

The role of microglia in synaptic stripping and synaptic degeneration: a revised perspective

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Cite this article as: Perry VH and O'Connor V (2010) The role of microglia in synaptic stripping and synaptic degeneration: a revised perspective. ASN NEURO 2(5):art:e00047.doi:10.1042/AN20100024

ABSTRACT

Chronic neurodegenerative diseases of the CNS (central nervous system) are characterized by the loss of neurons. There is, however, growing evidence to show that an early stage of this process involves degeneration of presynaptic terminals prior to the loss of the cell body. Synaptic plasticity in CNS pathology has been associated with microglia and the phenomenon of synaptic stripping. We review here the evidence for the involvement of microglia in synaptic stripping and synapse degeneration and we conclude that this is a case of guilt by association. In disease models of chronic neurodegeneration, there is no evidence that microglia play an active role in either synaptic stripping or synapse degeneration, but the degeneration of the synapse and the envelopment of a degenerating terminal appears to be a neuron autonomous event. We highlight here some of the gaps in our understanding of synapse degeneration in chronic neurodegenerative disease.

Key words: synapse, degeneration, microglia, synaptic stripping, chronic neurodegeneration.

INTRODUCTION

The focus of much research into pathological processes underlying both acute and chronic neurodegenerative disease has been on the factors that trigger death of the neuronal cell body and the mode of cell death (Friedlander, 2003; Saxena and Caroni, 2007). However, it is likely that by the time the cell body enters a phase of recognizable degeneration, be it by apoptosis or other processes, the pathology is

far advanced and the neuron beyond salvage. It seems unlikely that inhibiting the death of the cell body, which may only take a number of hours or days, is a practical or useful proposition in a chronic degenerative disease that might have progressed over a period of years or even decades. Recently, there has been a shift in emphasis in this debate to determine the earliest events in these diseases: it is thought that this may allow interventions to protect the nervous system from the ravages of degenerative disease. There is evidence that degeneration of synapses is one of the earliest pathological features in several chronic neurodegenerative diseases prior to the subsequent loss of neuronal cell bodies. It is unclear, however, whether the degenerative disease process is an incremental sequence in which there is synaptic dysfunction, synaptic degeneration followed by degeneration of the cell soma, with early synaptic events occurring independently of communication with the cell body, or whether there is continual simultaneous degeneration-related communication between the different compartments of the neuron.

It is also well known that non-neuronal cells of the CNS (central nervous system) play an intimate role in synaptic communication and that processes of astrocytes are well placed to influence synaptic transmission and survival. The so-called tripartite synapse, which comprises pre- and post-synaptic elements and an astrocytic process, demonstrates the intimate relationship of astrocytes and synapses (Perea et al., 2009). The resident macrophages of the CNS, the microglia, have long been implicated in synaptic remodelling in the adult brain. Recent evidence suggests that microglia may selectively contact synapses in their vicinity (Wake et al., 2009) and, unlike astrocytes, these cells make dynamic contacts with the synaptic subcompartment of the neuron. The intimate interactions between microglia and synapses have been hypothesized to bring about the so-called synaptic stripping (Blinzinger and Kreutzberg, 1968), a process in which microglia selectively remove synapses from injured

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Abbreviations: ALS, amyotrophic lateral sclerosis; CNS, central nervous system; CSP, cysteine string protein; ER, endoplasmic reticulum; hAPP, human amyloid precursor protein; LTP, long-term potentiation; NAA, N-acetylaspartate; nNOS, neuronal-nitric oxide synthase; PNS, peripheral nervous system; PrP^{Sc}, abnormal disease-specific conformation of PrP; VAMP-2, vesicle-associated membrane protein-2.

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neurons. We discuss here what is known about the process of synaptic degeneration in the CNS and focus on whether there is evidence that microglia precipitate or participate in this process.

MICROGLIA

Microglia, the resident macrophages of the CNS, are distinct from other tissue macrophage populations on account of their unusual morphology and phenotype, commonly referred to as down regulated or quiescent (Ransohoff and Perry, 2009). The CNS microenvironment has a profound effect in regulating the phenotype of microglia and in recent years some of these molecular interactions have been elucidated. There is a growing list of molecules expressed or secreted by neurons that bind to receptors on microglia. For example, CD200 expressed on neurons binds to the CD200R receptor on microglia, a receptor that contains an ITIM (inhibitory tyrosine immunomodulatory motif). This motif initiates a signalling cascade in macrophages that leads, in turn, to down-regulation of the state of activation of the macrophage (Barclay et al., 2002). In mice lacking CD200, microglia show evidence of being morphologically activated, and these mice are prone to a more rapid onset of clinical symptoms in EAE (experimental allergic encephalomyelitis) (Hoek et al., 2000). CX3CL1 is an example of a molecule expressed and secreted by neurons that regulates the microglia phenotype (Ransohoff and Perry, 2009). The importance of neurons, and likely also astrocytes, in controlling the microglia level of activation is critical to understanding changes in the microglia phenotype in neurodegenerative disease. The demise of the neuron or its processes will lead to loss of regulation of the microglia phenotype, unless of course there are adaptive changes in the expression of either ligands or their receptors.

