Autophagic activity in neuronal cell death

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As post-mitotic cells with great energy demands, neurons depend upon the homeostatic and waste recycling functions provided by autophagy. In addition, autophagy also promotes survival during periods of harsh stress and targets aggregate-prone proteins associated with neurodegeneration for degradation. Despite this, autophagy has also been controversially described as a mechanism of programmed cell death. Instances of autophagic cell death are typically associated with elevated numbers of cytoplasmic autophagosomes, which have been assumed to lead to excessive degradation of cellular components. Due to the high activity and reliance on autophagy in neurons, these cells may be particularly susceptible to autophagic death. In this review, we summarise and assess current evidence in support of autophagic cell death in neurons, as well as how autophagy dysregulation commonly seen in neurodegeneration can contribute to neuron loss. From here, we discuss potential treatment strategies relevant to such cell death pathways.

Keywords: autophagy; autophagic cell death; programmed cell death; apoptosis; necrosis; autosis; neurodegeneration

Introduction

Autophagy is an intracellular process of self-eating, which provides homeostatic maintenance through the capture and degradation of aggregate-prone proteins and dysfunctional organelles. More specifically, autophagy encompasses three separate mechanisms- microautophagy, chaperone-mediated autophagy and macroautophagy, but only the latter will be discussed in this review, and shall be referred to as simply 'autophagy' herein[1].

Briefly, autophagy proceeds through the capture of portions of cytoplasm containing target material inside expanding membranes, which finally enclose to form double-membrane vesicles called autophagosomes. Fully formed autophagosomes are shuttled along microtubules to lysosomes, whereupon fusion and degradation occurs[2, 3]. This removal and recycling serves as an emergency energy supply during starvation, but autophagy has also been linked to a diverse range of other protective roles[4-7]. These include the capture of invading pathogens[8], context-dependent tumour suppressive and tumourogenic qualities[9-12], and the removal of toxic aggregate-prone proteins often linked to neurodegeneration[13-15]. From these findings, interest in autophagy research has surged over the past decade or so[16].

Despite these pro-survival roles, autophagy has also been implicated as a mechanism of programmed cell death (PCD)[17-19]. Numerous studies have reported instances of dying cells displaying accumulated autophagosomes, which engulf large portions of the cell's cytoplasm and which have been presumed to lead to excessive destruction of vital components[20, 21]. However, this notion of 'autophagic cell death' (ACD) has met with some scepticism, with critics arguing that these accumulations of autophagosomes may represent a failed rescue response to a lethal stress, as opposed to a direct lethal mechanism in its own right[22-24].

Neurons have high energy demands, and as post-mitotic cells, quality control and homeostasis maintenance is vital[25]. From these traits, one would assume neurons to rely heavily on autophagy. Yet, these cells typically display very few autophagosomes, suggesting little autophagic activity. However, impairing lysosomal function leads to the accumulation of autophagosomes, revealing that autophagy is highly efficient in neurons, with a quick progression from vesicle formation to

degradation[26, 27]. Due to this high autophagic flux, it is possible that neurons are particularly sensitive to ACD. As pathologies like neurodegeneration are associated with a progressive loss of neurons, an appreciation of the diverse mechanisms of death in these cells may aid the design of future protective and preventative treatments for disease. In this review, after briefly covering the autophagy machinery, we will compare the seemingly paradoxical roles autophagy plays in both promoting cell survival and death in neurons. From there, we will address the implications these have for our understanding of ACD, as well as potential applications for neuron therapy.

Regulation of the Autophagy Machinery

The autophagy machinery is highly conserved. The AuTophaGy-related (Atg) genes were first observed in yeast[28, 29], but many of the 30+ members of this group have mammalian homologues[30]. The different Atg genes regulate each stage of autophagosome formation - from initiation of the process, to the nucleation of the target membrane, and finally its subsequent elongation and fusion, forming the complete vesicle[31]. Various origins of the membrane have been suggested; with sites including the endoplasmic reticulum (ER)[32, 33], plasma membrane[34-36], mitochondrial membrane[37] and Golgi apparatus[38] all receiving support as sources.

