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REVIEW ARTICLE

Neurophagy, the phagocytosis of live neurons and synapses by glia, contributes to brain development and disease

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It was previously thought that neurons were phagocytosed only when dead or dying. However, it is increasingly clear that viable synapses, dendrites, axons and whole neurons can be phagocytosed alive (defined here as neurophagy), and this may contribute to a wide range of developmental, physiological and pathological processes. Phagocytosis of live synapses, dendrites and axons by glia contributes to experience-dependent sculpting of neuronal networks during development, but excessive phagocytosis of synapses may contribute to pathology in Alzheimer's disease, schizophrenia and ageing. Neurons can expose phosphatidylserine or calreticulin, which act as 'eat me' signals provoking phagocytosis via microglial receptors, whereas sialylation of neuronal surfaces acts as a 'don't eat me' signal that inhibits phagocytosis and desialylation can provoke phagocytosis. Opsonins, such as complement components and apolipoproteins, are released during inflammation and enhance engulfment. Phagocytosis of neurons is seen in multiple human diseases, but it is as yet unclear whether inhibition of phagocytosis will be beneficial in treating neurological diseases. Here we review the signals regulating glial phagocytosis of live neurons and synapses, and the involvement of this phagocytosis in development and disease.

Phagocytosis and intercellular phagocytic signalling

Phagocytosis is the cellular process of engulfment and digestion of large (> 0.5 μm), extracellular particles, including other cells or parts of cells. The expression 'phagocytosis of neurons' is ambiguous, but in this review will mean neurons being eaten by something else, while 'neuronal phagocytosis' will mean neurons eating something else. So, 'microglial phagocytosis of synapses' means that microglia are eating synapses, and not the other way around.

Cell types specialised for phagocytosis are known as phagocytes, and the main, professional phagocytes in the brain are microglia [1]. Microglia can protect the brain by phagocytosing pathogens, dead cells, dying cells, debris and protein aggregates. As outlined below, they also shape development by phagocytosing excess synapses, dendrites, axons and neurons or neuronal precursors. However, excessive microglial phagocytosis of synapses and neurons may contribute to pathology. Astrocytes can also phagocytose synapses [2], and this is increased by apolipoprotein E [3], brain ischaemia [4] and sleep loss [5]. Astrocytes may also contribute to phagocytosis of myelin debris [6]. In *Drosophila*, astrocytes can also apparently engulf viable axons,

Abbreviations
CR3, complement receptor 3; DAP12, DNAX-activating protein of 12 kDa; Gas6, growth arrest-specific 6; LRP, low density lipoprotein receptor-related protein; MEGF10, multiple epidermal growth factor-like domains protein 10; MerTK, MER receptor tyrosine kinase; MFG-E8, milk fat globule epidermal growth factor factor 8; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; PGRN, progranulin; SIRP, signal-regulatory protein; Syk, spleen tyrosine kinase; TDP-43, TAR DNA-binding protein 43; TREM2, triggering receptor expressed on myeloid cells 2; VNR, vitronectin receptor.

dendrites and entire neurons during development [7]. During inflammation monocytes and neutrophils can enter the brain and may contribute to phagocytosis [8].

Phagocytosis has three main steps: recognition, engulfment and digestion. Recognition (and often engulfment too) is mediated by phagocytic receptors (such as the vitronectin receptor (VNR), MER receptor tyrosine kinase (MerTK) or complement receptor 3 (CR3) on phagocytes) that recognise 'eat me' signals (such as phosphatidylserine or calreticulin), or opsonins (such as growth arrest-specific 6 (Gas6), milk fat globule epidermal growth factor factor 8 (MFG-E8) or complement factors) on the surface of the target cell (see Fig. 1). An opsonin is a normally soluble protein that when bound to the surface of a cell encourages a phagocyte to phagocytose that cell. Classic opsonins include antibodies and complement components C1q and C3b, but also bridging proteins that bind both exposed phosphatidylserine and phagocytic receptors, including MFG-E8, which activates VNR, and Gas6, which activates MerTK. Apolipoprotein E also opsonises by binding both exposed phosphatidylserine and the phagocytic receptors low density lipoprotein receptor-related protein (LRP) or triggering receptor expressed on myeloid cells 2 (TREM2) [9]. The engulfment phase of phagocytosis may require activation of the P2Y6 receptor on phagocytes by its ligand, UDP, coming from neurons [10]. A variety of other receptors can mediate phagocytosis, but there is no clear evidence for the involvement of Fc receptors, scavenger receptors and C-type lectin receptors in neurophagy.

