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Neuronal Autophagy: Characteristic Features and Roles in Neuronal Pathophysiology

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Abstract

Autophagy is an important degradative pathway that eliminates misfolded proteins and damaged organelles from cells. Autophagy is crucial for neuronal homeostasis and function. A lack of or deficiency in autophagy leads to the accumulation of protein aggregates, which are associated with several neurodegenerative diseases. Compared with non-neuronal cells, neurons exhibit rapid autophagic flux because damaged organelles or protein aggregates cannot be diluted in post-mitotic cells; because of this, these cells exhibit characteristic features of autophagy, such as compartment-specific autophagy, which depends on polarized structures and rapid autophagy flux. In addition, neurons exhibit compartment-specific autophagy, which depends on polarized structures. Neuronal autophagy may have additional physiological roles other than amino acid recycling. In this review, we focus on the characteristics and regulatory factors of neuronal autophagy. We also describe intracellular selective autophagy in neurons and its association with neurodegenerative diseases.

Key Words: Neurons, Autophagy, Characteristic, Selective autophagy, Neurological disorder

INTRODUCTION

Autophagy is a tightly regulated cellular degradation pathway by which defective or superfluous cytosolic proteins, organelles, and other cellular constituents are sequestered in autophagosomes and delivered to lysosomes for degradation and recycling (Xie and Klionsky, 2007). Autophagy can be induced under stress conditions, such as nutrient starvation, hypoxia, and accumulation of toxic proteins or damaged organelles to maintain cellular homeostasis by providing nutrients to the cell (Williams *et al*., 2006; Maiuri *et al*., 2007; Mazure and Pouyssegur, 2010). In addition, autophagy is required for cell survival by removing toxic proteins or damaged organelles.

Autophagy is regulated by mammalian target of rapamycin (mTOR) signaling, which is a canonical regulator of starvation-induced autophagy. The other major mediators of autophagy are autophagy-related genes (ATGs) and microtubule-associated protein 1 light chain protein 3 (LC3), which are necessary for autophagosome formation (Gabryel *et al*., 2012; Bar-Yosef *et al*., 2019). During autophagy, autophagosomes are formed, which fuse with lysosomes. Double-membrane bound vesicles (autophagosomes) are initially formed and elongate enough by nucleation of the phagophore (a precursor of autophagosome) and acquisition of lipids to engulf and seal all components that will be digested (Bailly, 2013; Bernard and Klionsky, 2013). ATG proteins and LC3 are recruited to autophagosomal membranes to form autophagosomes. In addition, p62/sequestosome 1, an adaptor protein complex that transports polyubiquitinated protein aggregates for degradation, is localized to autophagosomes by the LC3-interacting region (Lim *et al*., 2011). Therefore, ATG proteins, LC3, and p62 are markers of autophagosomes (Suzuki *et al*., 2007). Autophagosomes can be fused with lysosomes to form autophagolysosomes. Autophagosomes or lysosomes are transported along microtubules using dynein for fusion (Cheng *et al*., 2015). In addition, fusion is mediated by soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors, Rab proteins, and adenosine triphosphatase, which are required for autophagosome maturation (Eskelinen, 2005; Furuta *et al*., 2010). Lysosomes contain acid hydrolases, such as proteases, nucleases, and lipases; which break down the engulfed proteins into individual amino acids. Autophagolysosomes are required to degrade and clear misfolded proteins and damaged organelles, and the breakdown products of the digested materials are reused by cells (Bailly, 2013). This process is essential for cell survival and adaptation to starvation in most mammalian cells (Nikole-

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topoulou *et al*., 2015).

Classical autophagy is classified as macroautophagy, microautophagy, and chaperone-mediated autophagy. Furthermore, organelle-selective autophagy has also been reported, such as autophagy of the endoplasmic reticulum (ER, ER-phagy), mitochondria (mitophagy), peroxisomes (peroxiphagy), and ribosomes (ribophagy) (Saito and Sadoshima, 2015; Ktistakis and Tooze, 2016).

In neurons, autophagy is involved in neuronal development, aging, survival, and death (Azarnia Tehran *et al*., 2018; Kulkarni *et al*., 2018; Liang and Sigrist, 2018; Wallings *et al*., 2019). As neurons are long-living post-mitotic cells, they are prone to accumulating misfolded proteins or damaged organelles that are typically diluted through cell division in proliferating cells. In addition, rapid turnover of synaptic proteins in response to repetitive electrical stimuli leads to the accumulation of damage in neurons. Thus, controlling protein quality by autophagy is critical for neuronal function. Neuron-specific loss of autophagy induces progressive neuronal dysfunction and accumulation of abnormal proteins, leading to neurodegeneration (Hara *et al*., 2006), and loss of autophagy regulatory genes results in abnormal behavior of mice (Tang *et al*., 2014). These studies indicate that autophagy is important for neuronal function. In this review, we summarize the characteristics of neuronal autophagy, and discuss the characteristics triggering factors of neuronal autophagy. We also discuss intracellular selective autophagy in neurons and its implications in neuronal physiology and diseases.