Under normal conditions, autophagy proceeds at a relatively low basal rate. Multiple regulators of autophagy have been identified, but in mammals the best characterised is the mammalian Target of Rapamycin (mTOR), which negates the initiation of autophagosome formation. However, upon certain stimuli such as starvation, mTOR is inactivated, allowing autophagy to proceed. The initial stages of the process are mediated by the un-coordinated 51-like kinase 1 (ULK1) complex, which activates the downstream Phosphatidylinositide 3-Kinase (PI3K) Class III complex[39]. Vps34, the only mammalian Class III PI3K, catalyses PI(3)P generation, allowing for recruitment of additional facilitators of autophagosome nucleation. Recently, PI(5)P has been shown to be able to substitute for PI(3)P in this regard, and this lipid is particularly important in responses to glucose starvation[40]. Membrane elongation is completed through the action of two ubiquitin-like conjugation processes: 1) ATG12-5; 2) LC3-phosphatidylethanolamine (PE)[41-43]. Although the ATG12-5-16L1 complex

dissociates from completed vesicles, the LC3-PE conjugate (LC3-II) remains, making it a commonly-used marker of autophagosomes[44]. At the end of the process, autophagosomes are shuttled to lysosomes and autophagosome-lysosome fusion occurs (Fig 1). Lysosomal enzymes like cathepsins degrade the vesicles and their cargo, and permeases release amino acids for recycling[45]. The successful progression from autophagosome formation to degradation is referred to as 'autophagy flux'.

Autophagy in neuronal survival

Perhaps the clearest demonstration of the importance of autophagy in survival is that complete knockout of several of the Atg genes (such as Atg3, 5, 7, 9 and 16L1) results in neonatal lethality in mice[30]. Neuron-specific Atg gene knockouts specifically reveal that basal rates of autophagy are required for normal neuronal survival[46, 47]. Autophagy is utilised as a protective mechanism in response to numerous stresses. As well as during harsh environmental cues such as starvation[48, 49] or hypoxia[50], autophagy action can also promote survival through the clearance of faulty intracellular material. For instance, the specialised subdivision of autophagy that targets mitochondria, mitophagy, serves as a form of quality control for these organelles. Defective mitochondria are targeted by a machinery including PTEN-induced kinase 1 (PINK1) and the ubiquitin ligase Parkin, which ubiquitinates proteins on their outer membrane, allowing for their selective engulfment in autophagosomes. The removal of damaged mitochondria limits the risk of further damage from reactive oxygen species (ROS) generation[51-53]. In the event of ROS production, autophagy is triggered by upstream activators like AMPK, or by increased activity of ATG proteins, again affording protection to cells[53]. In some circumstances, autophagy suppresses apoptosis and necrosis[54-57]. Given these roles, it is of little surprise that autophagy has emerged as one of the central targets in anti-ageing studies. Regimens that enhance the process have led to reductions in pathologies that manifest with age across several models[58].

One of the branches of age-related disease that autophagy has been shown to influence is neurodegeneration. A common feature shared across these pathologies is the progressive accumulation of toxic aggregate-prone proteins. The identity of the

aggregates varies between diseases: Alzheimer's disease (AD) features amyloid-β (Aβ) plaques and intracellular Neurofibrillary Tangles (NFTs) containing Tau aggregates[59, 60]; Parkinson's disease (PD) is characterised by Lewy body inclusions that have α-synuclein as a major constituent[61, 62]; and Huntington's disease (HD) is the result of polyglutamine expansions of the huntingtin (Htt) protein[13, 63]. As neurons are post-mitotic, 'in-house' modes of waste clearance are imperative to prevent the formation of these build-ups[25]. Autophagy seems vital in this regard, as the narrow entry to the proteasome precludes it handling oligomeric assemblies[15]. Therefore, autophagy dysfunction is likely a major contributor to the onset of neurodegeneration. Indeed, autophagic activity has been suggested to decrease with age in human tissues, including the brain[15, 64, 65]. In some cases, degenerating neurons show accumulations of non-degraded autophagosomes in addition to the aggregates, implicating a failure of the lysosomal clearance stage in these diseases[66-68]. An interesting exception is in the case of HD, where mutant Htt appears to reduce the recognition and capture of certain cargoes, as revealed by the recently discovered roles that the protein plays in autophagosome-substrate interactions[69, 70].

Consistent with these data, knockout of the autophagy regulators Atg5 or Atg7 in the mouse CNS results in pathologies comparable to the effects of neurodegeneration, including the presence of protein aggregates coupled with neuronal damage and loss[46, 47]. Beclin-1 (mammalian Atg6 homologue) activity decreases in ageing and neurodegenerative brains, and similarly, its loss enhances aggregate formation in models of AD, PD and HD[64, 71]. Strategies of beclin-1 overexpression complement these findings, with enhanced clearance of toxins and reduced neuronal damage[66, 72]. Mutations commonly associated with neurodegenerative pathologies have also been shown to affect autophagy. As examples, mutated Presenilin-1 in AD alters the acidification of lysosomes, causing a blockage to autophagosome degradation[73]. The AD PICALM locus is a well validated hit from genome-wide association studies and loss of this protein impairs autophagy and tau clearance and toxicity[74]. In PD, defects in Parkin and PINK-1 result in insufficient labelling of damaged mitochondria for mitophagy, increasing the risk of ROS generation and further neuron damage[59]. Furthermore, α-synuclein accumulation which characterises this disease impairs

autophagosome formation[75, 76], and the VPS35 D620N PD mutation has a similar impact on the pathway, which impairs autophagy substrate clearance[77].