The 'eat me' signal, phosphatidylserine, can be irreversibly exposed on apoptotic cells due to activation of a phosphatidylserine scramblase, Xk-related protein 8 (Xkr8) [11], and inhibition of phosphatidylserine translocases ATP11A and ATP11C [12] (see Fig. 2). However, viable cells can reversibly expose phosphatidylserine as a result of calcium activation of TMEM16 family phosphatidylserine scramblases and/or calcium inhibition of translocases ATP11A or ATP11C [13,14]. Glutamate can cause rapid and reversible phosphatidylserine exposure on neurons, provoking microglial phagocytosis of these neurons if microglia are present at the time of neuronal phosphatidylserine exposure, but such neurons survive long-term if activated microglia are not present [15]. Phosphatidylserine-exposed neurons are normally recognised either via the opsonin Gas6 and MerTK/Axl receptors on microglia [16] or the opsonin MFG-E8 and VNRs on microglia [17]. Calreticulin can act as an alternative 'eat me' signal when exposed on the cell surface [18], and this exposure can result, for example, from endoplasmic reticulum stress, and can evoke phagocytosis via LRP receptors on microglia [19]. C1q can act as an opsonin by binding to cell surface phosphatidylserine, calreticulin or a desialylated surface, and induces phagocytosis via generation of opsonin C3b or direct binding to the phagocytic receptor CR3 on microglia [20] or multiple epidermal growth factor-like domains protein 10 (MEGF10) on astrocytes [21].

Target cells (such as neurons) may also have 'don't eat me' signals (such as cell surface CD47) that inhibit phagocytosis of the target cell by engaging inhibitory receptors [signal-regulatory protein (SIRP) α in the case of CD47] on phagocytes [22]. In contrast, SIRPβ can stimulate microglial phagocytosis, but its extracellular ligand is unclear [22]. Sialylation of the neuronal

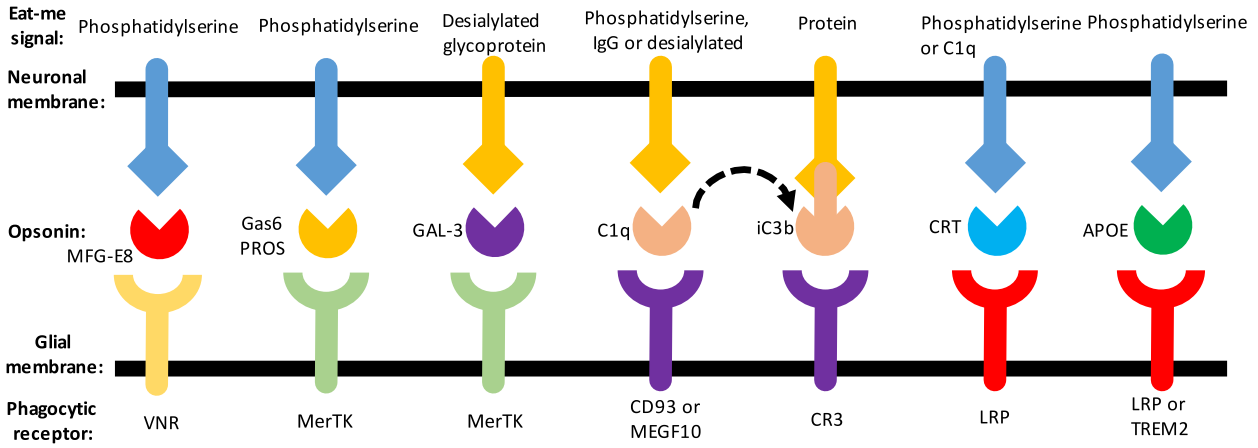

Fig. 1. Intercellular phagocytic signalling. Eat-me signals exposed on neuronal surfaces promote phagocytosis of that surface normally be binding opsonins that bridge between the neuronal surface and phagocytic receptors of phagocytes (microglia or astrocytes).