ROLE OF AUTOPHAGY IN NEURONAL PATHOPHYSIOLOGY

Neurons have highly polarized structures that include axons, dendrites, and synapses, and autophagy has been observed to occur in all compartments of neurons. In most cells, autophagy occurs at a low basal level. However, this process has been shown to have different features in neurons compared to non-neuronal cells (Mitra *et al*., 2009), such as constitutively active autophagy in neurons under basal conditions (Mizushima *et al*., 2004). Autophagy is required for neuronal development, neuronal homeostasis, and survival (Ban *et al*., 2013). Additionally, autophagy plays an important role in neuronal physiology, including axonal homeostasis, dendrite spine formation, and synapse formation. Loss of autophagy in neurons causes defects in cargo-specific axonal transport, which lead to axon swelling and axonal dystrophy (Komatsu *et al*., 2006; Lee *et al*., 2011) as well as over-formation of dendritic spine density due to deficits in synaptic pruning (Tang *et al*., 2014). Autophagosome biogenesis is observed near synapses (Stavoe *et al*., 2016), and autophagy is required for synapse development and neuronal plasticity (Shen and Ganetzky, 2009; Wang *et al*., 2019). Rapamycin-induced macroautophagy rapidly inhibits neurotransmitter release in dopaminergic neurons (Hernandez *et al*., 2012), and autophagy regulates presynaptic neurotransmission by controlling axonal ER calcium release in hippocampal neurons (Kuijpers *et al*., 2020). Furthermore, autophagy is involved in memory encoding, information processing, and cognitive function (Vijayan and Verstreken, 2017). These findings indicate that autophagy plays an essential role in neuronal function.

Impaired autophagy is associated with various neurodevelopmental disorders and neurodegenerative diseases (Lee *et al*., 2013). Patients with autism spectrum disorder exhibit impaired autophagy during neural development and malformation of neuronal structures resulting from an abnormal mTOR pathway, which is a negative regulator of autophagy (Tang *et al*., 2014; Winden *et al*., 2018). Fragile X syndrome, a genetic cause of autism spectrum disorder, is caused by mutation in the fragile X mental retardation 1 (*FMR1*) gene, which encodes for fragile X mental retardation protein (Lee *et al*., 2013). In *Fmr1*-KO mice, a model of human fragile X syndrome, reduced autophagy leads to hyperactivation of the mTOR pathway in hippocampal neurons, whereas activation of autophagy rescues synaptic and cognitive deficits in these mice (Sharma *et al*., 2010; Yan *et al*., 2018). Vici syndrome, a rare autosomal recessive neurodevelopmental disorder caused by mutations in the ectopic P-granules autophagy protein 5 (*epg-5*) gene that encodes an autophagy regulator is associated with impaired clearance of autophagosomes because of limited autophagosome-lysosome fusion (Hori *et al*., 2017). Furthermore, defective autophagy has been observed in several neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and Lafora disease (an atypical autosomal recessive neurodegenerative disorder) (Son *et al*., 2012; Zatyka *et al*., 2020). Atg proteins promote autophagosome formation, and the loss of these proteins in the mouse brain causes behavioral deficits, neurodegeneration, and neuronal death (Hara *et al*., 2006; Komatsu *et al*., 2006). Abnormal accumulation of misfolded proteins and defective organelles is a hallmark characteristic of neurodegenerative disorders. Thus, inducing autophagy by inhibiting the mTOR pathway represents a potential therapeutic strategy for treating neurodegenerative diseases (Metcalf *et al*., 2012).

Although autophagy is required to maintain neuronal function, it is not required under certain conditions. Excessive autophagy exacerbates brain injury in neonatal hypoxia-ischemia, whereas blocking autophagy attenuates brain injury (Carloni *et al*., 2010). Thus, inhibition of autophagy is a potential therapeutic strategy for perinatal asphyxia (Corti *et al*., 2020). Compared with the adult brain, the immature brain undergoes massive remodeling including neural cell proliferation and the formation and elimination of bulk synapses. In addition, the expression patterns of autophagy proteins differ between in immature and adult brains (Loeffler, 2019). Indeed, immature brains exhibit higher expression of autophagosome markers, including LC3 and beclin-1, and increased activity of lysosomes (Zhu *et al*., 2005; Shibata *et al*., 2006; Carmona-Gutierrez *et al*., 2016). Taken together, these studies indicate that homeostatic maintenance of autophagy balance is crucial to neuronal function; therefore, understanding neuronal autophagy and its precise regulation is important for developing novel strategies to treat several brain disorders.