Autophagy in cell death

Are increases in autophagy activity always beneficial for neuronal health? The concept of ACD has persisted from the early days of autophagy research, representing Class II death in the recently abandoned morphological classifications of PCD, alongside apoptosis (Class I), and necrosis (Class III)[23, 78]. Cells undergoing ACD are characterised by enhanced numbers of autophagosomes, resulting in extensive cytoplasmic vacuolisation[17, 20]. This has largely been attributed to increases in autophagosome synthesis and flux, causing excessive degradation of important cell components[78]. However, this concept has courted controversy across the literature, with some groups proposing these increases in autophagic vacuoles are representative of roles more in keeping with autophagy as a pro-survival system[22, 24]. Suggestions include autophagy up-regulation as a failing salvage effort against lethal stresses, or a clearance system of dying cells, rather than a direct route of cell death in and of itself. To try and provide more clarity on this issue, various guidelines have been suggested which themselves have attracted criticism for being overly stringent[19, 23]. It has been proposed that for an instance of cell death to be truly mediated by autophagy, then autophagy ablation by pharmacological or genetic inhibitors should provide some protection from lethality. In addition, suppression of apoptotic or necrotic processes should provide no such alleviation. Several cases of ACD have been strongly supported, such as large scale-clearance during development[79-81], and the actions of some chemotherapeutic treatments, at least *in vitro*[21, 82-85]. While a number of studies have supported the concept of autophagic cell death, for instance by showing that the death is attenuated by loss of autophagic genes[21, 79, 85, 86], the interpretations of such studies are not always straightforward. It is possible that one requires some autophagy to enable execution of cell death after certain insults, and such experimental paradigms using autophagy null states certainly support the concept that autophagy may be permissive in these scenarios. However, in order to test if the increased autophagy associated with certain forms of cell death is causal, one needs to ideally manipulate

autophagy back to normality and not to the null state. As this type of manipulation is very challenging, most studies have not excluded the possibility that the increased autophagy they observed is not causing the cell death but rather that some autophagy is required to execute cell death in a manner analogous to ATP being required for apoptosis. The extent of autophagy modulation of cell death may depend on cellular contexts and on the duration and strength of autophagy induction. Berry and Baehrecke[79] initially observed that both autophagy and caspase activity are required for the cell death in salivary glands during Drosophila development, and autophagy selectively degrades the caspase inhibitor dBruce to induce Drosophila ovary cell death[87]. These studies suggest that multiple possible mechanisms may be involved in ACD. In mammalian cells, the role of cellular contexts in ACD remains more elusive, whilst autophagy-relevant proteins such as beclin-1, Atg7 or DRAM were reported to play a role in cell death in a variety of tumour cell lines[88].

ACD in excitotoxic and ischaemic neuron stress

Some of the strongest support for ACD as a pathological process has been found from conditions of excitotoxicity and hypoxia-ischaemia, stresses that may result from traumatic injuries or stroke[89, 90]. Both conditions are potent inducers of autophagy, a response presumably associated with damage limitation and survival promotion[91]. Some have reported that pharmacological induction of autophagy with rapamycin reduces apoptotic and necrotic death during hypoxia, whilst inhibition with 3-methyladenine (3-MA) and wortmannin enhance this loss[50, 91, 92]. Contrary to this, multiple groups have reported that instead this increase in autophagy can contribute to lethality. Using the glutamate receptor activator kainate as a model of excitotoxicity results in death in rat cortical neurons which is largely independent of apoptotic caspase activation. However, this cell death is reduced by repression of autophagosome formation using the PI3K inhibitor 3-MA, or via genetic knockdown of Atg7 and beclin-1[93]. These observations have been supported in other excitotoxic models[94, 95]. Similar approaches during hypoxia-ischaemia have also aided in alleviating neuron loss both *in vitro* and *in vivo*. The administration of 3-MA has proven neuroprotective in multiple rodent hypoxia-ischaemia models[96, 97], although it

