Decreased regenerative capacity of oligodendrocyte progenitor cells (NG2-glia) in the ageing brain: a vicious cycle of synaptic dysfunction, myelin loss and neuronal disruption?

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Abstract

Oligodendrocytes are specialised glial cells that myelinate CNS axons. Myelinated axons are bundled together into white matter tracts that interconnect grey matter areas of the brain and are essential for rapid, integrated neuronal communication and cognitive function. Life-long generation of oligodendrocytes is required for myelination of new neuronal connections and repair of myelin lost through natural 'wear and tear'. This is the function of a substantial population of adult oligodendrocyte progenitors (OPs). Notably, there is white matter shrinkage and decreased myelination in the ageing brain, which is accelerated in dementia. The underlying causes of myelin loss in dementia are unresolved, but it implies a decline in the regenerative capacity of OPs. A feature of OPs is that they form neuron-glial synapses and respond to glutamate released by neurons via a range of glutamate receptors. Glutamate neurotransmission onto OPs is proposed to regulate their proliferation and differentiation into myelinating oligodendrocytes. Here, we discuss evidence that deregulation of glutamate neurotransmission in dementia and compromised generation of oligodendrocytes from OPs are key features of myelin loss and associated cognitive decline.

Key Words: Alzheimer's disease, dementia, oligodendrocyte, myelin, oligodendrocyte progenitor, white matter, glutamate

INTRODUCTION

The massive computing power of the human brain depends on bundles of myelinated axons that form the white matter which interconnects widely dispersed neuronal networks in the grey matter areas of the brain. Myelin is produced by specialised cells called oligodendrocytes that insulate nerve cell axons or fibres to form the white matter and is essential for cognitive functions [1]. Notably, myelin is generated throughout life by oligodendrocyte progenitors (OPs), but declines in humans after 50 years of age. White matter loss is among the earliest brain changes in Alzheimer's disease (AD), preceding the tangles and plaques that characterize neuronal deficits [2]. This has led to the hypothesis that myelin loss may be related to a failure in recruiting OPs in the ageing brain, which is accelerated in AD. Furthermore, synaptic activity is believed to be important in regulating both the expansion of OPs and their differentiation into oligodendrocytes. Here, we propose that disruption of synaptic signalling in AD may be important in the decreased capacity for OP regeneration.

Oligodendrocytes and myelin are generated throughout adult life

In humans, white matter volume steadily increases until the age of 50, but declines thereafter [3]. Studies in primates and rodents provide evidence that the generation of new oligodendrocytes in the adult is essential for continued growth and replacement of myelin loss through natural 'wear and tear' [4, 5]. Learning in adult humans and rodents results in structural changes in white matter and formation of new neuronal connections that depend on oligodendrocytes for myelination [6, 7]. Fate-map studies in mice demonstrate that new oligodendrocytes are generated from a pool of adult OPs [5, 8, 9]. Adult OPs are primarily identified by their expression of NG2 and PDGFRα and represent the largest proliferative

cell population in the adult brain [10]. Like stem cells, OPs divide asymmetrically to form 'sister OPs', one for self-renewal of the OP population and the other differentiates into an oligodendrocyte. In this way, adult OPs generate new oligodendrocytes at a slow rate throughout life and this is increased following CNS injury or demyelination, where proliferation of OPs is vital for regenerating oligodendrocytes and myelin [5, 11-15]. However, OP's capacity for self-renewal decreases with age [5, 16, 17]. This is concomitant with white matter shrinkage and an accumulation of white matter lesions in the ageing brain [18], which appears to be due to a combination of myelin loss and a decline in remyelination [19]. This has led to the hypothesis that decreased recruitment of OPs is central to the failure of myelination in the ageing brain [20-22]. Moreover, a failure of oligodendrocyte regeneration from OPs is a major feature of multiple sclerosis (MS), amyotrophic lateral sclerosis sclerosis (ALS), and stroke, emphasizing the criticality of regenerative processes in maintenance and functioning of the nervous system [20, 23, 24].