Microglia monitor the microenvironment

Although the microglia have commonly been described as down regulated, quiescent or resting, in keeping with their role as players in host defence, these cells continually survey their local microenvironment. Elegant *in vivo* imaging studies with two-photon confocal microscopy show that fluorescently labelled microglia continually move their fine processes so as to palpate or sample the surface of cells and interstitial fluid in their immediate vicinity (Davalos et al., 2005; Nimmerjahn et al., 2005). Within a few hours, the microglia processes will effectively make contact with the entirety of surfaces that lie within their territory. The microglia rapidly respond to the presence of a small focal laser lesion in the brain parenchyma and orient their processes towards the tissue damage (Davalos et al., 2005). The sensing of tissue damage by the microglia involves the purinergic receptors and these and other neurotransmitter receptors play a critical role in modulating microglia functions (Hanisch and Kettenmann, 2007).

Using mice that express both fluorescently labelled microglia and cortical pyramidal cells, it has been demonstrated that microglia make contact with synapses within their territory with a frequency of about once per hour, and the time of this contact is of the order of 5 min (Wake et al., 2009). The significance of these transient contacts with synapses is not clear but the release of molecules such as glutamate and ATP may modify these dynamic contacts (Hanisch and Kettenmann, 2007). Reducing synaptic activity in the visual cortex, either by removal of the sensory input or the use of tetrodotoxin, has an impact on the frequency of microglia contacts with synapses, reducing it to about one-third (Wake et al., 2009). On the other hand, microglia make extended contacts with synapses in the penumbra of an ischaemic lesion. These observations are consistent with the microglia playing a role in synaptic homeostasis and in synaptic pathology, but the extent to which these physical or chemical contacts are necessary or sufficient for synaptic dysfunction, degeneration and loss is not yet established.

Microglia in development

In the developing CNS, it has been shown that microglia play a role in the removal of cells undergoing apoptosis (Perry et al., 1985) and there is evidence from studies of slice cultures that microglia play an active role in the killing of supernumerary Purkinje cells (Marin-Teva et al., 2004). It has recently been suggested that microglia may also be involved in the removal of supernumerary synapses. Stevens et al. (2007) showed that mice lacking complement components C1q and C3 have deficits in developmental synaptic refinement in the visual system. They propose that C1q labels synapses that are destined to be removed and speculate that since microglia express receptors for complement components they may be involved in this process. As discussed elsewhere (Perry and O'Connor, 2008), it is at present unclear how a soluble protein such as C1q targets particular synapses, and the lack of involvement of microglia in synaptic stripping in pathology (see below) suggests that more needs to be done before concluding that microglia have a role in refining synaptic connectivity in development. The PU.1 mouse, which lacks microglia (McKercher et al., 1996), offers a way of addressing this issue. Even though the animals die a few weeks post-natally, they survive the period in which microglia have been implicated in synaptic refinement (Stevens et al., 2007).

Microglia and synaptic stripping

The classic work of Kreutzberg and colleagues demonstrated the exquisite sensitivity of microglia to disturbances of homeostasis (Kreutzberg, 1996) and the value of the facial nerve transection model for the study of glia reactivity in the CNS in response to a peripheral nerve injury (see Moran and Graeber, 2004 for references). It was from the study of this model that the concept of microglia involvement in 'synaptic stripping' evolved (Blinzinger and Kreutzberg, 1968).

Injury to the facial nerve results in a reaction of the motor neuron cell body and the retraction and loss of synaptic boutons from the surface of the cell body and proximal dendrites. The cell body response to injury is accompanied by the rapid proliferation of microglia, which then enwrap the cell body with their processes. These observations led to the suggestion that the microglia might be involved in the active removal or 'stripping' of these synaptic contacts. Similar observations on synaptic stripping have been made after injury to the hypoglossal nerve and during inflammation in the cortex (Svensson et al., 1993; Trapp et al., 2007). It is important to note that in the facial nucleus there is no evidence that the microglia are actually phagocytosing or digesting the synapses, it rather may be simply guilt by association. The fine microglia processes are interposed between the presynaptic element and the postsynaptic cell soma, with microglia processes encircling an apparently healthy presynaptic element (see Figure 2; Moran and Graeber, 2004).

To address whether the microglia are actively involved in the removal of motor neuron somatic synaptic contacts after peripheral nerve injury several groups have manipulated microglia proliferation. Inhibition of injury-induced microglia proliferation by the application of intraventricular cytosine arabinoside (Ara C), after transection of the hypoglossal nerve, had no impact on the retraction of synapses from the motor neuron cell body and, importantly, this treatment had no discernable impact on the subsequent success of regeneration of the peripheral nerve (Svensson and Aldskogius, 1993). Similarly, studies in the *op/op* mice, a strain that lacks functional macrophage CSF-1 (colony stimulating factor-1), showed that in the absence of this growth factor, although microglia do not proliferate after peripheral nerve transection, synaptic withdrawal or removal still takes place (Kalla et al., 2001). These studies show that microglia proliferation is not an essential process in the remodelling or withdrawal of presynaptic contacts from an injured neuron of the adult CNS; synaptic pathology induced plasticity proceeds without microglia synaptic stripping.