is worth bearing in mind that this agent inhibits multiple PI3 kinases and thus has many autophagy-independent effects. Interestingly, Atg7 loss in pyramidal neurons appears to suppress both caspase-dependent and -independent death, suggesting that apoptosis and ACD may both occur in neurons under hypoxia, with autophagy serving as a positive mediator of both processes[94]. The distinctive morphological changes and increases in autophagic vesicles following hypoxia of rat hippocampal neurons has even aided in the coining of a new subtype of ACD, autosis. Autosis has been characterised by an increase in both autophagosomes and autolysosomes, and displays other unique morphological characteristics, such as a mild extent of chromatin condensation and focal swelling of the perinuclear space[86, 98]. Notably, autotic death shows an independence from apoptosis and necroptosis, instead requiring the activity of the Na^+, K^+-ATPase. This pump can be blocked with cardiac glycosides. Neriifolin belongs to this class of compounds, and reduces cerebral infarct size in multiple rodent ischaemic models[99]. Importantly, these improvements in neuron survival are coupled with a decline in the number of autophagic vesicles, as well as the absence of other autotic features[86]. Therefore, autosis seems to be a distinct form of canonical ACD that occurs in neurons. The dependence of this phenomenon on the Na^+, K^+-ATPase may be relevant for the treatment of hypoxia-ischaemia, as many cardiac glycosides have well characterised safety profiles and are widely used in clinical medicine[99].

So, how can we explain these dramatically opposing results of autophagy activation on cell death susceptibility? One possibility is that the extent of autophagy induction dictates the outcome. Physiological levels likely still serve a protective role, and provide an energy source and relief from oxidative stress. However, over-activation may lead to destruction of cellular components, as well as exerting additional strains on the neuron through continued autophagosome formation[89, 100] (Fig 2). This form of ACD by excessive autophagosome stimulation has also been implicated in the neurotoxicity caused by drugs such as MDMA[101]. The involvement of other forms of PCD appears to vary under ischaemic stresses, with both caspase-dependent and -independent cases documented[94, 97]. Whilst neuron type seems an unlikely determinant of which scenario takes place (as both have been observed in the same population of pyramidal neurons[94]), the neuronal region or nature of the stress may shape the outcome[89]. It is important to consider that to date there is no evidence

that specific activation of autophagy induces cell death, and thus it is possible that other signalling pathways induced by different neuronal stressors may determine the impact of autophagic activity on cell survival. It has even been postulated that in certain circumstances, autophagy may promote apoptosis as a form of damage limitation against inflammatory necrosis[91].

Autophagy-lysosome dysfunction in neurodegenerative disease

Rather than over-activation of autophagosome synthesis, the combination of autophagosome and aggregate accumulations seen in neurodegenerative cells are frequently the result of impaired lysosome degradation[63, 102]. Therefore, in such instances it seems unlikely that ACD is occurring via excessive degradation of cellular components, as there will be impaired autophagic flux. However, these accumulations may still have detrimental effects on cell survival. Without a means of waste removal, the hallmark toxic aggregates associated with conditions like AD, PD and HD can accumulate[59]. Other important homeostatic processes, such as faulty organelle removal, will not be fulfilled either in these conditions, which may exacerbate damage. Neurotoxins like rotenone and MPTP mimic PD pathology by inhibiting complex I of the mitochondrial electron transport chain and causing ROS generation[103-105]. Defective mitochondria may disrupt the microtubule-dependent trafficking of autophagosomes to lysosomes further, decreasing clearance of aggregates and ROS even more[106]. These conditions also favour mitochondrial release of cytochrome c to the cytosol, promoting cell death by apoptosis[107]. Elevated ROS have a negative impact on lysosomes: decreasing both number and their membrane integrity. Lysosomal membrane permeabilisation (LMP) can lead to leakage of proteases like cathepsins with damaging consequences[104, 105]. Whilst these events can hardly be classified as a bona fide case of ACD, it still highlights how impairment of the system can lead to neuron death (Fig 3). Autophagy-lysosome dysfunction associated death is likely to be of particular relevance to conditions like AD and PD, which commonly display defective autophagy flux[15, 59, 104].

In such cases, efforts should focus on salvaging lysosomal function and boosting autophagy flux. Pharmacological autophagy inducers have been trialled across a variety of animal models, and can aid the removal of aggregates associated with AD,

PD and HD[59, 108, 109]. In some cases, these treatments provide a degree of cognitive restoration[71, 110]. Notably, a number of these therapies have been achieved with approved drugs, such as rapamycins[71, 111-114], rilmenidine and spermidine[110, 115].