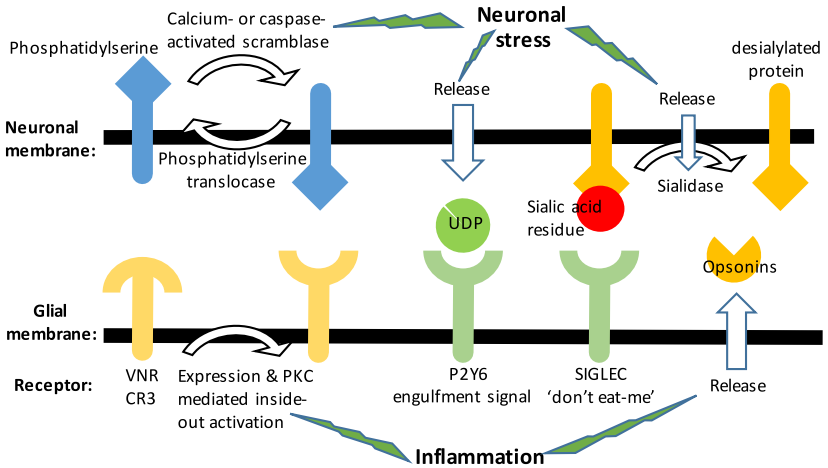

Fig. 2. Regulation of phagocytic signalling. Exposure of the 'eat me' signal phosphatidylserine is mediated by calcium- or caspase-activated scramblases, while re-internalisation is mediated by phosphatidylserine translocases inhibited by caspases, calcium, oxidants or ATP depletion. Neuronal stress may cause release of sialidase, which desialylates glycoproteins converting them from 'don't eat me' signals to 'eat me' signals. Neuronal stress also causes release of UDP, which activates the engulfment receptor P2Y6 on microglia. Glial inflammation causes release of opsonins, and expression and protein kinase C (PKC)-mediated inside-out activation of the integrin receptors VNR and CR3, which when bound to opsonins can also bind to DAP12 to mediate engulfment.

surface also acts as a 'don't eat me' signal, blocking phagocytosis via engaging microglial Siglec receptors [23]. Siglec receptors mostly inhibit phagocytosis, but Siglec-H can activate, for example mediating microglial phagocytosis of live glioma cells [24]. Desialylation also enables phagocytosis of neurons by enabling C1q to bind, promoting phagocytosis via CR3 [20], and potentially also galectin-3 opsonisation and phagocytosis via MerTK [25,26]. Stressed/damaged neurons can release the soluble 'find me' signals ATP and fractalkine that attract microglia by chemotaxis to such neurons via the microglial P2Y12 and fractalkine receptors, respectively [1,27].

The above describes the intercellular signalling regulating phagocytosis of neurons. Within the phagocytes, the phagocytic receptors initiate intracellular signalling to induce phagocytosis. Extracellular engagement of receptors CR3, VNR and TREM2 recruit and activate Src family kinases to phosphorylate tandem tyrosine residues in the intracellular immunoreceptor tyrosine-based activation motif (ITAM) domains of the receptors or associated transmembrane adaptor DNAX-activating protein of 12 kDa (DAP12). The phosphorylated ITAM domain then recruits and activates spleen tyrosine kinase (Syk), which phosphorylates Vav, which activates Rac1 or other G proteins to the GTP-bound form, which activates the cytoskeletal rearrangements of engulfment. VNR can also activate Rac1 via focal adhesion kinase. MerTK and Axl are receptor tyrosine kinases that can recruit Src, and activate phosphatidylinositol 3 kinases and phospholipase C, to activate phagocytosis. Engagement of LRP1 also activates Src and focal adhesion kinase. SIRPα and inhibitory Siglec receptors have intracellular immunoreceptor tyrosine-based inhibition motif domains that recruit and activate tyrosine phosphatase SHP-1, which reverses the phosphorylations mediated by Src and/or Syk, thereby inhibiting activation of phagocytosis [22,23].

Phagocytosis of viable synapses: synaptophagy

Synaptic pruning (also known as synaptic elimination) is the process of synapse elimination that occurs during development, which contributes to neuronal network refinement and learning between birth and late adolescence in humans [28]. Synaptic pruning includes loss of axons and dendrites, and in fact most models of synaptic pruning involve axonal loss or shortening [29,30]. Four mechanisms of synaptic pruning have been proposed: synaptic/axonal degeneration, synaptic/axonal retraction, synaptic/axonal shedding and synaptic/axonal engulfment [29,30]. Only the latter corresponds to synaptophagy, and therefore the terms cannot be used synonymously, and indeed equating synaptic pruning with synaptophagy has led to some confusion in the past.