In the facial nerve model, the lesion occurs distal to the synaptic contact and thus a signal must pass from the lesion to the neuronal cell soma and subsequently to the presynaptic contacts to initiate the synaptic withdrawal response. The signals from the lesion site to the motor nerve cell body, the signals that initiate chromatolysis, have been described recently and involve both a rapid electrophysiological and an importin-mediated injury signal (Rishal and Fainzilber, 2010): these signals activate intrinsic regeneration and repair programmes. Whether importins are involved in the rapid up-regulation of nNOS (neuronal-nitric oxide synthase) activity following nerve injury is unclear (Yu, 2002). However, the time course of expression of nNOS is co-extensive with the period of synaptic withdrawal from the cell body (Sunico et al., 2005) and pharmacological manipulation of nNOS shows that in the absence of NO presynaptic withdrawal is arrested. It was also demonstrated that the up-regulation of nNOS was associated with S-nitrosylation of elements adjacent to

the injured neuronal cell bodies. The authors speculate that changes in trophic factor expression, well documented in the injured facial nerve lesion model (Moran and Graeber, 2004), have a role to play in conjunction with changes in NO levels (Sunico et al., 2005). However, this group's recent work shows that NO leads to the phosphorylation of the myosin light chain, part of a cascade known to cause neurite retraction, and is therefore well suited to the synapse withdrawal process (Sunico et al., 2010). The role of NO as a retrograde messenger for synaptic withdrawal conceptually parallels studies demonstrating a role for NO and other retrograde signalling molecules in modifying synaptic efficacy in the LTP (long-term potentiation) paradigm (Garthwaite, 2008).

The evidence described strongly suggests that synaptic withdrawal from motor neurons following peripheral nerve injury is a neuron autonomous event and does not require active participation of the microglia. It is important to recognize, however, that in this pathology the synapses are not actually degenerating but are still part of an intact healthy presynaptic neuron with the capacity to re-establish functional connections once the peripheral neuron has regenerated. We now consider whether synaptic dysfunction and degeneration in chronic neurodegeneration involves microglia.

SYNAPSE DEGENERATION

There are important diseases of the CNS that are associated with the accumulation of misfolded proteins either extracellularly, such as Alzheimer's disease and prion diseases, or intracellularly, such as Parkinson's disease and ALS (amyotrophic lateral sclerosis) (Soto, 2003). A significant risk factor for many of these chronic neurodegenerative diseases is old age and with increasing life expectancy their prevalence is increasing. A major focus of research has been to understand the processes that link the presence of a misfolded protein and the degeneration of neurons, with the ultimate aim of preventing the neuronal degeneration. At present, this linkage is poorly understood (Aguzzi et al., 2008; Soto, 2003). Although there is evidence that an early and significant part of the pathology of these diseases is the dysfunction, degeneration or loss of synapses (Conforti et al., 2007; Saxena and Caroni, 2007), the demonstration that a particular synapse in the CNS becomes dysfunctional or degenerates prior to either changes in or the demise of the cell body of origin is not a simple matter; this is true of neuron populations in both the human brain and in experimental models. In Alzheimer's disease, synaptic degeneration is an important correlate of the degree of cognitive impairment (Scheff and Price, 2006) and the loss of dendritic spines from neurons is well documented. We do not know, however, precisely where synaptic degeneration was initiated and whether there are changes in the cell body or the axonal

transport of essential synaptic components prior to synaptic changes. The investigation of these early components of neurodegeneration, be they in the pre- or post-synaptic elements, is greatly aided by the development of animal models that simulate at least some components of the human neurodegenerative disease. The synaptic loss that has been documented in human neuropathology has been demonstrated in a number of these models (Table 1). Temporal investigation of these models is often consistent with a linear sequence in which the disease causing insult leads to synaptic dysfunction and loss that precedes eventual neuronal cell loss (Figure 1A), although alternative pathways to neuronal loss may also operate (Figure 1B). A simple progression from synapse dysfunction to degeneration and then cell loss should not, however, be assumed at the present time in all chronic neurodegenerative diseases.

A valuable system in which it has been shown that synaptic degeneration precedes the demise of the cell soma is the animal models of ALS. In transgenic animals, overexpression of mutated forms of the enzyme SOD-1 (superoxide dismutase-1) leads to the death of spinal motor neurons (Turner and Talbot, 2008). In these mice, the peripheral synapses in a defined muscle with their cell bodies of origin in the appropriate spinal cord segment are readily studied and it has been shown that synapses at the neuromuscular junction degenerate prior to the cell soma (Frey et al., 2000; Fischer et al., 2004). However, an elegant analysis of retrogradely labelled motoneurons demonstrates up-regulation of the ER (endoplasmic reticulum) stress pathway prior to the loss of neuromuscular junction synapses and eventual loss of the cell body (Saxena et al., 2009). Although these biochemical

changes at the cell body are the first outward sign of the impending demise of the synapse and cell body, microglia activation precedes even these changes. This paper highlights the importance of knowing the neuronal cell body of origin of synapses, which makes it possible to demonstrate cell body-related changes that may precede both degeneration of the synapse and morphological changes in the cell body. Importantly, it remains to be shown whether ER stress proteins in the neuromuscular junction synapses have also been up-regulated and, if they are, whether changes in expression at the synapse precede, follow or coincide with changes in the cell body.

In the case of protein misfolding, diseases dominated by the deposition of extracellular aggregates the evidence suggests a similar sequence of synapse dysfunction then synapse degeneration followed by neuronal loss. In mouse models of Alzheimer's disease that overexpress hAPP (human amyloid precursor protein) with pro-amyloidogenic point mutations, or in combination with other genes related to familial Alzheimer's disease such as presenilin-1, there are widespread deposits of amyloid in the brain. Many of these models show synaptic loss (Table 1), but limited evidence of neuronal loss, indicating that synaptic degeneration can precede the loss of the cell soma. The synaptic loss is associated with both electrophysiological deficits such as a reduction in LTP and changes in cognitive behaviour (Table 1).