CHARACTERISTICS OF NEURONAL AUTOPHAGY

Compartment-specific autophagy processes

Biogenesis and transport of autophagosomes at presynaptic terminals: Neurons are highly polarized cells with distinct structures for intracellular communication. Compartment-specific autophagosomes, including somatodendritic and axonal autophagosomes, have been observed in these

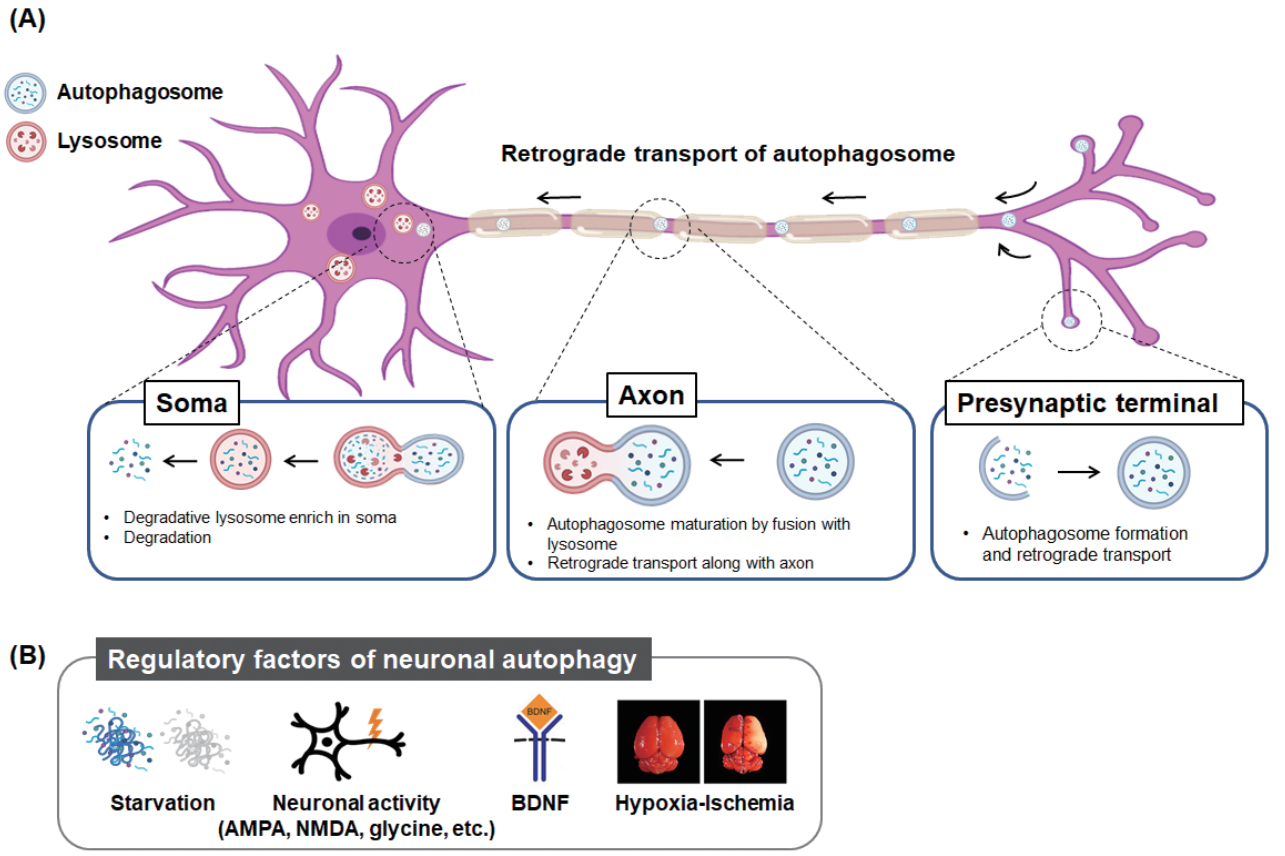

Fig. 1. Characteristics and of neuronal autophagy. (A) Compartment-specific autophagy in neurons. Biogenesis of autophagosome at presynaptic terminals, maturation of autophagosome by retrograde transport along the axon, and degradation of autophagosome in soma. (B) Regulatory factors of autophagy in neurons. Starvation, neuronal activity, BDNF, and ischemia regulates autophagy in neurons.

cells (Maday and Holzbaur, 2016). This compartment-specific regulation of autophagy is required to maintain synaptic integrity and neuronal function (Wang *et al*., 2015a). Autophagosomes are constitutively formed at the distal end of the axon and are less frequently observed in the soma or dendrites (Maday and Holzbaur, 2014). Autophagosomes are transported from the axon terminal to the soma, where they undergo maturation by fusing with late endosomes or degradative lysosomes (Fig. 1A) (Maday and Holzbaur, 2016; Farfel-Becker *et al*., 2019). Dynein, a specific cargo protein, facilitates retrograde transport of autophagosomes (Cheng *et al*., 2015). Once inside the soma, autophagosomes can freely diffuse between the soma and dendrite, whereas they cannot re-enter the axon. Autophagosomes can bind to the specific motor protein kinesin (Farias *et al*., 2015), or region-specific filter mechanisms at the axon initial segment (Song *et al*., 2009) may contribute to restricting autophagosomes in the soma, although the exact mechanism is unclear.

