stress response in axonal dystrophy and may participate in the remodeling of axon structures. In *pcd* mice, aberrant autophagymediated mitochondria clearance (mitophagy) contributes to the Purkinje cell degeneration and dendritic retraction. Cultured cortical neurons overexpressing G2019S mutant of *l*eucine-*r*ich *r*epeat *k*inase 2 (LRRK2) displayed dendritic retraction due to calcium dysregulation and induction of dendritic mitophagy [54, 55]. Furthermore, autophagy inhibition prevents the neuritic retraction, whereas autophagy stimulation potentiates LRRK2-induced neuritic shortening in SH-SY5Y cells [56]. The data suggest that excessive mitochondrial removal could be one of the contributing factors for dendritic retraction (Figure 2a). Nonetheless, the involvement of mitophagy in axonal dystrophy remains to be further clarified.

Synaptic pathology

Synaptic pathology, accompanied by abnormal accumulation of autophagosomes, is found in the hippocampus of young Alzheimer's model mice [40]. In G2019S LRRK2 transgenic mice, an animal model for PD, autophagosomes appear in synaptic terminal in cerebral cortex at advanced age (17–18 months) [57]. Synaptic loss is an early and major feature of the brain pathology induced by prion diseases [58]. Prion infection induced accumulation of autophagosomes within synaptic terminals in various brain regions of infected hamsters and mice [59]. In brain biopsies from the patients with prion diseases, autophagosomes are also found within damaged synapses [27]. The evidence links dysfunctional autophagy and synaptic pathology.

A recent report reveals that induction of autophagy by rapamycin elevated the number of autophagosomes in prejunctional dopaminergic axons, concomitant with decreased axonal profile volumes, synaptic vesicle numbers, and evoked dopamine release, suggesting the critical role of autophagy in regulation of presynaptic structure and neurotransmission [60]. In addition, autophagosomes are located in the postsynaptic cells that selectively degrade cell surface receptors, such as $GABA_A$ receptor in *C. elegans* [61]. In *Drosophila*, autophagy and ubiquitin-proteasome systems converge to regulate synaptic development [62]. Moreover, decreased autophagic activity is associated with reduced number of neuromuscular junction synapses in *Drosophila dynein light chain 1* (*ddlc1*) mutant [63]. The neuron-specific synaptic v-soluble NSF attachment protein receptor (v-SNARE) n-*syb* (*neuronal Synaptobrevin*) plays an important role during synaptic vesicle exocytosis. Increased autophagy might represent a secondary response to a primary vesicle trafficking defect downstream of endocytosis in *Drosophila* loss of n-*syb* [64]. Hence, autophagy may contribute to the clearance of the synaptic proteins and receptors and thus involved in the pathology of synapses (Figure 2b).

Beading and swelling formation

Beadings and swellings are thought to be the early features of axonal or dendritic degeneration, often associated with jamming of intracellular organelles [65–67] and accumulation of autophagosomes [8, 68] (Figure 2c). Methamphetamine (METH) selectively elicits pathogenic effect in the neurites of dopamine neurons without inducing cell death. METH was reported to promote the formation of autophagosomes, particularly in neuritic swellings and, ultimately, within cell bodies of dopaminergic neurons [5]. The disruption of organelle trafficking induced by lysosomal proteolysis inhibition caused

autophagosomes to accumulate in axons, especially within focal swellings [31]. Lee et al. detected the cathepsin-containing vesicular organelles were sequestered in axonal swellings during lysosomal proteolysis disruption [31]. In addition, nonvesicular axonal constituents, such as mitochondria, ubiquitin and phosphorylated neurofilaments, were also enriched in swellings with LC3 accumulation [31, 48]. Despite the above observation, the pathological significance of beading and swelling formation is poorly understood. It may result from neuritic transport failure of organelles, or from a failure of autophagy in the axons because ablation of *atg7* specifically in Purkinje cells initially induces the axonal swellings [25].

Autophagy in neurite protection and degeneration

Autophagy induction in diverse experimental models of neurite injury

A variety of experimental injury models have been applied to study the mechanism of axonal and dendritic degeneration *in vitro* (Table 1). Induction of autophagy, characterized by an increase in autophagosome synthesis, is detected in dopaminergic axons in anterior axotomy and posterior axotomy models [69]. Conditional deletion of the essential autophagy gene *atg7* in adult mice achieves striking axon protection in this acute model of retrograde degeneration [69], suggesting that autophagy may in fact contribute to axonal degeneration. The exact mechanism whereby autophagy is involved in axonopathy is unclear. In rats with root avulsion or distal axotomy, autophagy is initiated in motor neurons independently of the distance and severity of the lesion [13]. Administration of 3-MA or downregulation of *atg7* prevents neuritic degeneration of cultured SCG neurons following the transection [68]. Enhanced autophagic activity was also described in various types of neurons during neuritic degeneration induced by pharmacological toxins, including METH [5], zinc depletion [50], NMDA [6], serum deprivation [34, 70] or growth factor withdrawal [68]. A similar pattern of neuritic dystrophy accompanied by autophagy activation is observed when lysosomal degradation is inhibited by deleting one or more cathepsins [71] or by using cysteine protease inhibitors or general lysosomal enzyme inhibitors [72, 73].