Our own experience has involved prion disease in mice. In these studies, we initiate degeneration by selectively introducing the prion agent into the hippocampus and analyse the evolution of disease pathology associated with the accumulation of misfolded prion protein. The loss of

Table 1 Human pathology and animal models: the case for compartmentalized neuronal degeneration with chronic neurodegeneration following a sequential degeneration process or a simultaneous degeneration of all compartments
The evidence for synapse loss and astrocytic/microglia activation in human disease are designated: ++ good evidence; + some evidence; +/- conflicting evidence. A similar scoring system is used to indicate whether experimental data support synaptic dysfunction, synaptic loss and cell loss in selected animal models of indicated human diseases. In cases where animal models have been assayed temporally, the case for chronic neurodegeneration generating a sequential (Type A) or simultaneous form of degeneration (Type B) is based on the simplified schema depicted in Figure 1. The key references supporting the tabulated conclusions are indicated.

Disease	Human disease		Animal models			
	Synapse loss	Microglial activation	Synaptic dysfunction	Synaptic loss	Neuronal loss	Degeneration type
Alzheimer's (A-beta)	++ (Masliah and Terry, 1994)	++ (Striet, 2004)	++ (Shankar and Walsh, 2009)	++ (Koffie et al., 2009)	+/- (Duyckaerts et al., 2008)	Type A
Alzheimer's (tauopathy)	++ (Mukaetova-Ladinska et al., 2000)	+/- (Striet, 2004)	++ (Polydoro et al., 2009)	++ (Thies and Mandelkow, 2007)	+/- (Ding and Johnson, 2008)	Type A (Yoshiyama et al., 2007)
Prion	++ (Clinton et al., 1993)	++ (Eitzen et al., 1998)	++ (Chiti et al., 2006)	++ (Jeffrey et al., 2000)	++ (Gray et al., 2009)	Type A (Gray et al., 2009)
Huntington's	++ (DiProspero et al., 2004)	+/- (Möller, 2010)	++ (Smith et al., 2005)	++ (Smith et al., 2005)	+/- (Yamada et al., 2008)	Type A/Type B
Parkinson's	++ (Schulz-Schaeffer, 2010)	+/- (Banati et al., 1998)	++ (Bagetta et al., 2010)	++ (Helton et al., 2008)	+/- (Kahle, 2008)	Type B (Sulzer, 2007)
Motor neuron diseases (ALS)	++ (Fischer et al., 2004)	+ (Kawamata et al., 1992)	++ (Geracitano et al., 2003)	++ (Pun et al., 2006)	++ (Pun et al., 2006)	Type A (Saxena et al., 2009)

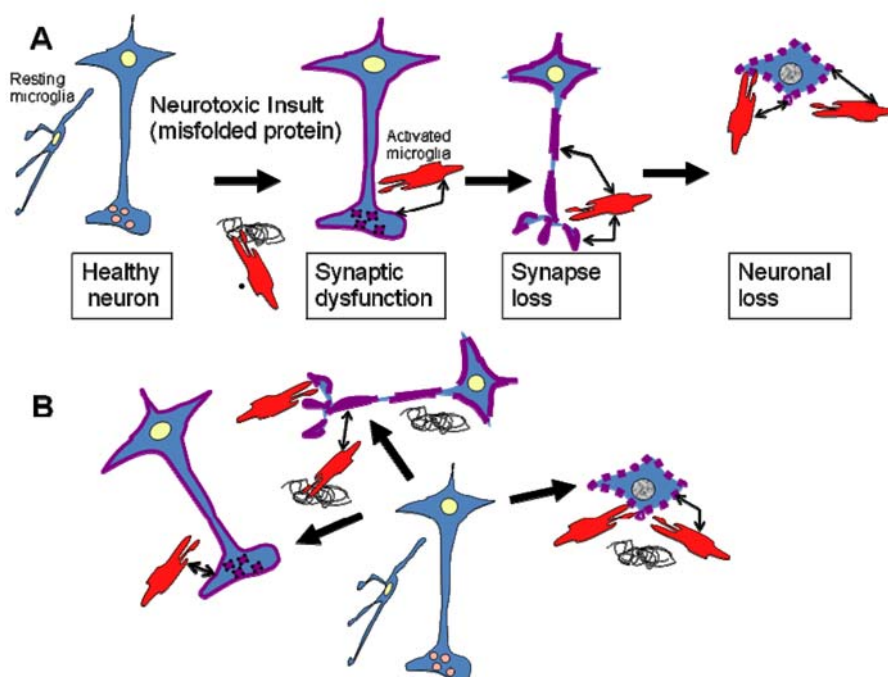


Figure 1 Schemes to illustrate the temporal relationship between the presence of an accumulating neurotoxic misfolded protein and the sequence of events leading to neuronal loss in chronic neurodegeneration