Maturation and degradation of autophagolysosomes in neurons: Lysosomes are synthesized in the nucleus and transported anterogradely along the axon with the help of dynein (Farfel-Becker *et al*., 2019). Lysosomes fuse with autophagosomes at the axon terminal, and the autophagolysosomes are retrogradely transported and subsequently degraded within the soma (Kulkarni and Maday, 2018; Hill and Colon-Ramos, 2020), which is a structure enriched in degradative lysosomes and proteases (Fig. 1A) (Padamsey *et al*., 2017; Yap *et al*., 2018). Autophagosomes accumulate only in the soma, but not in the axons or dendrites following treatment with bafilomycin A1 (Maday and Holzbaur, 2016), a molecule that blocks acidification of lysosomes by inhibiting vacuolar-type adenosine triphosphatase activity (Yoshimori *et al*., 1991). Therefore, the soma is the primary site of degradation in neurons, and this high degradative capacity may be important for amino acid recycling.

Recent studies showed that degradative lysosomes are present at the axon terminal with distinct motility compared with non-degradative lysosomes, and their anterograde trafficking is associated with autophagy stress (Farfel-Becker *et al*., 2019). Additionally, activity-dependent recruitment of lysosomes to dendritic spines regulates synaptic plasticity (Goo *et al*., 2017). Altogether, these studies indicate that neurons exhibit compartment-specific autophagy regulation; however, their role in neurons remains to be investigated.

Rapid autophagy flux in neurons

Autophagy flux is a measure of autophagic degradation. Atg proteins mediate autophagosome formation by converting LC3 into the active form (LC3-II) and promoting conjugation of inactive LC3 (LC3-I) to phosphatidylethanolamine (Kabeya *et al*., 2000). As lipid conjugation of LC3 is increased during autophagosome elongation, LC3-II is a representative marker protein for tracking autophagy initiation. Although Atg proteins

are highly expressed in neurons, and autophagosomes are constitutively generated, biochemical and structural studies have shown that LC3-II expression is lower in neurons than in non-neuronal cells, and autophagosomes are rare in healthy neurons (Nixon *et al*., 2005; Shehata *et al*., 2012). Starvation and some chemicals that can enhance LC3-II levels in non-neuronal cells have been shown to marginally increase LC3-II levels in neurons (Benito-Cuesta *et al*., 2017). This indicates that autophagy flux and clearance of autophagosomes by fusion with lysosomes are rapid in neurons (Boland *et al*., 2008; Ariosa and Klionsky, 2016).

TRIGGERING FACTORS OF NEURONAL AUTOPHAGY

Starvation

Starvation and starvation-related pathways are known to induce autophagy through the mTOR pathway, both in non-neuronal and neuronal cells (Fig. 1B). Nutrient deficit and short-term fasting induce autophagosome formation and reduce mTOR activity in neurons both *in vitro* and *in vivo* (Young *et al*., 2009; Alirezaei *et al*., 2010). In addition, rapamycin and other mTOR inhibitors induce autophagosome formation to promote synaptic growth and axon elongation (Shen and Ganetzky, 2009; Hernandez *et al*., 2012; Ban *et al*., 2013), as well as regulate local autophagosome formation and presynaptic neurotransmission in dopaminergic neurons (Hernandez *et al*., 2012). Interestingly, other studies showed that starvation or mTOR pathway suppression failed to induce autophagy in neurons (Mizushima *et al*., 2004; Fox *et al*., 2010; Tsvetkov *et al*., 2010). Rather, constitutive formation of autophagosomes is observed in distal axons, even without starvation (Maday and Holzbaur, 2016). Short-term nutrient deficit suppresses mTOR activity, whereas long-term starvation activates mTOR activity (Zhu *et al*., 2019); therefore, the duration of starvation may differentially regulate autophagy in neurons. In addition, alternative mechanisms of autophagy regulation may be utilized in neurons, and there may be differences in the physiological roles of autophagy in neurons, such as regulation of neuronal development, maintenance of structural components, and neuronal homeostasis (Tang *et al*., 2014; Yamamoto and Yue, 2014; Wang *et al*., 2015a).