A variety of strategies aimed at a number of targets have adopted this concept, with some encouraging findings. Agents like the small molecule GTM-1 and natural product Arctigenin both enhance autophagic clearance, and are associated with reductions in toxic aggregates and improved cognition in AD mouse models[116, 117]. Boosting cathepsin activity also provides similar benefits, although the incidence of lysosomal membrane permeabilisation in some disease states may make this mechanism unfeasible[118]. Glucosylceramide (GlcCer) has been associated with reductions in lysosomal activity, and inhibitors of GlcCer appear to provide the desired restorative effects for the organelles and improve PD pathology[119, 120]. Niemann-Pick Type C (NPC) is another neurodegenerative disease that bears similar pathology to AD[121]. In NPC mice, enhancing degradation aids neuron survival[122]. An exciting candidate that has emerged in recent years is the Transcription Factor EB (TFEB). TFEB is a positive regulator of a number of lysosomal and autophagy related genes, with its expression associated with enhanced lysosome biogenesis and substrate clearance[123]. As brains of neurodegenerative mice models have been reported to show reductions in TFEB, the effects of elevating its expression have been investigated[25, 67]. Importantly, increased TFEB aids in the degradation of misfolded Tau[124], α-synuclein[67] and mutant Htt[25] both *in vitro* and *in vivo*. There has been less clear support for Aβ plaque removal[124]. Pharmacological autophagy inducers like rapamycin and trehalose also activate TFEB, and aid in the clearance of protein aggregates in neurodegenerative mouse models, as well as reduce the damage associated with neurotoxins like rotenone and MPTP[67, 104, 105]. Table 1 summarises selected pharmacological agents/strategies used for autophagy flux restoration in neurodegenerative models.

Concluding Remarks

The role of autophagy in neuronal survival appears complex. Whilst the homeostatic functions of autophagy seem vital for survival through protection against stress and the removal of toxins, imbalances in the pathway can promote lethality. Interestingly,

it appears that both over-activation and inactivation of autophagy can lead to neuronal death. Support for ACD by autophagy over-activation has come from observations made during periods of harsh stress like excitotoxicity and hypoxia. In these situations, it is possible autophagy acts as an out-of-control protective response, and induces death by excess capture and destruction of intracellular components. Genetic ablation of the autophagy machinery can alleviate this phenomenon. However, it is important to consider that the experimental paradigms that led to the conclusions of autophagic cell death generally use autophagy null states or chemical inhibitors like 3-MA or wortmannin which have multiple autophagy-independent effects. Thus, autophagy may be permissive for certain forms of cell death, but may not be sufficient – we are not aware of any data showing that "specific" autophagy hyper-activation by overexpressing a complete ATG gene induces cell death. Lysosome dysfunction can also lead to cellular aberrant autophagosome accumulation through a block of their degradation, and is associated with neurodegenerative pathologies. In such cases there is reduced autophagic flux and the failure to successfully clear intracellular protein aggregates and ROS means the toxins can propagate unchecked and cause further damage, ultimately leading to cellular demise. These differing mechanisms will influence treatment strategies. Whilst autophagy inhibitors may improve survival in ACD by autophagosome biogenesis over-activation, their application is likely of little use in lysosomal dysfunction. For the latter instance, therapies should instead focus on restoration of lysosomal function and autophagy flux. Therefore, it is clear that targeting autophagy for cell death prevention is not a case of 'one size fits all', and rather, careful consideration is needed before selecting a treatment strategy.

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FIGURE LEGENDS

Fig 1. The schematic diagram of autophagy process

The autophagy process includes autophagosome biogenesis, autophagosome-lysosome fusion and cargo degradation. The ULK1/2-Atg13-FIP2000 complex, negatively regulated by mTOR, senses the signals for autophagosome initiation. The class III PI3-kinase complex, containing Vps34, beclin-1/Atg6, p150/Vps15, and Atg14, generates PI3P required for autophagosome nucleation. Autophagosome elongation involves two ubiquitin-like (UBL) conjugations: the conjugation of the UBL protein Atg12 to Atg5, and the conjugation of the UBL protein LC3 to phosphatidylethanolamine (PE). Atg5-Atg12 conjugation is catalysed sequentially by E1-like enzyme Atg7 and E2-like enzyme Atg10. The Atg5-Arg12 complex then form a larger complex with Atg16 and the site of Atg16 recruitment is enabled by its binding to WIPI2, which, in turn is recruited to membranes enriched in PI3P or PI5P. The Atg5-12 conjugate (Atg5-12) is an E3 ligase that catalyses LC3-PE (LC3-II) conjugation that also requires E1-like Atg7 and E2-like Atg3. LC3-II, the lipidated from of LC3, is required for the expansion and completion of pre-autophagosomal membranes.

Fig 2. One hypothetical model where the extent of autophagy dictates the fate of neurons under stress

When faced with a harsh stress, neurons rely on autophagy induction as a means of protection and damage limitation, suppressing cell death and promoting survival. This means an inefficient or inhibited level of autophagy can be detrimental for neuron health. On the other end of the spectrum, an excessive autophagic response may result in degradation of vital cellular components, culminating in autophagic cell death (ACD). Therefore, it seems in order for autophagy to exert its protective effects, a balance needs to be maintained to avoid neuron death.