Given that protein synthetic and degradative machineries may vary between different neuron types in response to insults [51], it is plausible that autophagy may play differential roles in axonal and dendritic degeneration under different experimental paradigms.

Compartmentalized regulation of autophagy - an example of RGC neuron

Axons and dendrites may also differ from the soma in their autophagy response to various insults. Autophagy inhibition by 3-MA potentiates the harmful effect of axotomy in cultured RGCs, and thus, autophagy induction may serve as a survival mechanism in this model [74]. In addition, a recent report reveals that autophagy promotes the survival of RGCs after optic nerve axotomy in mice [75]. Genetic inhibition of autophagy, for example, knockout of *atg4B* in mice or specific deletion of *atg5* in RGCs, reduces cell survival following optic nerve transection, while pharmacological induction of autophagy by rapamycin *in vivo* increases the number of surviving cells [75]. Therefore, autophagy may supply energy by self-digestion when the neurons re-program their mode to initiate regeneration. Conversely, an *in vivo* study shows that blocking autophagic activity with 3-MA results in slower disintegration of RGC axons compared to the control within 360 min postcrush [12]. It appears that, in some scenarios, axon degeneration is a self-destructive program, which has a distinct mechanism from the cell body death [2, 67]. Emerging evidence suggests that molecularly distinct degenerative pathways underlie the destruction of RGC soma and axons [76]. It remains possible that autophagy may participate in a compartmentalized program that regulates axonal or dendritic degeneration distinct from that of the soma.

Distinct role of autophagy in neuritic degeneration associated with neurological disorders

The available evidence that autophagy acts in both pro-survival and pro-death pathways suggest that autophagy is a double-edged sword in health and disease [77]. The appearance of autophagic structures in dying neurites has led to the initial hypothesis that autophagy play a causative role in neurite damage or degeneration induced by acute or chronic injuries, while recent evidence shows protective autophagy as a homeostatic response of neurites required to preserve their integrity. Despite the increase in autophagosome and autolysosome number widely observed in various disease brains, the precise role of autophagy in regulating neuritic degeneration remains unclear (Table 2). Disease-relevant cell and animal models are important for the dissection of the molecular mechanism underlying the exact function of autophagy in neuritic degeneration in the neurological disorders.

In LRRK2 G2019S-transefected SH-SY5Y cells, it was suggested that autophagy contributed to the progression of neuritic degeneration [56]. Knockdown of *lc3* or *atg7* reverses the toxic effects of LRRK2 G2019S expression in neuritic length, whereas rapamycin potentiates these effects [56]. Similarly, in deep cerebellar nuclei (DCN) of *pcd* mutant mice, an animal model of inherited ataxias, Purkinje cell axons frequently contain autophagosomes [9]. In addition, treatment of rapamycin accelerates the motor neuron degeneration and shortens the life span of the ALS mice [11]. The above evidence therefore suggests that aberrantly hyperactive autophagy may lead to accelerated neuritic degeneration under specific conditions.

On the other hand, autophagy may act as a quality control pathway that prevents neuritic degeneration in neurodegenerative disorders. The autophagy-lysosome system clears disease-related proteins particularly in the form of aggregates or inclusions, such as APP in AD [31], α-synuclein in PD [18, 19], and N-terminal fragments of huntingtin (htt) in HD [78]. Genetic disruption of autophagy specifically in midbrain dopaminergic neurons results in the axonal and dendritic degeneration, the presynaptic accumulation of α-synuclein and LRRK2, and the formation of ubiquitinated protein aggregates, recapitulating some of the pathologic features of PD [79, 80]. Moreover, conditional knockout of *atg7* in mouse forebrain neurons is sufficient to promote the development of spontaneous seizures [81]. Therefore, basal autophagy is neuroprotective by constant removal of ubiquitinated or aggregated proteins.

Dysfunctional neuritic mitophagy in neurological disorders

Injured mitochondria with depolarized membrane potential either occurring spontaneously or induced by pro-death signals can be targeted for selective elimination by autophagic processes [82]. In the axons of DRG neurons, approximately 90% of the depolarized mitochondria with high potential were transported towards the growth cone, while about 80% of the depolarized mitochondria with low potential were transported towards the cell body [83]. Emerging evidence has implicated dysfunctional mitophagy in the pathogenesis of AD and PD [84, 85]. For instance, several studies show the importance of mitophagy in the clearance of dysfunctional mitochondria during neuritic remodeling. A number of molecules, including Atg32, NIP3-like protein X (NIX), phosphatase and tensin homolog (PTEN)-induced putative kinase 1 (PINK1) and parkin, participate in mitophagy [86] (Figure 3). Parkin was shown to mediate Beclin 1-dependent autophagic clearance of defective mitochondria and ubiquitinated amyloid β peptide (Aβ) in AD models [87]. Moreover, compared to mitophagy in non-neuronal cells, neuronal mitophagy is a slower process that may be compartmentally restricted or coupled with reduced anterograde mitochondrial transport [88]. Parkin-targeted mitochondria accumulate in the somatodendritic regions of cortical neurons where mature lysosomes are predominantly