Neuronal activity

Although the precise mechanism is not clearly understood, neuronal and electrical activities have been implicated in several steps of autophagy (Vijayan and Verstreken, 2017). It has been shown that *N*-methyl-*D*-aspartic acid (NMDA), a glutamate agonist, or potassium chloride (KCl)-induced depolarization promotes autophagosome formation in cerebellar granules and hippocampal neurons (Fig. 1B) (Katsumata *et al*., 2010; Shehata *et al*., 2012; Wang *et al*., 2015b). KCl-induced autophagosome formation is suppressed by DL-2-amino-5-phosphonovaleric acid (AP5), an NMDA receptor (NMDAR) blocker (Shehata *et al*., 2012). Moreover, neuronal activity regulates autophagosome and lysosomal trafficking as well as lysosome-mediated degradation. Retrograde transport of autophagosomes is enhanced by KCl-induced depolarization or presynaptic activity (Wang *et al*., 2015b). Furthermore, application of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and glycine, which activate the NMDAR, recruits lysosomes to dendritic spines in cultured hippocampal neurons, and activation of a single spine with 4-methoxy-7-nitroindolinyl-glutamate uncaging recruits lysosomes to dendritic spines in hippocampal organotypic cultures (Goo *et al*., 2017). This activity-dependent trafficking of lysosomes was blocked by AP5 treatment. NMDA activation and neuronal activity induce autophagy, which promotes AMPA receptor (AMPAR) internalization and lysosome-mediated degradation, leading to a reduced number of AMPARs at synapses (Schwarz *et al*., 2010; Shehata *et al*., 2012). Overall, neuronal activity contributes to autophagosome formation, trafficking, and lysosome-mediated degradation in the local region. However, further studies are required to reveal the mechanistic link between neuronal activity and autophagy and the physiological role of autophagy in neuronal homeostasis.

Brain-derived neurotrophic factor (BDNF)

Brain-derived neurotrophic factor (BDNF), a member of the mammalian neurotrophin family, has been suggested as a promising candidate for treating several brain disorders and cognitive dysfunction. BDNF signaling promotes neuronal survival and prevents neurodegeneration and is initiated by the binding of BDNF to its receptor, tropomyosin receptor kinase B (TrkB) (Reichardt, 2006). BDNF signaling was shown to suppress autophagy by regulating the phosphatidylinositol 3-kinase/protein kinase B (Akt) pathway in the mouse forebrain. BDNF-induced suppression of autophagy promotes synaptic remodeling and memory enhancement (Fig. 1B) (Nikoletopoulou *et al*., 2017). In addition, BDNF-mediated suppression of autophagic flux promotes neuronal cell survival (Smith *et al*., 2014). BDNF induces the phosphorylation of mTOR and Akt proteins, which in turn promotes dendritic protein synthesis (Briz *et al*., 2013). Interestingly, to promote neuronal survival and prevent neurodegeneration, BDNF/TrkB complexes are internalized and BDNF/TrkB-containing autophagosomes are trafficked retrogradely (Ginty and Segal, 2002; Philippidou *et al*., 2011). Reduced retrograde transport of the BDNF/TrkB complex in mice leads to decreased BDNF levels, neuronal complexity, and neurodegeneration (Kononenko *et al*., 2017). These studies indicate that a reciprocal relationship exists between BDNF and autophagy in neurons. Further studies are needed to clarify the mechanistic link between BDNF and autophagy and its pathophysiological role in the brain.

Hypoxia-ischemia (H-I)

Previous studies reported that hypoxia and ischemia induce autophagy in the brain (Fig. 1B). Ischemia induces neuronal autophagy by increasing the levels of reactive oxygen species and intracellular Ca^{2+} and decreasing the levels of intracellular ATP, amino acids, and insulin (Gabryel *et al*., 2012). Hypoxia and ischemia enhance the formation of autophagosomes and lysosomes in neonatal and adult animal models, leading to neuronal death (Adhami *et al*., 2006; Rami *et al*., 2008; Puyal *et al*., 2009). Knockdown of Atg5 or Atg7, the core proteins of autophagy, prevents ischemia-induced neuronal death (Koike *et al*., 2008; Grishchuk *et al*., 2011). Similarly, lysosomal inhibitors reduce ischemia-induced neuronal injury (Wen *et al*., 2008). This suggests that autophagy plays a detrimental role in ischemia-induced neuronal death. Although the precise role of autophagy in ischemia-induced brain damage is controversial, a protective role for autophagy in brain ischemia has been reported. Rapamycin, an autophagy inducer, enhances LC3 and