Evidence that phagocytosis contributes to synaptic pruning includes the following. Astrocytes have been shown to continuously phagocytose synapses in both developing and adult mice, and knockout of the

phagocytic receptors MEGF10 and MerTK has been shown to block synaptic pruning [2]. MEGF10 can bind C1q enabling astrocytic phagocytosis of C1q opsonised cells [21]. Schwann cells have been found to continuously phagocytose synapses at the developing neuromuscular junction [31]. Microglia have been found to engulf synapses during pruning, and the engulfment was dependent upon neural activity, the complement opsonin C3 and the complement receptor CR3 [32]. The complement opsonin C1q localises to synapses, and knockout of C3 also prevent synaptic pruning [33]. Note however that C1q-like protein 1 (C1ql3) regulates synaptic density via the receptor brain-specific angiogenesis inhibitor 3 (BAI3) [34] independent of phagocytosis, so in principle C1q and C3 may also act by mechanisms other than phagocytosis. Neurons can express and release fractalkine (CX3CL1), which recruits microglia via its receptor (CX3CR1), and knockout of this receptor prevents synaptic pruning of synapses by microglia in the hippocampus [27], resulting in deficits in social behaviour in mice [35].

'Synaptic stripping' originally referred to a loss of presynaptic terminals on spinal cord motor neurons as a result of damage to the motor neurons (e.g. after axotomy), and is now used to refer to a secondary loss of synapses onto damaged neurons, or more generally to synaptic loss in a pathological context. For example, injection of dead bacteria into the brain causes synaptic loss mediated by microglia [36]. Synaptic stripping preferentially removes excitatory (rather than inhibitory) synapses, and thus has been suggested to be protective against excitotoxicity [37]. Again, because synaptic loss can occur by multiple mechanisms, synaptic stripping is not synonymous with synaptophagy. The main suggested mechanism of synaptic stripping is physical displacement of the pre- from postsynaptic elements by microglia or astrocytes. However, there is some limited evidence supporting a role of phagocytosis in synaptic stripping including C3 knockout mice having greatly reduced synaptic loss [38].

There is evidence that excessive phagocytosis contributes to synaptic loss in some pathologies. In mouse (amyloid) models of Alzheimer's disease, there is evidence of microglial phagocytosis of synapses, and inhibition or knockout of C1q, C3, or CR3 reduced synapse loss [39,40]. Similarly, viral-induced synaptic loss was accompanied by localisation of complement opsonin C1q to synapses and microglial phagocytosis of synapses, while knockout of complement opsonin C3 or depletion of microglia reduced synaptic loss [41]. HIV-induced synaptic loss is accompanied by microglial phagocytosis of synapses [42]. Knockout of C3 prevented ageing-induced loss of both synapses and neurons in the hippocampus [43]. Microglia lacking TAR DNA-binding protein 43 (TDP-43) have increased phagocytosis, and mice lacking TDP-43 in microglia have drastic synapse loss [44]. Variants of the complement component C4 genes predispose to schizophrenia in humans, and C4 catalyses C3 deposition and contributes to synaptic pruning during development in mice, suggesting that excessive phagocytosis of synapses may contribute to schizophrenia [45]. Note, however, that dozens, perhaps hundreds of genes, contribute to the genetic risk of schizophrenia. A high fat diet can induce loss of hippocampal synapses in mice, accompanied by microglial activation, and microglia isolated from these mice had increased phagocytosis of isolated synapses [46].

The signalling between cells that mediates synaptic loss during development, neuronal injury and neurodegeneration in each case appears to involve the complement pathway C1q–C3–CR3 (see above), where C1q is deposited on synapses, causing C3 derivatives to accumulate on the same synapses, inducing microglia or astrocytes to phagocytose these synapses. However, it is unclear what causes C1q to be deposited on these synapses – candidates include phosphatidylserine, calreticulin and desialylation. And C1q, C3 and CR3 could in principle cause synaptic damage independent of phagocytosis.