located [88]. In cortical neurons, overexpression of PD-linked LRRK2 mutants caused shortening dendrites, concomitant with enhanced mitophagy likely due to imbalanced calcium homeostasis [54, 55]. The evidence for axonal mitophagy was also reported. For example, in DRG neurons, autophagosomes move along axons toward the cell body carrying engulfed mitochondria cargo, although autophagosomes positive for mitochondrial fragments are scarce under basal condition [30]. Additionally, overexpression of PINK1 and Parkin was shown to decrease mitochondrial movement in rat hippocampal axons [89]. Despite the available evidence, the exact role of mitophagy in regulating the compartmentalized degeneration and regeneration of neurons requires further investigation.

Potential molecular mechanisms underlying autophagy-regulated neuritic degeneration

Neuritic degeneration has been recognized as a self-destructive program distinct from the soma apoptosis [2]. Current evidence shows that autophagy acts as a downstream target of calcium influx following mechanical axonal damage [12] (Figure 3). In non-neural cells, calpain mediates the cleavage of Atg5, which is required for the formation of autophagosomes, and subsequently provokes apoptotic cell death [90]. However, further study is needed to clarify whether Atg5 cleavage by calpain activation occurs in, and contributes to, neuritic degeneration.

Although caspase activation may contribute to the cell body death, some types of neuritic degeneration do not involve local caspase activation because caspase inhibitors fail to block neuritic degeneration induced by various insults [68, 91]. However, in PS/APP mouse brain, impairment of lysosomal proteolysis by leupeptin enhances caspase-3 immunolabeling within autophagosomes of dystrophic neurites [72]. Hence, we propose that the absence of apoptotic activation in dystrophic axons and dendrites may reflect autophagic removal of local caspases *via* retrograde transport. In the spinal cord motor neurons of ALS model mice, rapamycin elevates the caspase-3 activity and induces the transfer of cleaved capase-3 into the nucleus [11]. Compared to the controls, rapamycin-treated ALS mice exhibit an elevated ratio of Bax/Bcl-2 (a marker of apoptosis activation), implying that autophagy-triggered apoptotic pathway may contribute to the motor neuron degeneration in ALS mice [11].

Mitogen activated protein kinase/extracellular signal regulated protein kinase (MAPK/ERK) kinase (MEK) pathway is thought to play crucial roles in regulating axonal degeneration [92]. Pharmacological inhibition of MEK pathway by U0126 reverts the protective effect of proteasome inhibition on axonal degeneration [92]. In contrast, U0126 has been shown to reduce LRRK2-induced neuritic autophagy and neuritic shortening, implicating the involvement of MAPK/ERK-related signaling pathway in autophagy-regulated neuritic degeneration.

Concluding remarks and future perspectives

Neuritic autophagy is a constitutive process consisting autophagosome formation at the distal end, transport and maturation of autophagosomes along the length of processes, and degradation and clearance of autophagosomes at the soma. Recent studies have revealed the dynamic autophagic activity in the axons and provided compelling evidence for the essential role of autophagy in the maintenance of axonal homeostasis and normal functions. Moreover, autophagy serves a self-defense mechanism in many disease conditions, notably in proteinopathies and specific axonal injuries, and contributes to the protection of the axons and dendrites. On the other hand, insufficient or excessive autophagy often causes axonal and dendritic degeneration through disrupting the homeostatic balance of critical proteins

and membranes in the highly specialized compartments. A critical question is how exactly autophagy is altered and participates in diverse pathophysiological processes associated with axonal or dendritic degeneration (Box 1).

In fact, many basic questions regarding axonal or dendritic autophagy remain unanswered (Box 1), such as molecular mechanisms that control axonal autophagosome biogenesis, cargo packing, transport, maturation and clearance. In addition, the source of the autophagosome membrane, the fusion with endosomes/lysosomes and the cross-talk with apoptotic machinery have yet to be characterized in the axons. Future experiments will also be needed to understand the difference in the signaling and regulation of autophagy between the soma and neurites (especially the axons). Furthermore, because of the known non-specific effects of the autophagy "enhancers" (e.g., rapamycin) [93] or "inhibitors" (e.g., 3-MA) [94], caution should be taken when interpreting the results in the studies using these compounds. Thus development of highly specific and potent small chemicals that stimulate or inhibit autophagy is desirable.