Fig 3. Dysfunctional lysosomal clearance of autophagy promotes the accumulation of toxic aggregates associated with neurodegeneration

Efficient autophagic clearance is required to remove potentially toxic aggregate-prone proteins in neurons. In instances of lysosomal dysfunction, these accumulations can form, and, coupled with the loss of protective autophagy, generate further damage to neurons. Ultimately, these stresses can equate to neuron loss and neurodegeneration. Strategies of restoring lysosome integrity and function allow autophagy degradation to resume, and may provide a means of prevention against these pathologies occurring.

Table 1: Selected strategies of autophagy flux restoration in neurodegenerative models (mammalian where available)

Strategy	Neurodegenerative Disease	Changes to Pathology	Reference
Pharmacological			
Rapamycin	Alzheimer's Disease	Autophagy induction; reductions in Aβ and cognitive recovery in AD mice	[71],[112]
	Huntington's Disease	Reductions in Htt aggregate formation, improvements in behavioural tests in mice	[113],[114]
	Parkinson's Disease	Reductions in α-synuclein accumulations, alleviations to neurodegenerative behaviour in mice	[111]
Rilmenidine	Huntington's Disease	Autophagy induction; enhanced clearance of mutant Htt, improved motor performance in mice	[110]
Spermidine	Parkinson's Disease	Autophagy induction; Improved motor performance in fruit fly, Reduced dopaminergic neuron loss in nematodes	[115]
Arctigenin	Alzheimer's Disease	Autophagy induction; Reduction in Aβ plaques through inhibition of formation and enhanced clearance, improved memory in mice	[117]
GTM-1	Alzheimer's Disease	Autophagy induction and increased flux; Removal of Aβ oligomers, cognitive improvements in mice	[116]
Glucosylceramide inhibitors	Niemann-Pick Type-C 1	Corrections to autophagy flux; Improved clearance of cholesterol and autophagic vesicles in mouse and feline models, prolonged neuron survival	[119],[120]
Genetic TFEB	Alzheimer's Disease Huntington's Disease Parkinson's Disease	Upregulation of lysosomal and autophagy genes; Enhanced clearance of Tau, α-synuclein and mutant Htt aggregates	[124] [25] [67]