Phagocytosis of viable axons and dendrites

Phagocytosis of axons and dendrites includes the phagocytosis of synapses, but phagocytosis of axons and dendrites also results in a larger scale reorganisation of the architecture of neurons and their connections (Fig. 3). Axon pruning removes exuberant neuronal connections during development, and as outlined above there is evidence that phagocytosis contributes to this process [28–30]. Developmental axon loss at the neuromuscular junction is accompanied by Schwann cell phagocytosis of axonal ends [7,31]. In *Drosophila*, glia engulf viable axons during development, and disruption of phagocytosis delays axonal pruning [7,47]. Nerve cell injury resulted in microglia phagocytosis of both injured and uninjured myelinated axons, prevented by blocking the microglial P2Y12 receptor [48]. Wallerian degeneration of axons is accompanied by axonal phosphatidylserine exposure that encourages phagocytosis of the degenerating axons [49], but it is unclear whether blocking this phagocytosis is beneficial or detrimental.

Neurite pruning may in some cases be triggered by sub-toxic caspase activation [50], and sub-toxic caspase

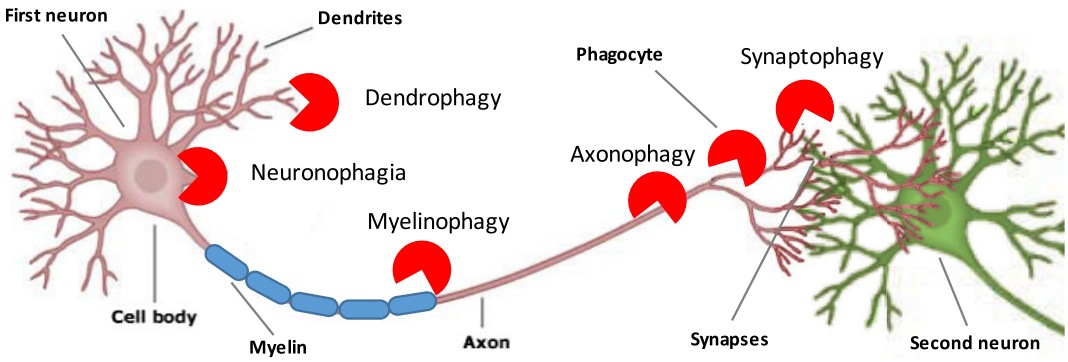

Fig. 3. Types of neurophagy and related processes.

activation can provoke phagocytosis of mammalian cells [51] and in *Caenorhabditis elegans* [52–54]. Alternatively, in *C. elegans*, caspase-activated exposure of phosphatidylserine on the axon enables re-fusion of transected axons [55].

In culture, desialylation of neurons (by adding a sialidase) results in microglia phagocytosing live neurites (dendrites and axons), because (a) sialic acid residues on the surface of neurons activate Siglec receptors on microglia to inhibit microglial phagocytosis, and (b) C1q binds to desialaylated neurons enabling microglial phagocytosis via CR3 [20,23,56,57]. In *Drosophila*, astrocytes can also apparently engulf dendrites during development [7].

Phagocytosis of part of an axon or dendrite might in principle cut across the axon or dendrite to generate fragments. Thus in principle axonal or dendritic pruning or stripping, or axotomy might be mediated by phagocytosis cutting across the axon or dendrite. However, no such action has been reported.

Phagocytosis of intact myelin

Intact myelin is part of oligodendrocytes or Schwann cells, not neurons, so phagocytosis of intact myelin is not neurophagy, but as myelin is structurally and functionally associated with neurons, we briefly review it here. 'Demyelination' refers to loss of myelin in a variety of pathologies, such as multiple sclerosis. Demyelination is generally presumed to result from degeneration of myelin, followed by phagocytosis of myelin debris. And because myelin debris can promote inflammation, which delays remyelination, it has been thought beneficial to promote phagocytosis of myelin. However, in human multiple sclerosis lesions, early myelin damage is accompanied by opsonisation of the myelin by complement and anti-myelin antibodies, together with myelin outfoldings, surrounded by phagocyte processes [58]. This suggests the possibility that demyelination is driven by phagocytosis of intact myelin encouraged by these opsonins. In peripheral nerve cell injury, apolipoprotein D is released and apparently acts as an opsonin to promote macrophage phagocytosis of intact myelin [59]. Injury of spinal cord neurons apparently results in microglia phagocytosing intact myelin, which is prevented by blocking the microglial P2Y12 receptor [48]. Viral infection of microglia can apparently activate them to phagocytose intact myelin sheath [60].