Finally, understanding how autophagy executes or protects against neuritic degeneration will be critical for the development of novel effective therapeutic or preventive interventions for neurodegenerative diseases. It remains to be determined whether altered autophagy is causative or secondary to the pathological processes of the axons. Specific autophagy-modifying tools should be developed to test the ideas that manipulation of autophagy offers opportunity to protect against axonal or dendritic degeneration in neurodegenerative diseases.

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Glossary

Autophagosome	A double-membrane vesicle that forms at an early stage of the autophagic pathway and fuses with endosomes and lysosomes for degradation of its contents [100].
Autolysosome	A single-membrane vesicle that forms at a late stage of the autophagic pathway by fusion of autophagosomes with endosomes or lysosomes [101].
Axonal dystrophy	characterized by either focal dilations that interrupt the continuity of axons or terminal end bulbs in dysfunctional or degenerating neurons.
Dying back degeneration	degeneration initiates from the distal ends of axons, following by distal-to-proximal progression.
Anterograde transport	from the cell body to the axonal or dendritic terminals, is carried out mostly by kinesin superfamilies, which are plus-end-directed motors [102].
Retrograde transport	from the axonal or dendritic terminals to the cell body, is carried out mostly by dyneins, which are minusend-directed motors [102].
Mitophagy	selective removal of mitochondria by autophagy [103].

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Box 1

Outstanding questions

- What are the molecular mechanisms that control axonal autophagosome biogenesis, autophagic cargo packing, transport, maturation and clearance?
- By what mechanisms does autophagy contribute to neuritic protection and degeneration in diverse pathophysiological conditions?
- What is the exact role of mitophagy in regulating the compartmentalized degeneration of neurons?
- What is the cross-talk between autophagy and apoptosis in regulating axonal and dendritic degeneration?
- How does autophagy participate in synaptic proteins or vesicles turnover and regulate synaptic activity?

Highlights

- Neuritic degeneration is a pathological feature of many neurodegenerative diseases.
- Autophagy regulates protein and organelle homeostasis in the axons and dendrites.
- Insufficient or excessive autophagy contributes to neuritic degeneration.