Fig 1

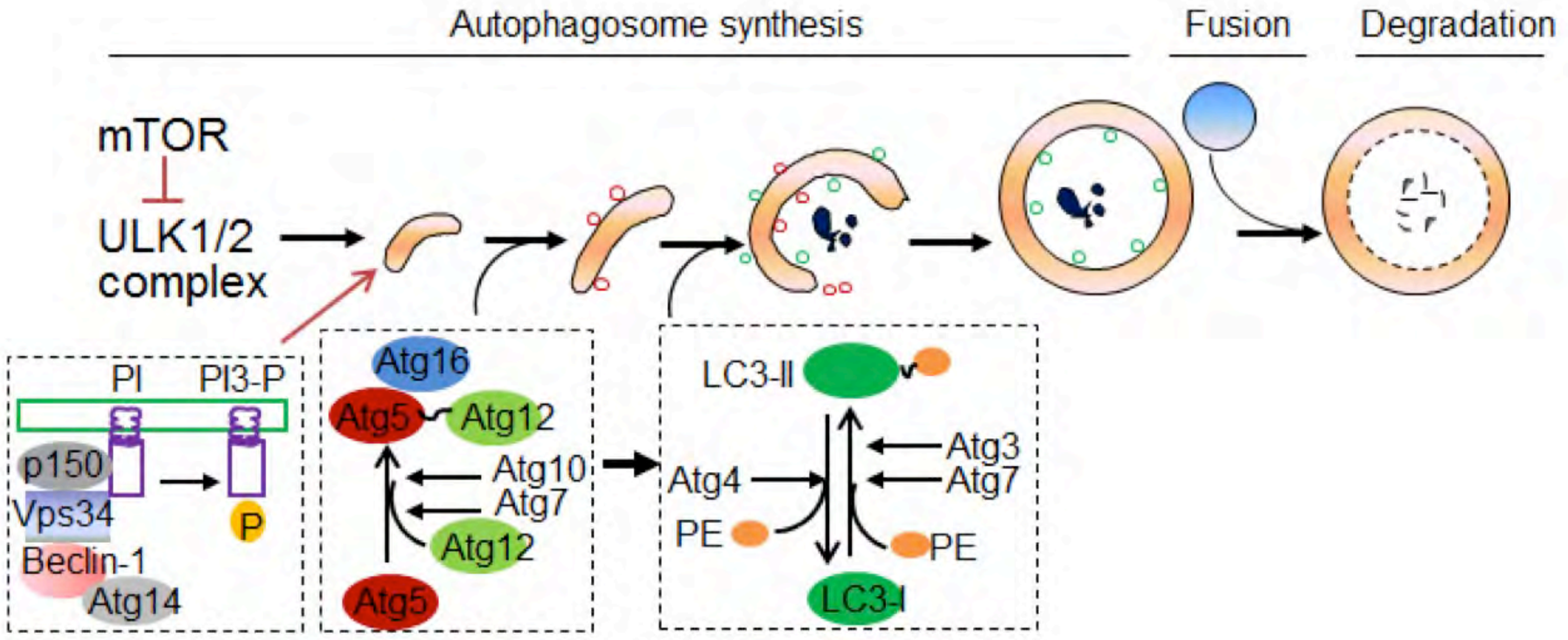

Autophagosome synthesis
Fusion
Degradation
mTOR
ULK1/2
complex
PI
PI3-P
p150
Vps34
Beclin-1
Atg14
P
Atg16
Atg5
Atg12
Atg10
Atg7
Atg12
Atg5
LC3-II
Atg4
PE
Atg3
Atg7
PE
LC3-I