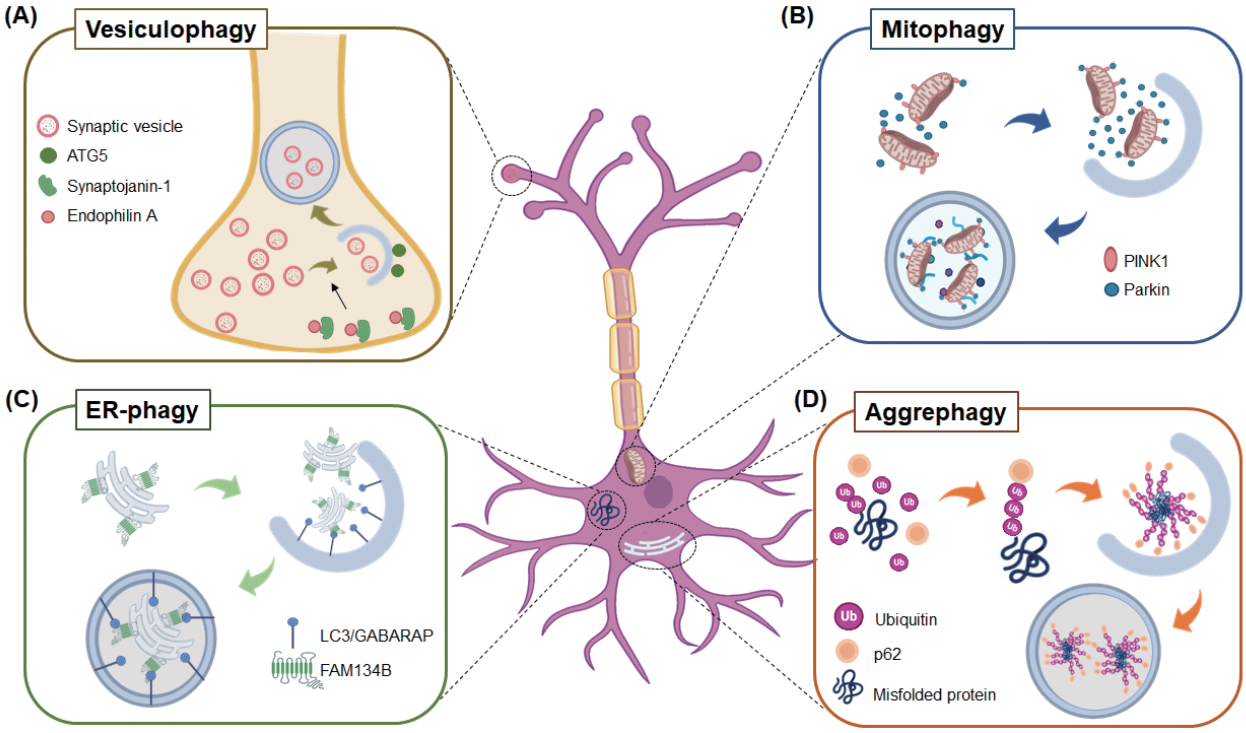

Fig. 2. Subcellular selective autophagy in neurons. (A) Vesiculophagy, degradation of synaptic vesicles by autophagy at presynaptic terminal in neurons. Synaptojanin-1 and Eedophilin A complex and ATG5 induce presynaptic autophagy. (B) Mitophagy in neurons. PINK1 and Parkin regulate mitophagy by initiating autophagosome formation around the damaged mitochondria. (C) ER-phagy in neurons. FAM134B is an ER-phagy adaptor that induces ER-phagy by recruiting GABA type A receptor-associated protein (GABARAP). (D) Aggrephagy in neurons. Ubiquitin and p62 promote aggrephagy to selectively remove misfolded proteins.

beclin-1 expression and reduces H-I-induced neuronal death and injury to the hippocampus and cerebral cortex through the Akt/cyclic adenosine monophosphate-response element binding protein signaling pathway (Carloni *et al*., 2008, 2010). Considering the conflicting reports on the role of autophagy in ischemia-induced brain damage in different animal models (Sheng and Qin, 2015) and regional differences in ischemia-induced autophagic neuronal death (Ginet *et al*., 2009; Puyal *et al*., 2009), the exact role of neuronal autophagy in ischemia-induced brain damage should be further investigated.