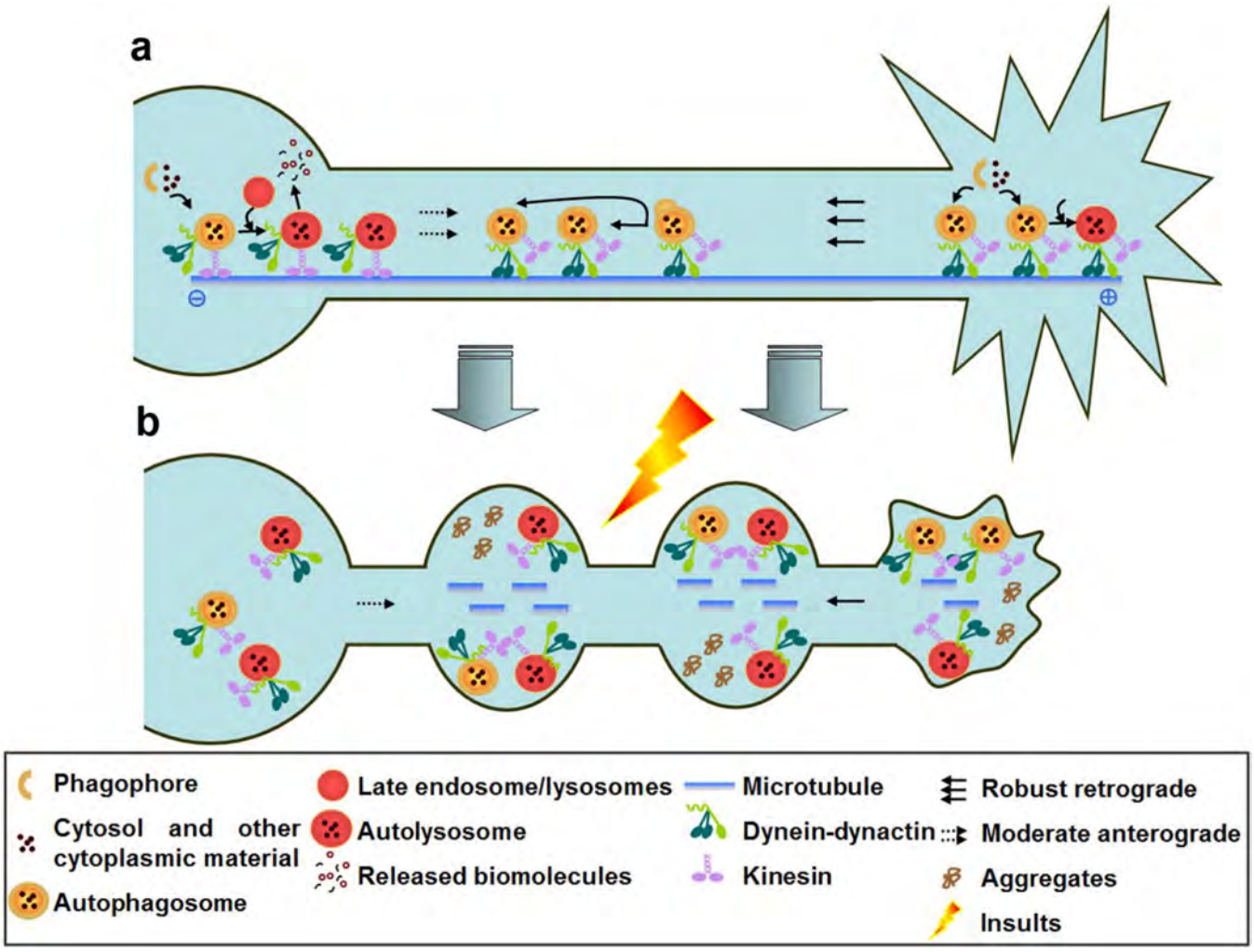

Figure 1.

Biogenesis, maturation and dynamic transport of autophagosomes and autolysosomes in healthy and degenerating axons. (a) Under normal conditions, autophagosomes may arise in the distal ends of healthy axons, become acidified (matured), and initiate retrograde transport along axons depending on microtubules and dynein/dynactin complex. Mature autophagosomes (autolysosomes) switch to bidirectional motility and initiate the proteolytic clearance of the autophagy substrates. (b) Upon certain insults, autophagosomes are induced in a large number, exceeding the rate of clearance. As result, autophagosomes/autolysosomes accumulate in axonal swellings or beadings associated with protein aggregates and collapsed cytoskeleton.

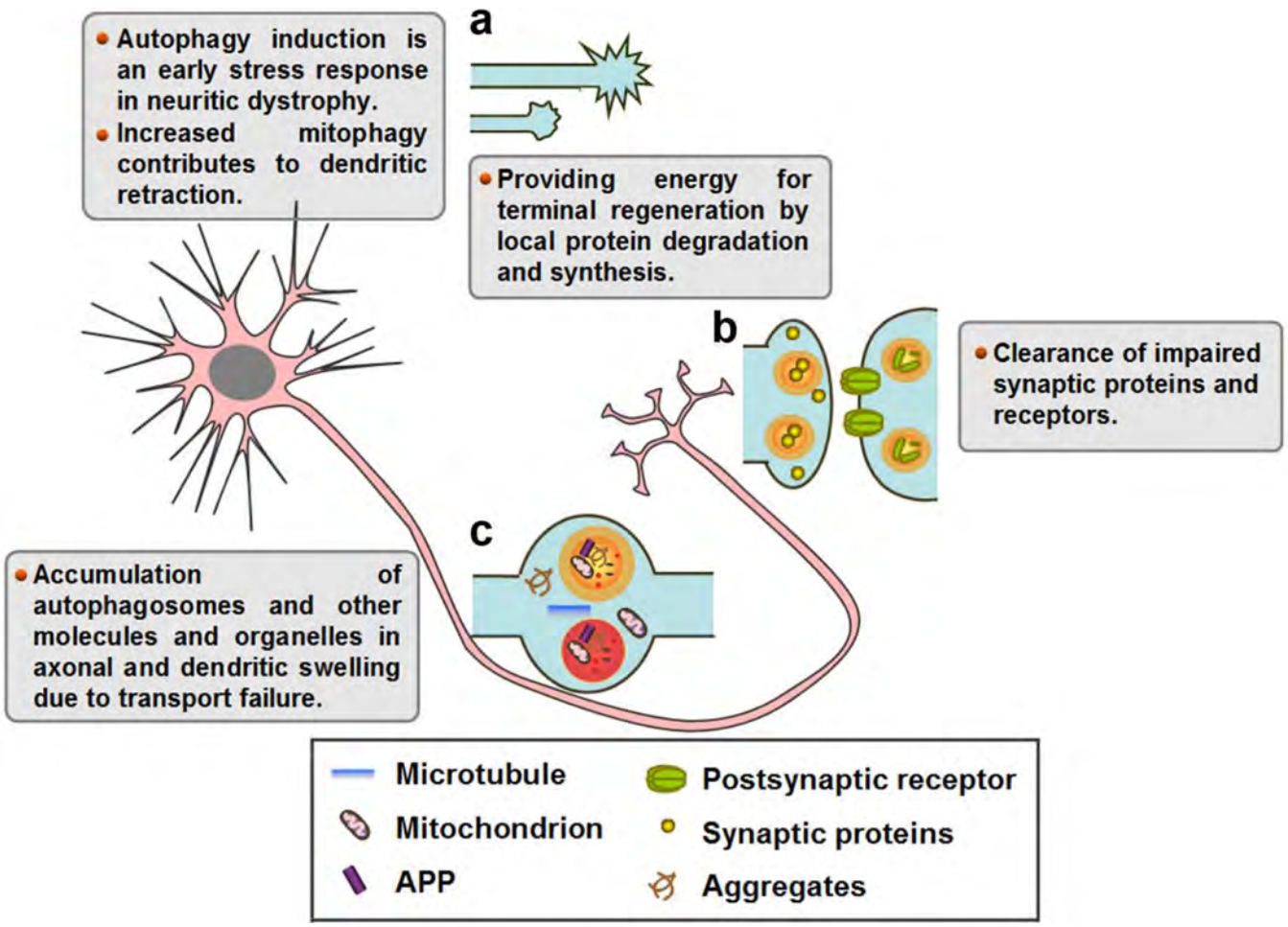

Figure 2.