(A) The sequential model. The disease-associated neurotoxic agent (e.g. a misfolded protein) has an impact on neurons that first causes synaptic dysfunction, illustrated here by disrupted synaptic vesicle integrity. This disruption progresses through to synaptic loss in which the synaptic subcompartments and intracellular organelles disappear and in turn compromise the survival of the remaining neuronal structure. The fragmentation of the axon is illustrated, but similar disintegration has been noted in dendritic processes. The loss of synapses, axon and dendrites progresses to a stage in which the cell soma undergoes necrosis, apoptosis or other form of cell loss. Each step, including the neurotoxic insult, synaptic dysfunction, synaptic degeneration and cell loss, has the potential to produce signalling molecules that lead to the activation of microglia: activation of microglia may in turn lead to the synthesis of neurotoxic molecules by the microglia. This two-way interaction is indicated by the double-headed arrows. (B) The simultaneous model. The neurotoxic agent simultaneously impacts on all compartments of the neuron, leading to signalling dysfunction or failure due to simultaneous effects on the synapse, the axon and cell body. The simultaneous injury of all these compartments is most easily visualized as the loss of the cell soma. Activation of microglia by either the misfolded protein or the injured neuron may in turn lead to the synthesis of neurotoxic molecules by the microglia. This two-way interaction is indicated by the double-headed arrows. Few studies have carried out the detailed temporal investigations that allow these distinct possibilities to be directly addressed. Further, it should be noted that reality may lie some where between these two extremes.

synapses has been reported in mouse models of prion disease prior to the onset of neuronal loss. Electron microscopy studies show that as many as 25% of the synapses in the stratum radiatum are lost at a time when there is no detectable neuronal loss from CA3, the neurons of origin of the vast majority of these glutamatergic synapses (Jeffrey et al., 2000; Siskova et al., 2009). The loss of synapses is proposed as the substrate of behavioural deficits at early stages of disease (Cunningham et al., 2003), but it is unclear whether the defects in either behaviour or LTP (Chiti et al., 2006) can be accounted for by synaptic dysfunction prior to synaptic degeneration.

Morphology of synaptic degeneration

Although there are numerous reports documenting synaptic loss in particular brain regions during chronic neurodegenerative disease, what we know about the degeneration process *per se* and the events that initiate it is much more

limited. A significant change in thinking about synaptic degeneration has come about from the discovery of the *Wld^s* mouse (Lunn et al., 1989; Mack et al., 2001). Transection of an axon in either the PNS (peripheral nervous system) or CNS leads to the rapid degeneration of the distal synapses and the axon, Wallerian degeneration. In the *Wld^s* mouse Wallerian degeneration of synapses and axons in both the PNS and CNS is dramatically delayed although, as in wild-type mice, CNS Wallerian degeneration and loss of the ability of axons to conduct action potentials is slower than in the PNS (Lunn et al., 1989; Perry et al., 1991). Prior to the discovery of this mouse, there was little reason to believe that the degeneration of the axon and synapses was anything other than the passive degeneration of processes isolated from the cell soma, their source of support. The discovery that the chimaeric *Wld^s* protein, which arose as a spontaneous mutation in a substrain of C57BL mice, leads to dramatic slowing of Wallerian degeneration (Mack et al., 2001), implies that in wild-type animals there must be biochemical

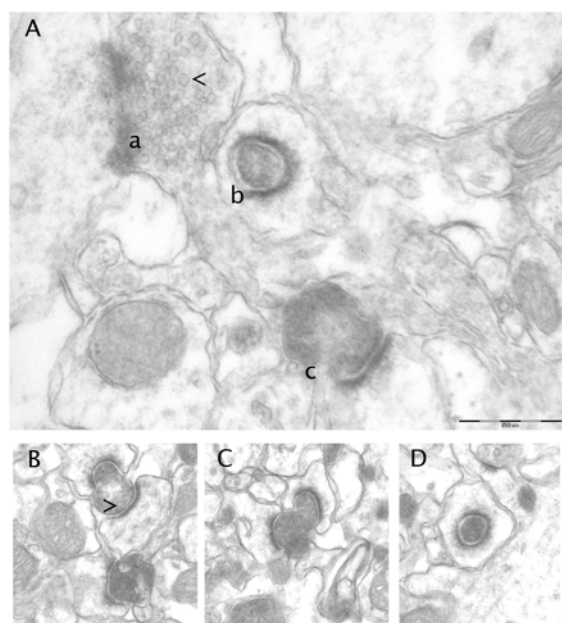


Figure 2 Electron micrographs to illustrate synapse degeneration in the stratum radiatum of a mouse affected by prion disease

(A) The image shows a normal symmetric synapse with well-defined individual vesicles (arrowhead) (a), while in degenerating synapses (b and c) the cytoplasm is electron dense and the integrity of the synaptic vesicles is lost. (B–D) Images of degenerating synapses with varying degrees of envelopment of the dark degenerating presynaptic compartment by the PSD of the dendritic spine. Note that in (B) it is possible to resolve the outlines of some vesicles (arrowhead), but this is lost in (C) and (D). Scale bar in A=500 nm.

processes to both activate and inhibit the axon and synapse degeneration pathways. The current concept of a neuron with distinct compartmentalized degeneration has been reviewed elsewhere (Gillingwater and Ribchester, 2001) and the molecular basis of the mode of action of Wld^s is being hotly pursued (Coleman and Freeman, 2010). If synapse degeneration is an active process, it is critical to understand the sequence of events involved.