SELECTIVE AUTOPHAGY IN NEURONS

Vesiculophagy in neurons

Precise elimination of synapses in the adult brain is mediated by microglial cells or astrocytes (Chung and Barres, 2012; Stephan *et al*., 2012). Within neurons, ubiquitin-mediated proteasomal degradation contributes to eliminating synapses or synaptic proteins at both presynaptic and postsynaptic sites (Lee *et al*., 2004; Jiang *et al*., 2010). Autophagy also regulates the clustering of synaptic vesicles, and clearance of synaptic vesicle proteins has been termed as “vesiculophagy” (Fig. 2A) (Binotti *et al*., 2015). Various biological molecules regulate protein proteostasis at the presynaptic terminal via vesiculophagy. At presynaptic terminals, light-activated reactive oxygen species generators rapidly induce autophagy to clear damaged synaptic vesicle proteins, thus maintaining synaptic function (Hoffmann *et al*., 2019). A novel role for presynaptic proteins that regulate autophagy at presynaptic terminals has been reported. Synaptojanin-1 and its binding partner endophilin A, which are enriched at presynaptic terminals, facilitate membrane remodeling during synaptic vesicle recycling (Watanabe *et al*., 2018) and promote presynaptic autophagy (Soukup *et al*., 2016; Vanhauwaert *et al*., 2017). Moreover, loss of bassoon, a presynaptic active zone protein that regulates synaptic autophagy by interacting with Atg5, is crucial for the formation of autophagosomes and decreases the synaptic vesicle pools and synaptic vesicle proteins by promoting presynaptic autophagy (Okerlund *et al*., 2017; Hoffmann-Conaway *et al*., 2020). Recent studies showed that Rab26, a member of the Rab-guanine triphosphatase superfamily, regulates synaptic vesicle clustering by recruiting the autophagosome proteins Atg16L1, LC3B, and Rab33B (Binotti *et al*., 2015). Pleckstrin homology containing family member 5 (Plekhg5) is required for Rab26 activity, and *Plekhg5*-deficient mice exhibit impaired synaptic vesicle autophagy to cause accumulation of synaptic vesicle proteins and late-onset motor neuron disease (Luningschror *et al*., 2017). These studies suggest a role for vesiculophagy in regulating presynaptic proteins or synaptic vesicles. Additional studies suggested that autophagosome-related proteins contribute to synaptic transmission by regulating synaptic vesicle pools and proteins.

Mitophagy in neurons

Selective autophagy is responsible for the degradation of

specific subcellular organelles and other cellular cargo (Anding and Baehrecke, 2017). To date, defects in several types of selective autophagy, including mitophagy (degradation of mitochondria), ER-phagy (degradation of the endoplasmic reticulum), and aggrephagy (degradation of aggregated proteins) have been identified in non-neuronal cells and have been linked to various brain disorders, such as Alzheimer's disease, Parkinson's disease, Huntington's disease, and others. Neurons consume large amounts of energy, and maintaining mitochondrial activity or clearance of damaged mitochondria is critical for neuronal homeostasis (Nicholls and Budd, 2000; Erecinska *et al*., 2004). Impaired mitophagy has been shown to be associated with several neurodegenerative diseases (Martinez-Vicente, 2017). Mitochondrial damage can occur because of several reasons, including oxidative stress, production of reactive oxygen species, and exposure to toxins such as rotenone and phthalate, and mitophagy, which is responsible for eliminating damaged mitochondria (Zolkipli-Cunningham and Falk, 2017). Phosphatase and tensin homolog-induced serine/threonine kinase 1 (PINK1) and E3 ubiquitin ligase Parkin have been shown to regulate mitophagy in neuronal and non-neuronal cells (Evans and Holzbaur, 2020) and mediate quality control of the mitochondria (Ge *et al*., 2020). Upon mitochondrial damage due to oxidative stress or exposure to compounds such as protonophore carbonyl cyanide 3-chlorophenylhydrazone or rotenone, PINK1 phosphorylates ubiquitin, thus inducing translocation of Parkin to the outer membrane of damaged mitochondria (Joselin *et al*., 2012; Koyano *et al*., 2014), leading to the recruitment of LC3 and initiation of autophagosome formation around the damaged mitochondria (Fig. 2B) (Wong and Holzbaur, 2014; Moore and Holzbaur, 2016). Interestingly, glutamate excitotoxicity induces mitophagy in neurons by enhancing the accumulation of Parkin in mitochondria, whereas NMDA inhibitors block Parkin translocation (Van Laar *et al*., 2015), indicating that neuronal activity plays a regulatory role in mitophagy. Mechanisms known to eliminate damaged mitochondria in neurons include ubiquitin-, lipid-, and receptor-mediated mitophagy (Evans and Holzbaur, 2020), and further studies are required to understand their physiological or pathological roles in neurons.

ER-phagy in neurons

Although ER-phagy is not as well-studied as a form of mitophagy, it is a selective process that contributes to neuronal homeostasis through quality control of the ER (Grumati *et al*., 2018). Depending on the location of autophagosome formation and fusion with lysosomes, three different types of ER-phagy have been reported: macro-ER-phagy, micro-ER-phagy, and vesicular delivery (Chino and Mizushima, 2020). ER-phagy is mediated by ER-phagy adaptors, such as FAM134B, an ER-resident protein containing a reticulon-homology domain that enables the binding of LC3 and GABA type A receptor-associated protein (GABARAP) leading to ER fragmentation (Fig. 2C) (Bhaskara *et al*., 2019). Knockdown of FAM134B causes structural changes to the *cis*-Golgi compartment, and mutations in *FAM134B* are associated with sensory and autonomic neuropathy in humans (Kurth *et al*., 2009). Several ER-phagy adaptors have been reported in mammals and yeast (Chino and Mizushima, 2020); however, their roles in neuronal ER-phagy remain unclear.