Link of autophagy and diverse neuritic degenerative features. Autophagic process may be involved in a variety of neuritic degenerative features, including terminal degeneration (a), neuritic retraction (a), synaptic dysfunction (b) and neuritic swelling (c). APP, amyloid precursor protein.

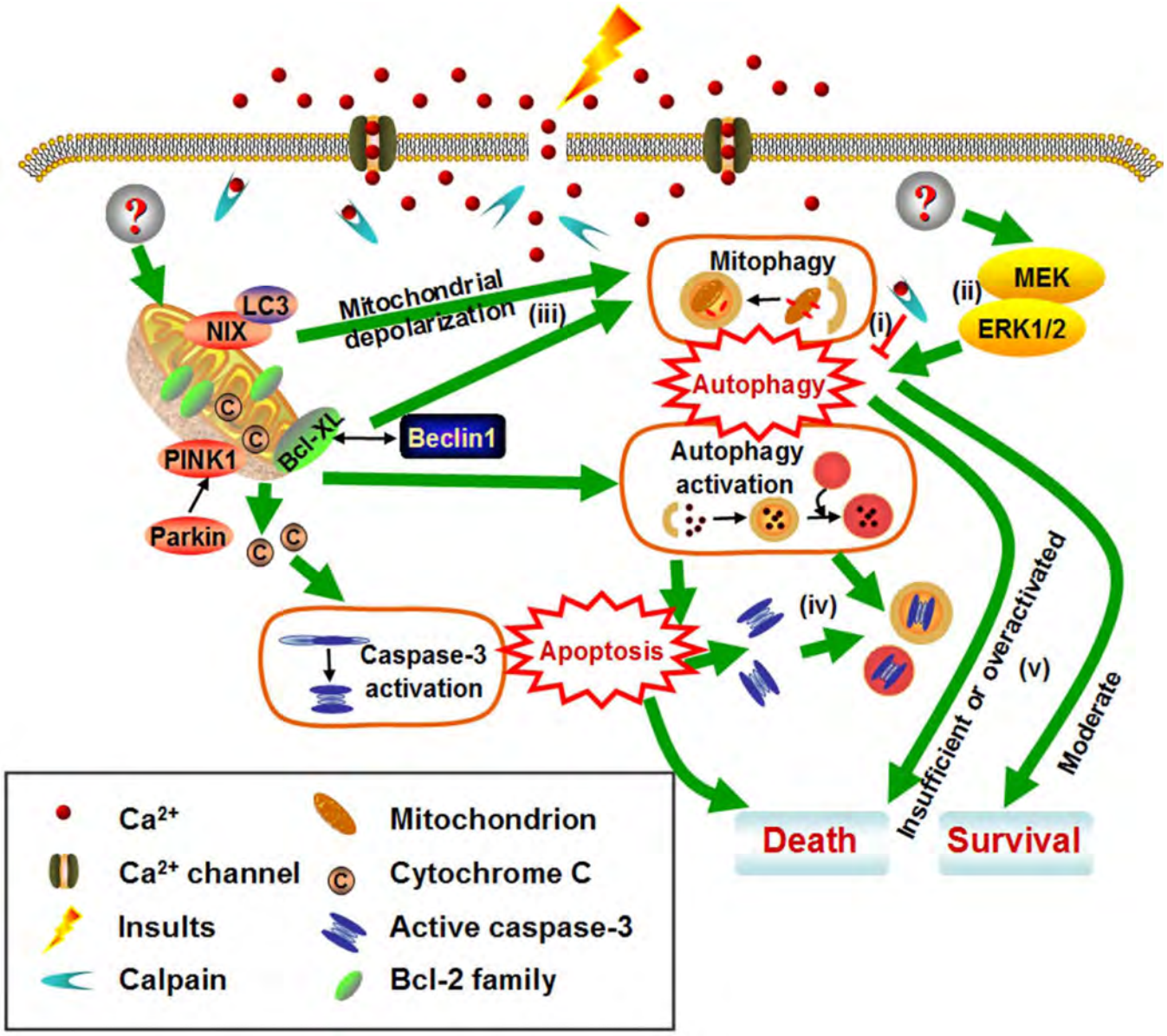

Figure 3.