In the PNS, when synapses at the neuromuscular junction undergo Wallerian degeneration, there is a withdrawal of the presynaptic ending from the postsynaptic sites, with preservation of both the presynaptic membrane and the synaptic vesicles (Winlow and Usherwood, 1975). Detailed analysis of the degeneration process is facilitated in Wld^s mice with slowed degeneration, and electron microscopy studies show that withdrawal of the presynaptic terminal is associated with retention of synaptic vesicle content, invasion of the terminal by neurofilaments and envelopment of terminal fragments by Schwann cells (Gillingwater et al., 2003). The retraction of the synapse during PNS Wallerian degeneration has clear parallels with modelling of the neuromuscular junction synapse in development. The retracting synapses shed small organelle-rich membrane-bound portions of the axon, so-called axosomes, which are engulfed by Schwann cells (Bishop et al., 2004). Lysosomes in the withdrawing axon and the

Schwann cell have been described and are likely involved in degrading the terminal (Song et al., 2008).

In contrast to the PNS, during Wallerian degeneration in the CNS, degenerating synapses do not withdraw from the PSD (postsynaptic density). The presynaptic terminal has electron dense cytoplasm with the apparent loss of integrity of synaptic vesicles and other organelles, but the presynaptic membrane remains intact and closely adhering to the PSD membrane (Lund and Lund, 1971; Nadler et al., 1980). Studies of Wallerian degeneration in the corticostriatal pathway in both wild-type and Wld^s mice show that the morphology of the degeneration process is identical, although delayed, in the Wld^s mice (Gillingwater et al., 2006). In a small number of instances, the portion of the axon immediately pre-terminal to the degenerating synapse was morphologically intact, consistent with the idea that degeneration of the synapse is indeed a compartment-specific event.

It is clear from the available data that degeneration of CNS synapses induced by an acute injury is distinct from that seen in the PNS at the neuromuscular junction. During acute degeneration of synaptic terminals in the PNS there is involvement of the Schwann cells that phagocytose some of the degenerating material, while in the CNS there is little evidence of direct involvement of either the microglia or astrocytes. Despite their lack of involvement in synapse phagocytosis, microglia rapidly respond to the presence of synaptic degeneration with increased or *de novo* expression of different proteins and a change in morphology (Rao and Lund, 1993; Jensen et al., 1999). The nature of signals from degenerating synapses that lead to activation of the microglia are not known although there are many potential candidates including neurotransmitters and other low-molecular mass mediators (Hanisch and Kettenmann, 2007). While the activation of microglia and the subsequent degeneration of synapses are coincident in time and space, the association may not be a causative one.

Synaptic degeneration in chronic neurodegeneration

In chronic neurodegenerative diseases in humans, the study of synapse degeneration is limited by the availability of tissue with the appropriate tissue fixation and preservation. Animal models of protein misfolding disease offer an opportunity to study these events, in particular, in circuits where the cell body of origin is well defined. Prion disease in mice is a highly tractable laboratory model of chronic neurodegeneration caused by the presence of a misfolded protein (Aguzzi et al., 2008). Unlike many other models, the precise timing and locus of the site of initiation of disease is under the control of the experimenter. The appearance of the first behavioural deficit in the ME7 prion model appears in hippocampal-dependent tasks (Guenther et al., 2001) and, since the circuitry of the hippocampus is well known, this offers the opportunity to dissect the anatomical and electrophysiological substrate of these behavioural deficits. At the time of appearance of the first behavioural deficit there

is no loss of neuronal cell bodies, but there is significant loss of synapses from the stratum radiatum of the hippocampus, as detected by synaptophysin immunocytochemistry (Cunningham et al., 2003). The axons of the CA3 pyramidal cells form en passant synapses on the CA1 pyramidal cells and the varicosities, presumed synapses, are aligned like a string of beads along the axon. The cell bodies of origin of these synapses, the CA3 pyramidal cells, do not degenerate during the course of the disease, but during late stages of the disease they shrink and show abnormal vacuolation of their dendrites (Belichenko et al., 2000; Gray et al., 2009). Quantitative electron microscopy studies have confirmed the loss of the synapses (Jeffrey et al., 2000; Siskova et al., 2009) and allow a study of the morphological events.

The degenerating synapses are recognized by the presence of an electron dense cytoplasm, and the loss of definition of the vesicle integrity (Figure 2) (Siskova et al., 2009). The appearance of the presynaptic element is very similar to that seen in Wallerian degeneration but with a notable difference. In the animals with prion disease, the presynaptic membrane not only remains intact in close apposition with the PSD, but now the PSD also appears progressively curved around the degenerating presynaptic element (Figures 2A–2D) and, in advanced stages, it appears that the PSD has almost completely enveloped the degenerating terminal (Figures 2A and 2C). The progressive darkening of the presynaptic terminal has also been reported in hAPP transgenic mice and there is a notable increase in dark organelles that may be either lysosomes or autophagic vacuoles (Adalbert et al., 2009). This is in contrast to the presynaptic endings seen in prion disease and also in Wallerian degeneration, where there is no apparent increase in lysosomes or autophagic vacuoles. We should also be cautious about drawing too close a parallel between synapse degeneration in Wallerian degeneration and degeneration mediated by accumulation of an extracellular misfolded protein. Wld^S mice do not show prolonged survival when challenged with prion disease (Gultner et al., 2009; VH Perry, H Scott and D Boche, unpublished results).