Phagocytosis of viable neuronal precursors

Microglia have been found to phagocytose presumably live neural precursor cells in rat and monkey cortex [61], and knockout of the phagocytic receptors MerTK and Axl increased the number of neuronal precursors in mouse brain [62]. In *Drosophila*, astrocytes can also apparently engulf neurons during development [7]. During development of *C. elegans*, there is indirect evidence that some neuronal precursors and new-born neurons are phagocytosed alive by neighbouring cells, contributing to programmed cell death during development [52,53].

Phagocytosis of viable neurons resulting in neuronal death and neurodegeneration

Cell death by phagocytosis (called phagoptosis for short) refers to cell death resulting from a cell being phagocytosed by another cell. Dead or apoptotic cells are rapidly phagocytosed (so called secondary phagocytosis or efferocytosis), and inhibition of phagocytosis

in this case will not prevent cell death, but rather cause dead or apoptotic cells to accumulate. However, in some circumstances, viable cells are phagocytosed and digested, resulting in their death (so called primary phagocytosis, synonymous with phagoptosis), and in this case inhibition of phagocytosis will prevent cell death, and this property can be used to diagnose phagoptosis [10]. Note, however, that inhibition of phagocytosis may in some cases have other effects, such as inflammation, so it is important to consider whether any such effects may impact on cell death independent of phagocytosis.

We found that inflammatory activation of microglia by lipopolysaccharide, tumour necrosis factor-α or amyloid-β induced neuronal loss via microglial phagocytosis of viable neurons over several days in culture [15,17,63,64]. These activated microglia released oxidants that caused reversible exposure on neurons of phosphatidylserine, which evoked their phagocytosis by microglia [15]. Preventing phagocytosis by blocking phosphatidylserine, calreticulin, MFG-E8, VNR, MerTK, or P2Y6 receptor inhibited neuronal loss induced by microglial activation in culture and *in vivo* [15–17,63,64]. Injection of lipopolysaccharide into rodent brain caused microglial phagocytosis of neurons and neuronal loss, prevented by inhibition or knockout of opsonin MFG-E8 or VNR or P2Y6 receptors [17,63]. Similarly, transient brain ischaemia caused microglial phagocytosis of neurons and neuronal loss, prevented by knockout of MFG-E8 or MerTK [16]. Note, however, that knockout of a different phagocytic receptor, TREM2, was detrimental in brain ischaemia [65].

Phagoptosis has also been implicated in the loss of hippocampal neurons with ageing, as genetic ablation of the opsonin C3 prevented this loss [43]. And loss of retinal rod cells (sensory neurons) in a model of retinitis pigmentosa was prevented by removing microglia or blocking VNRs [66]. In models relevant to Parkinson's disease, loss of dopaminergic neurons in the substantia nigra was decreased by reducing phagocytosis by (a) knockout of complement component C3 in a peripheral lipopolysaccharide model [67], (b) inhibition of the VNRs in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) model [68], (c) Rho-associated protein kinase in an MPTP model [69], (d) knockout of the phagocytic adaptor protein DAP12 in a 6-hydroxydopamine model [70], and (e) blocking exposed phosphatidylserine with annexin V in a histamine model [71]. Knockout of the phagocytic receptors MerTK and Axl increased survival in an α-synuclein model of Parkinson's disease [62]. However, as noted above, in all these models, it is possible that knockout of phagocytic receptors or opsonins may impact on neuronal death by means other than phagocytosis.

Knockout of the complement opsonin C3 reduced neuronal loss and memory impairment, despite increased amyloid plaques in a mouse model of Alzheimer's disease accompanied by microglial activation [40]. Mutations in (and aggregates of) TDP-43 are associated with frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Depletion of TDP-43 in microglia is found to increase phagocytosis and induce synaptic loss [44]. Mutations/polymorphisms in the progranulin (*PGRN*) gene are associated with frontotemporal lobar degeneration, Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis, and inactivation of PGRN causes increased phagocytosis of apoptotic neurons, suggesting the possibility that neurodegeneration may be due to excessive phagocytosis normally prevented by PGRN [72].