Putative molecular pathways involved in autophagy-associated neuritic degeneration. Although the underlying mechanism of autophagy-associated neuritic degeneration is still elusive, several pathways might contribute to this process: (i) Autophagy acts as a downstream target of calcium influx following mechanical axonal damage. Calpain may block autophagy activation by mediating the cleavage of Atg5. (ii) MEK/ERK may act as upstream signals of autophagic induction. (iii) Several molecules, including NIX, PINK1 and parkin, may participate in mitophagy. (iv) Autophagy may also mediate the clearance of cleaved caspase-3, reflecting the cross-talk between autophagy and apoptosis in the execution of cell death. (v) Moderate autophagic activation may contribute to neuritic survival, while insufficient or overactivated autophagy lead to neuritic degeneration. ERK, extracellular signal regulated protein kinase; LC3, microtubule-associated protein light chain 3; MEK, mitogen activated protein kinase/ERK kinase; NIX, NIP3-like protein X; PINK1, phosphatase and tensin homolog (PTEN)-induced putative kinase 1.

Table 1

Neuritic accumulation of autophagosomes and autolysosomes in diverse experimental models of neurite injuries.

Insult	In vivo/ in vitro	Tissues/cell type/cell line	Effects	Phenotype	Refs
Mechanical injury					
Root avulsion/distal axotomy	*In vivo*	Motor neuron	Undetermined.	Autophagy is initiated independently of the distance and severity of the lesion.	[13]
Anterior/posterior axotomy	*In vivo*	DA	Harmful.	Conditional deletion of Atg7 in adult mice offers axon protection.	[69]
Axotomy	*In vivo*	RGC	Harmful.	Autophagy inhibition prevents axonal degeneration.	[12]
Posterior transection	*In vitro*	SCG	Harmful.	Autophagy inhibition prevents neuritic degeneration.	[68]
Pharmacological toxin exposure					
METH	*In vitro*	DA	Undetermined.	Degradative autophagic vacuoles accumulate in neuritic varicosities.	[5]
Zinc depletion	*In vitro*	SCG	Harmful.	Autophagy inhibition prevents terminal neurites from degeneration.	[50]
NMDA	*In vitro*	CGN	Harmful.	Autophagy inhibition prevents neuritic degeneration.	[6]
Nutrition withdrawal					
NGF deprivation	*In vitro*	SCG, PC12	Harmful.	Autophagy inhibition prevents neuritic degeneration.	[68]
Serum deprivation	*In vitro*	PC12	Undetermined.	Autophagosomes and autolysosomes accumulate in degenerating neurites.	[34, 70]
Lysosome inhibitors or inactivation of degradation					
Vinblastine	*In vitro*	Cortical neurons, SCG	Harmful.	Autophagosomes and autolysosomes accumulate in degenerating neurites. Autophagy inhibition prevents neuritic degeneration.	[7, 68]
Cathepsins deletion	*In vivo*	Brain tissues	Undetermined.	Autophagosomes accumulate in degenerating neurites.	[71]
Cysteine protease inhibitors	*In vivo*	Hippocampus	Beneficial.	Activated caspase-3 and autophagic vacuoles colocalize in degenerating neurites, which accelerates apoptosis.	[72]

Insult	In vivo/ in vitro	Tissues/cell type/cell line	Effects	Phenotype	Refs
Cathepsins inhibitors	*In vitro*	Hippocampal neurons	Undetermined.	Lysosomes and fused dense bodies (FDBs) composed of secondary lysosomes, postlysosomal compartments, and autophagic vacuoles accumulate selectively in the axon hillock and initial segment of the axon.	[73]

3-MA, 3-methyladenine; CGN, rat cerebellar granule neuron; DA, Dopamine neurons; METH, methamphetamine; NGF, nerve growth factor; NMDA, N-methyl-D-aspartate; RGC, retinal ganglion cell; SCG, superior cervical ganglion.

Table 2

Examples of autophagy alteration in degenerating neurites in different neurodegenerative disorders.