Perhaps the most striking feature of the prion-induced synaptic pathology is the increased curvature of the PSD. This is a remarkable morphological change that requires profound remodelling of the proteinaceous cytomatrix structure that acts as an organizer of postsynaptic signalling and is the core of the transynaptic process that ensures the tight junction-like association of the pre- and post-synaptic specialization. It is clear that the PSD structure is susceptible to changes in the biochemical composition that impact on plasticity. The idea that the pathological plasticity represents a biologically relevant pathway is reinforced by observations highlighting similar morphological changes in PSDs in hippocampal synapses following the induction of LTP (Connor et al., 2006) and also during reactive synaptogenesis (Marrone et al., 2004). It is suggested that the change in curvature is a compensatory mechanism by which the probability of transmitter release is increased to improve synaptic efficacy (see Marrone et al., 2004 for references).

The presence of degenerating synapses enveloped by the dendritic spine raises a number of interesting questions such as what happens to the dendritic spine and to the axon. We have not yet addressed in the prion disease model whether the degenerating bouton or some of this material is internalized into the spine or dendrite cytoplasm but there are precedents for the envelopment of large structures by neighbouring cells in both pathology and development. Most remarkable is the process of entosis in which whole cells, likely cancerous in nature, are engulfed by neighbouring cells (Overholtzer et al., 2007). In the nervous system, the envelopment of synapses by the neuronal cell body both in normal development and following axon injury has been described (Borke, 1982; Ronnevi, 1979) as has phagocytosis of diverse materials by neurons (Bowen et al., 2007). In prion disease, there is a loss of dendritic spines from hippocampal pyramidal cells, but the relationship with the presynaptic changes is not established (Belichenko et al., 2000). *In vivo* imaging studies of cortical pyramidal cells during prion disease progression reveal that dendritic spines are retracted over a period of several days, with the appearance of dendritic varicosities (Fuhrmann et al., 2007). A further intriguing question is whether the degeneration of a synapse along the Schaffer axon leads to the loss of only that synapse or to the degeneration of the axon distal to the degenerating synapse as well. Does the spine envelopment compromise the survival of the distal axon? Studies are in progress to try and resolve this matter.

Microglia in synaptic degeneration

The electron microscopy studies allow us to address the issue of whether microglia are involved in the phagocytosis of degenerating synapses. In the prion model, there is no evidence at any stage during the envelopment of the synaptic terminal by the PSD that processes of microglia, or indeed other glia, are directly involved in the degeneration process (Figures 2A–2D). Three-dimensional reconstructions from both conventional and dual-beam electron microscopy rule out the presence of non-neuronal processes between the presynaptic and postsynaptic elements (Siskova et al., 2009). In other models of either Wallerian degeneration in the CNS or chronic degeneration, the synaptic boutons do not appear to be enveloped by microglia processes (Adalbert et al., 2009; Scott et al., 2010). The microglia respond early to the presence of prion pathology in the hippocampus (Betmouni et al., 1996) and although the microglia are able to phagocytose latex beads delivered to the hippocampus of prion-diseased animals (Hughes et al., 2010) they do not engulf the degenerating synapses.

The microglia in the hippocampus of mice with prion disease have an activated morphology but are associated with an anti-inflammatory phenotype, dominated by the presence of transforming growth factor- β and prostaglandin- E_2 , akin to that seen in macrophages that have phagocytosed apoptotic cells (Perry et al., 2002; Savill et al., 2002). Given the suggestion that synapse degeneration is an active compartmentalized

auto-destructive process (Conforti et al., 2007; Gillingwater and Ribchester, 2001) one hypothesis would be that this phenotype arises as a consequence of phagocytosis of the degenerating synapses. This is clearly incorrect, since the microglia do not seem to be involved. However, at this stage, we cannot rule out the possibility that the conspicuous shrinkage of the presynaptic element is associated with the shedding of exosomes, nanoparticle-sized membrane vesicles, which has been described in the PNS (Bishop et al., 2004) and also for CNS neurons in culture (Faure et al., 2006). It has been suggested that supernumerary synapses in development and synapses in neurodegenerative conditions might be opsonized by complement prior to phagocytosis by microglia (Stevens et al., 2007). It is unclear whether the complement cascade is activated or decorates the degenerating synapses in the early stages of the evolution of prion disease. The possibility that the slow accumulation of misfolded protease-resistant prion protein [PrP^{Sc} (abnormal disease-specific conformation of PrP)] activates the microglia is difficult to establish *in vivo*, but it is notable that in peripheral tissues, such as the spleen where PrP^{Sc} is also deposited, the local macrophages do not show an activated phenotype (Cunningham et al., 2005). The factors that lead to the morphological activation of the microglia with an anti-inflammatory phenotype are yet to be identified.

Biochemical events in synaptic degeneration

In addition to morphological studies of synapse degeneration, some attempts have been made to investigate the biochemical events associated with the morphological changes. Although a number of studies have shown the loss of synaptic proteins in late-stage disease in both human and animal models, these are not particularly informative, since the loss of synaptic proteins is unsurprising if there is also neuronal degeneration. We have attempted to address this issue by studying the time points in prion disease when there is ongoing synaptic loss prior to detectable neuronal loss. The quantification of the synaptic proteins from an isolated region of the diseased brain is not straightforward, since it is not immediately clear what a particular synaptic protein should be quantified relative to. The usual housekeeping proteins used in many studies are of little intrinsic value in a condition where the non-neuronal cells are dramatically changing in number and changing their proteome (see Gray et al., 2009 for discussion). We have thus used a method to quantify the absolute protein loading on Western blots and then studied both pre- and post-synaptic protein levels at different stages of disease evolution. A number of proteins associated with the synaptic vesicle membrane were the first to show reduced levels of expression, including VAMP-2 (vesicle-associated membrane protein-2), synaptophysin and the chaperone CSP (cysteine string protein) (Gray et al., 2009), and these proteins were also reduced when compared with the levels of PSD-related proteins. The loss of presynaptic proteins is consistent with the early morphological changes in which there is a loss of vesicle integrity but an apparently morphologically intact PSD.