In mice, knockout of WD repeat domain 45 (WDR45), a beta-propeller scaffold protein and one of the mammalian homologs of Atg18, leads to impaired synaptic transmission and cognitive function with ER protein accumulation and enhanced ER stress (Wan *et al*., 2020). WDR45 plays a regulatory role in autophagy because it acts as a sensor of ER stress (Tsuyuki *et al*., 2014; Mollereau and Walter, 2019). Furthermore, patients with *de novo* mutations in *WDR45* exhibit neurodegeneration with brain iron accumulation and Rett syndrome-like features (Haack *et al*., 2012; Ohba *et al*., 2014).

In neurons, constitutive formation of autophagosomes is observed near the ER at presynaptic terminals and axons (Maday and Holzbaur, 2014; Hill and Colon-Ramos, 2020). Neuronal autophagy regulates excitatory synaptic transmission by controlling calcium release from the ER at the presynaptic terminal (Kuijpers *et al*., 2020). These studies suggest that ER-phagy is crucial for maintaining neuronal physiology and is associated with pathological conditions that are derived from impaired quality control of the ER.

Aggrephagy in neurons

Accumulation of protein aggregates within neurons is a hallmark of several neurodegenerative diseases. Aggregate-prone proteins are degraded by the ubiquitin-proteasome system, although larger protein aggregates induce selective autophagy in neurons, a process known as aggrephagy, which is a specific form of macroautophagy that selectively removes protein aggregates (Fig. 2D) (Berger *et al*., 2006; Corrochano *et al*., 2012). Like mitophagy, several adaptors, such as p62, optineurin (OPTN), and autophagy-linked FYVE protein (Deng *et al*., 2017) are known to regulate aggrephagy in neurons. In humans, genetic mutations of p62 or OPTN are correlated with ALS and frontotemporal lobar degeneration, which are progressive neurodegenerative diseases involving the development of inclusion bodies in neurons (Neumann *et al*., 2006) and are associated with impaired selective autophagy. Although p62 promotes protein degradation by autophagy and prevents the formation of protein aggregates (Falcon *et al*., 2018), p62 deficiency leads to protein aggregation with neurodegenerative disease phenotypes (Ramesh Babu *et al*., 2008). Similarly, deletion of OPTN increases cytoplasmic vacuolar formation, protein aggregation, and abnormal myelination in sciatic nerves (Kurashige *et al*., 2020). Adaptor proteins contain a conserved LC3-interacting region, which is important for the association of LC3 with the autophagosomal membrane (Wild *et al*., 2014). During aggrephagy, adaptor proteins interact with LC3 and facilitate the formation of surrounding autophagosomes, leading to the sequestration and subsequent degradation of protein aggregates. Diverse regulatory mechanisms of adaptor protein activity have been shown to enhance aggrephagy, such as phosphorylation of specific residues (Richter *et al*., 2016; Turco *et al*., 2019), oligomerization (Wurzer *et al*., 2015), and post-translational modifications (McEwan and Dikic, 2011). Stimulating aggrephagy by modulating adaptor protein activity would be a beneficial approach for treating neurodegeneration.

CONCLUSION

Neurons exhibit constitutive formation and rapid turnover of autophagosomes. These autophagosomes may be derived from damaged intracellular organelles. It is well-known that misfolded or aggregated proteins cannot be diluted in

post-mitotic cells; therefore, neurons are particularly vulnerable to autophagy inhibition. Loss of autophagy in neurons causes neurodegeneration via the accumulation of protein aggregates (Hara *et al*., 2006; Komatsu *et al*., 2006). High autophagic flux is a key feature of neurons. Moreover, neurons exhibit compartment-specific autophagy that depends on polarized structures, such as compartmentalized biogenesis and transport of autophagosomes and lysosomes (Fig. 1A). Consistent with this, blocking acidification of lysosomes induces autophagosomes in the soma alone and not in the axons or dendrites. The physiological role of autophagy likely differs among the subcellular compartments of neurons. Autophagy is not only responsible for recycling amino acids but is also required for neuronal homeostasis and maintaining neuronal functions. Recent studies showed that degradative lysosomes have distinct features compared to lysosomes; thus, studies are needed to investigate the role of degradative lysosomes in neurodevelopmental and neurodegenerative diseases. Although diverse factors and signaling pathways trigger autophagy in neurons, the role of some of these factors remains controversial, and their mechanistic link to autophagy requires further investigation. Because autophagy is variously observed in brain regions and cell types and during brain development, an understanding of autophagy steps, including biogenesis, transport of autophagosomes, and fusion with lysosomes, would be beneficial for understanding the precise mechanism of autophagy in neurons. Selective autophagy is highly associated with neurodegenerative diseases because it is involved in the clearance of damaged organelles and protein aggregates (Fig. 2). Adaptor proteins also contribute to the clearance of damaged intracellular organelles. The link between selective autophagy and neuronal physiology and its pathological role in neurodevelopmental disorders must be further evaluated. An in-depth understanding of neuronal autophagy will facilitate the development of novel strategies for treating several neurological disorders.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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