Disease (Organism/animal or cell model)	Tissues/cells	Effects	Phenotype	Refs
AD				
Human patients	Neocortex	Undermined.	Autophagosomes and other prelysosomal autophagic vacuoles accumulate in degenerating neurites.	[14]
	Hippocampus and inferior temporal cortex	Undermined.	Enhanced immunoreactivity of Atg12 in tau-immunopositive dystrophic neurites and some neurofibrillary tangles.	[15]
	Hippocampus	Undermined.	APP accumulates in the lysosomal system of the degenerating neurites present in senile plaques.	[16]
	Biopsied brain material	Undermined.	PS1 immunoreactivity is identified in autophagic vacuoles in degenerating neurites.	[17]
APP, PS/APP transgenetic mice	Cerebral cortex, hippocampus	Beneficial.	Autophagosomes and autolysosomes accumulate in degenerating neurites. Recovery of lysosomal proteolysis reverses axonal dystrophy and enhances maturation of accumulated autophagosomes.	[17, 31, 40, 72]
Lysosomal proteolysis inhibition	Mouse cortical neurons	Beneficial.	Lysosomal dysfunction disrupts axonal transport of degradative organelles and causes Alzheimer-like neurite dystrophy.	[31]
ALS				
SOD1 mutant mice	Spinal cord motor neurons	Harmful.	Autophagic vacuoles accumulated in degenerating axons. Autophagy stimulation accelerates the motor neuron degeneration.	[11]
Human patients	Motor neurons	Undermined.	The autophagy features were also found in close association with the characteristic inclusions of ALS.	[95]
Epilepsy				
Atg7 conditional knockout mice	Forebrain neurons	Beneficial.	Atg7-deficiency promotes the development of spontaneous seizures.	[81]
Human TSC patients	Brain tissues	Undermined.	Autophagy is suppressed in brains.	[81]
HD				
Human patients	Striatum	Beneficial.	Beclin1-positive inclusion-like deposits accumulate in axon or neuropil of HD samples. However, striatal neurons in normal control brains show diffuse immunoreactivity for Beclin1.	[20]
$Hdh^{140Q/\Delta Q}$ mice	Striatum	Beneficial.	LC3 colocalizes with neuropil htt aggregates in the $Hdh^{140Q/\Delta Q}$ striatum at 1 and 2 years of age, and such phenomenon is absent in the $Hdh^{140Q/+}$ striatum.	[78]
Inherited ataxias				
Human patients	Cerebral and brainstem	Undermined.	Widespread axonal aggregates, immunopositive for autophagy associated shuttle protein p62, appear in fiber	[96]

Disease (Organism/animal or cell model)	Tissues/cells	Effects	Phenotype	Refs
			tracts known to undergo neurodegeneration in SCA3 brains.	
Lurcher mice, *atg5* and *atg7* conditional knockout mice	DCN	Tentatively Beneficial.	Autophagy serves as an early stress response in axonal dystrophy. Atg7 and Atg5 are both required for the maintenance of axonal homeostasis and the prevention of axonal degeneration.	[8, 25, 97]
pcd mutant mice	DCN	Harmful.	Autophagosomes accumulate in Purkinje cell axons. Increased or aberrant mitophagy may contribute to the Purkinje cell degeneration in *pcd* mice.	[9]
PD				
Human patients	SNpc	Beneficial.	LC3 immunoreactivity is detected in a-synuclein-positive Lewy neurites, a neuropathological feature of PD indicative of axonal pathology.	[18, 19]
G2019S LRRK2 mutant mice	Cerebral cortex, striatum	Undermined.	Early and late autophagosomes accumulate in axons and synapses in animals with advanced age.	[57]
Atg7 conditional knockout mice	Midbrain DA neurons	Beneficial.	Disrupted autophagy leads to dopaminergic axon and dendrite degeneration and promotes presynaptic accumulation of a-synuclein and LRRK2 in the brain.	[79, 80]
G2019S LRRK2 overexpression	Mouse cortical neurons	Harmful.	LRRK2 mutants selectively cause dendrite retraction due to calcium dysregulation and induction of dendritic mitophagy.	[54,55]
G2019S LRRK2 overexpression	SH-SY5Y cells	Harmful.	Autophagosomes accumulate in neurites. Autophagy inhibition prevents neuritic retraction, whereas autophagy stimulation potentiates LRRK2-induced neuritic shortening.	[56]
MPP^+ intoxication	Mouse DA neurons	Undermined.	Neuritic degeneration and autophagy occurs before cell body loss.	[98]
Prion disease				
Human patients	Brain tissues obtained from CJD patients	Undermined.	Autophagosomes appear in the affected synapses.	[27]
CJD-infected hamsters or GSS-infected mice	Parietal cortex, corpus callosum, hippocampus, thalamus, cerebellum, brainstem	Undermined.	The major target of autophagy is dystrophic neurites, mostly dendrites but also axonal terminals and preterminals.	[59]
Scrapie prion strain infection	Mouse brain aggregate	Beneficial.	The LC3-II immunoreactivity is seen as deposits scattered throughout the neuropil.	[99]

AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; APP, amyloid precursor protein; CJD, Creutzfeldt-Jakob disease; DA, dopamine; DCN, deep cerebellar nuclei; GSS, Gerstmann-Sträussler-Scheinker; HD, Huntington's disease; $Hdh^{140Q/\Delta Q}$, full-length htt lacking its polyglutamine stretch in a knockin mouse model for HD; htt, huntingtin; LRRK2, leucine rich repeat kinase 2; MPP^+, 1-methyl-4-phenylpyridinium ion; *pcd*, Purkinje cell degeneration; PD, Parkinson's disease; PS1, presenilin 1; PS/APP, presenilin/amyloid precursor protein; SCA3, spinocerebellar ataxia type 3; SNpc, substantia nigra pars compacta; TSC, tuberous sclerosis complex.