The changes in CSP expression are of particular interest, as it has been shown that deletion of the CSP gene in mice (CSP^{-/-} mice) leads to a synapse degeneration phenotype and dramatically shortened lifespan of the mice (Fernandez-Chacon et al., 2004). Whether microglia or astrocytes are involved in any stage of synaptic degeneration in the CSP^{-/-} mice is not known. The degeneration phenotype of CSP^{-/-} mice can be rescued by overexpression of the synaptic protein α -synuclein (Chandra et al., 2005), suggesting an important interaction between these two proteins. Hence, one might expect that the deletion of this gene would lead to accelerated synaptic degeneration in chronic neurodegeneration. A comparison of prion disease progression by behavioural, biochemical and anatomical methods surprisingly revealed no difference in disease progression in mice with or without α -synuclein (Asuni et al., 2010). A number of *in vitro* models have interesting parallels with the loss of presynaptic proteins and the changes in LTP described in the prion model (Chiti et al., 2006). Parodi and colleagues (Parodi et al., 2010) have shown that chronic treatment of neurons with A β oligomers leads to the loss of presynaptic proteins and reduced spontaneous activity. In another model, overexpression of human α -synuclein in hippocampal neurons has a profound effect in down-regulating the expression of a number of important proteins in synaptic vesicles including VAMP-2, piccolo and synapsin-1 (Scott et al., 2010). The authors report abnormally large synaptic vesicles, suggesting a fusion of vesicle membranes.

At the neuromuscular junction, the Wallerian degeneration of synapses is not associated with a loss of synaptic vesicles but there may be a loss of integrity of mitochondria (Gillingwater et al., 2003). The idea that oxidative stress contributes to chronic neurodegenerative disease is widespread, but how it might specifically contribute to degeneration of the synaptic compartment has not been addressed. We have investigated whether mitochondria in the hippocampus of prion-diseased mice with synaptic loss are also affected. We found that although the mitochondria density remains normal, the mitochondria show subtle morphological abnormalities and a reduction in cytochrome c oxidase activity (Siskova et al., 2010). The change in mitochondrial function is consistent with magnetic resonance spectroscopy measurements showing a reduction of the NAA (N-acetylaspartate)/choline and NAA/creatine ratio in the hippocampus of prion-diseased mice at the time of synaptic loss (Broom et al., 2007). NAA is widely accepted as a marker of neuronal functional integrity and is closely linked with a bio-energetic role in neuronal mitochondria (Moffett et al., 2007). It is at present not clear whether the mitochondrial changes are a cause or a consequence of the synaptic degeneration and the events leading to mitochondrial cytochrome c oxidase impairment are not known. However, during the period of early synaptic loss and mitochondrial abnormalities, there is an enhanced expression and activity of nNOS in the stratum radiatum (Picanco-Diniz et al., 2004). This could, as discussed earlier, modulate synaptic function and additionally impair mitochondrial function.

CONCLUSION

Despite the emerging and obvious importance of synaptic degeneration in chronic neurodegenerative diseases of the CNS, it is surprising how little we know about the molecular events that underlie this process. There is a growing body of evidence to show that synaptic degeneration takes place without overt morphological changes at the cell body, but it remains to be proven that synaptic degeneration takes place in a truly pre- or post-synaptic compartment-specific fashion independent of communication from the cell body. It is clear that at least in some circuits the demise of the presynaptic element precedes the degeneration of the postsynaptic component, but this may not be universal. The degeneration of synapses in the CNS during both Wallerian degeneration and during chronic neurodegeneration is distinct from Wallerian degeneration-induced synapse retraction at the well-studied peripheral neuromuscular junction. The evidence suggests that in the CNS, the loss of synaptic vesicle integrity is an early stage in the degeneration of the presynaptic terminal and is associated with mitochondrial dysfunction, but there is little evidence for the direct involvement of microglia and astrocytes. It is apparent that the degeneration of the CNS synapse is very different from a simple reversal of developmental CNS synapse formation where the immature pre- and post-synaptic partners find each other and establish an initial contact, which stabilizes and matures if the connection is functionally appropriate (Jin and Garner, 2008). The synapses that are lost in major neurodegenerative diseases will have undergone the maturation steps that lead to the formation of a tightly adherent synaptic specialization. In view of the functional co-reliance of presynaptic and post-synaptic components of mature synapses, it is perhaps not surprising that the degeneration process does not involve the synaptic elements separating and going their separate ways. Uncovering the sequence of events involved in the dysfunction, disassembly and degeneration of these mature CNS synapses is an important area for future research involving this fundamental component of chronic neurodegenerative diseases.

FUNDING

The work in the authors laboratory is supported by The Wellcome Trust, Medical Research Council (UK), Alzheimer's Disease Society and The European Union.

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Received 13 July 2010/17 September 2010; accepted 22 September 2010

Published as Immediate Publication 22 September 2010, doi 10.1042/AN20100024