Fig 2

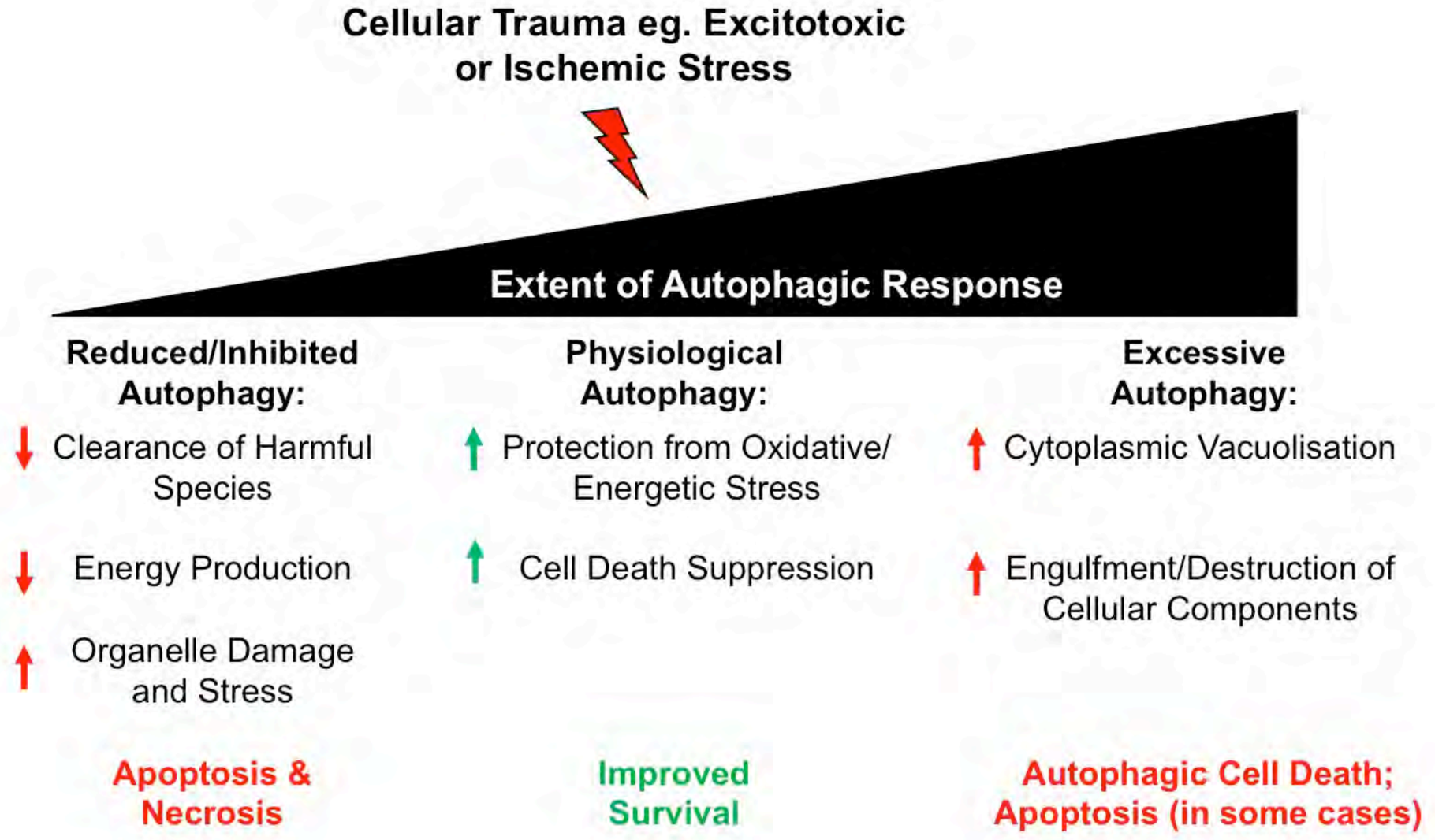
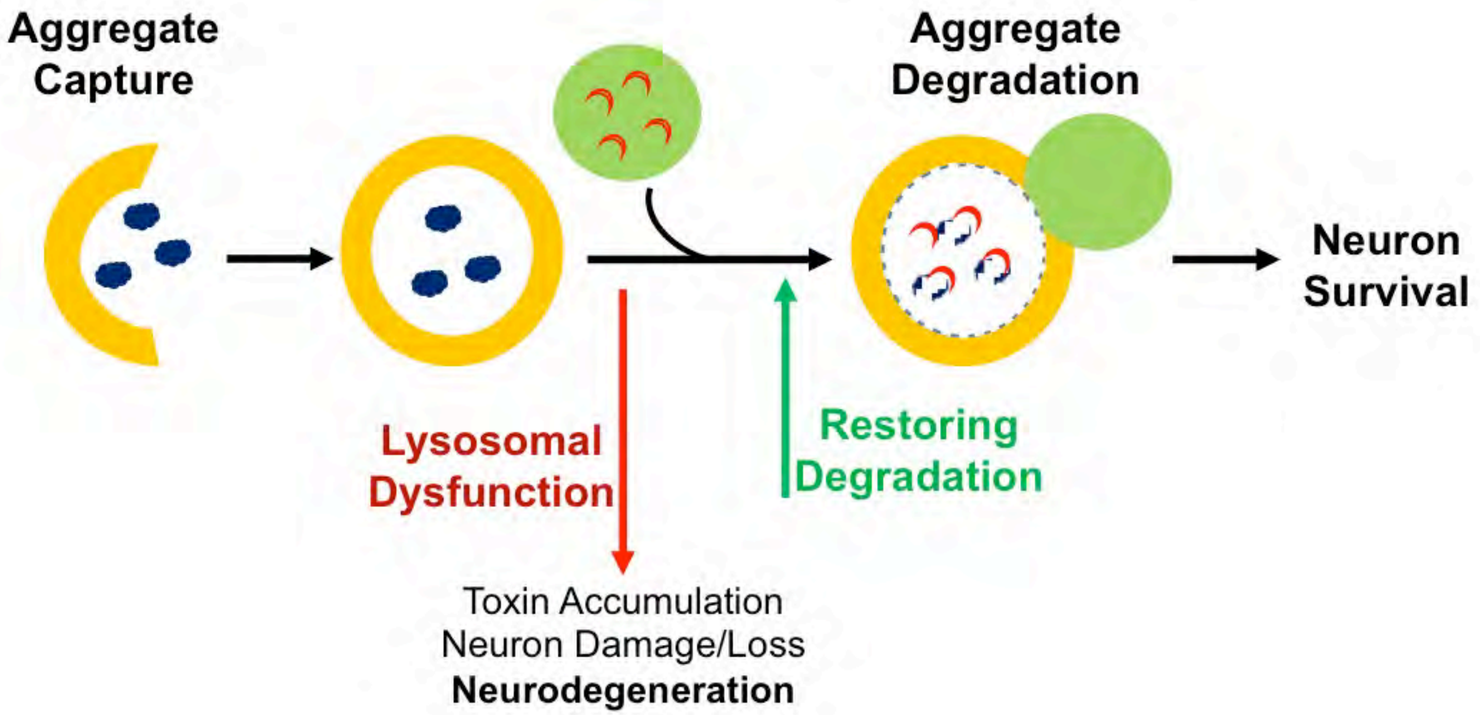

Cellular Trauma eg. Excitotoxic or Ischemic Stress
Extent of Autophagic Response
Reduced/Inhibited Autophagy:
Clearance of Harmful Species
Energy Production
Organelle Damage and Stress
Apoptosis & Necrosis
Physiological Autophagy:
Protection from Oxidative/ Energetic Stress
Cell Death Suppression
Improved Survival
Excessive Autophagy:
Cytoplasmic Vacuolisation
Engulfment/Destruction of Cellular Components
Autophagic Cell Death; Apoptosis (in some cases)

Fig 3


Aggregate Capture
Aggregate Degradation
Neuron Survival
Lysosomal Dysfunction
Restoring Degradation
Toxin Accumulation
Neuron Damage/Loss
Neurodegeneration