As noted above, in *C. elegans* phagocytosis contributes to programmed cell death of neuronal precursors during development [52,53]. However, in *C. elegans* phagocytosis also causes the death of adult cells subjected to sub-toxic insults [54] or cells exposing phosphatidylserine due to the expression of mutant phosphatidylserine translocases [73].

Thus there is evidence that neuronal cell death by phagoptosis is common during development and pathology. Phagocytosis may result in cell death if the cell is engulfed whole. However, a cell can be killed as a result of partial phagocytosis of the cell [74], for example, phagocytosis of dendrites might damage plasma membrane integrity to the extent that the cell necroses. Or phagocytosis might result in axotomy that kills the cell by Wallerian degeneration, although there is no evidence for this. Or, in principle, excessive phagocytosis of synapses could result in secondary neuronal death of the pre- or postsynaptic neuron. Phagocytosis specifically of excitatory synapses could in principle protect by preventing excessive activation and neuronal death by excitotoxicity, whereas phagocytosis specifically of inhibitory synapses could in principle result in excessive activation and neuronal death by excitotoxicity. Thus, neurophagy could result in neuronal death (or neuroprotection) by multiple mechanisms.

Neuronophagia

'Neuronophagia' is a term introduced in the 1890s by the neuropathologist Georges Marinesco when describing fixed brain sections from patients and observing glial cells apparently phagocytosing neurons.

Marinesco used 'neuronophagia' to refer to neurons being destroyed by phagocytosis [75], but the term is nowadays used by neuropathologists to refer to phagocytes eating neurons without signifying whether the neurons are eaten dead or alive and whether phagocytosis is the cause of death, because pathologists cannot currently determine whether a neuron is/was being eaten dead or alive in a fixed brain section. Thus 'neuronophagia' and 'neurophagy' differ in meaning in that the former currently means phagocytosis of neurons (dead or alive) while the latter refers to phagocytosis of live (viable) neurons and/or their component parts. Some reported cases of neuronophagia may be neurophagy. For example, there are multiple reports of neuronophagia in brains infected with different viruses, including the hand-foot-and-mouth disease enterovirus 71, where viral infection resulted in exposure of calreticulin on neurons that were apparently eaten alive by glia [76]. This raises the possibility that one of the functions of neuronophagia and neurophagy is to remove and destroy virally infected neurons to prevent spread of the virus. Neuronophagia has been reported in other human brain pathologies and animal models of disease, including stroke with diabetes [77], epilepsy [78], ageing [79], and models of ageing [80] and multiple sclerosis [81].

Phagocytosis of dead and dying neurons and debris

In addition to eating live neurons, microglia phagocytose dead and dying (apoptotic) neurons, and neuronal debris [82]. This phagocytosis is mediated by neuronal exposure of phosphatidylserine, detected by all the opsonins and receptors targeting phosphatidylserine depicted in Fig. 1. Thus the phagocytosis of dead neurons overlaps with the phagocytosis of live neurons. However, while (a) the complement system of C1q, C3 and CR3, (b) the UDP and P2Y6 system, and (c) neuronal desilaylation can mediate microglial phagocytosis of live neurons or neuronal parts, it is currently unknown whether they mediate phagocytosis of dead neurons. This is important if disease treatments are to be designed to block phagocytosis of live neurons, without preventing phagocytosis of dead neurons or debris.

Perspectives

To what extent does phagocytosis of synapses, dendrites, axons and neurons share common mechanisms? The answer to this question is unclear because the mechanisms have not been compared. However, we do know that the mechanisms overlap. Complement components C1q, C3b and CR3 are known to be involved in phagocytosis of synapses, dendrites and whole neurons (see above). Exposure of phosphatidylserine or surface desialylation can apparently induce phagocytosis of any neuronal component.

There is an enormous amount that we do not know about neurophagy, including the following: (a) What signals on synapses, axons and dendrites promote and inhibit phagocytosis? (b) What role does neurophagy play in physiology and pathology? And (c) if and when is it beneficial to block neurophagy in particular diseases? In order to answer these questions, we need to increase our ability to: (a) image neurophagy *in vivo* and *in vitro*, and (b) detect neurophagy (or some marker thereof) in fixed brain sections.

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Conflicts of interest

The authors have no conflicts of interest in relation to this article.

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