Oligodendrocyte and myelin pathology are major factors in AD

The loss of myelin with age is central to cognitive decline in dementia [3]. MRI studies indicate that myelin loss contributes to the cognitive deficits observed in dementia through disruption of rapid transmission and the loss of synchronization of higher cognitive functions [25]. Ultrastructural analysis of the normal ageing brain has confirmed a 20% decrease in the number of myelinated nerve fibres of cerebral white matter fibre tracts associated with frontal lobe areas critical in cognitive processing [26]. Post-mortem studies have identified myelin loss from frontal and temporal lobes as a key feature of vascular dementia, dementia with Lewy bodies and AD [27]. Furthermore, myelin disruption has been demonstrated as an early feature of animal models of AD, correlating with the earliest appearance of Aβ

accumulation in the 3×Tg-AD mouse model [28]. There is clear evidence of Aβ toxicity in oligodendrocytes [29, 30], and myelin disruption is correlated with increased levels of Aβ in human AD [31]. The close correlation between myelination defects and learning/memory deficits implicate loss of oligodendrocytes early in disease-related cognitive decline.

Synaptic control of myelination

There is evidence that OPs receive instructive signals from axons for myelination in an activity dependent manner via glutamate signalling [32, 33]. A key feature of OPs is that they form synapses with neurons and respond to glutamate released by neurons at grey matter synapses and along white matter axons [34-39]. OPs have prominent expression of both AMPA- and NMDA-type glutamate receptors, with evidence that activation of AMPA receptors regulates OP proliferation and migration [2, 6], and NMDA receptors mediate activity-dependent myelination [7, 32]. Axons have mechanisms for vesicular release of glutamate during action potential propagation [37, 38, 40] and glutamate signalling provides a mechanism of adaptive myelination of electrically active axons [7, 32]. Blockade of axonal electrical activity slows down OP proliferation and myelination, whereas stimulation increases OP proliferation and myelination [41-43]. In addition, AMPA and NMDA receptors are expressed by mature oligodendrocytes and myelin, which has led to the hypothesis that communication between axons and myelin represents a new type of 'axon-myelin synapse' [44]. After demyelination, neuron-OP synapses are formed during spontaneous remyelination, suggesting that synaptic glutamate signalling is important in the early stages of remyelination [45]. Thus, myelination/remyelination is dependent on axonal electrical activity and glutamatergic signalling even in adulthood.

Neurotransmission is altered in ageing white matter

Many studies have demonstrated that in AD synaptic activity is impaired in grey matter, e.g. in the 3xTg-AD mouse [46]. However, white matter has been largely overlooked in this context. We have used a whole genomic approach to examine neurotransmission in the ageing mouse optic nerve, one of the most commonly studied white matter preparations, which we and others have shown has prominent glutamate-mediated signalling [47]. Microarray analysis highlights the prominent expression of transcripts for vesicular release machinery in CNS white matter, with significant changes in the SNAP-SNARE complex, from SNAP23/Synaptobrevin2/Synaptotagmin4/syntaxin6,7 in the mature adult nerve to SNAP25/Cellubrevin/Synaptotagmin4/syntaxin12 in the 18-month ageing optic nerve (Figure 1). Differential regulation of synaptic proteins in the frontal and temporal cortex is a feature of the ageing brain and AD [48]. Our analysis of the optic nerve indicates similar important age-related changes in synaptic signaling in CNS white matter, which may play a role in white matter loss in AD. Moreover, altered synaptic signaling is implicated in other neuropathologies in which OP regeneration is compromised, including MS, ALS, and stroke, further emphasizing how critical these mechanisms for the maintenance of CNS function [20, 23, 24, 47]. Microarray analysis of transcripts for the different glial cell types indicates a clear reduction of oligodendrocyte/myelin genes with age (Figure 2A). Notably, there was a marked decrease in the OP marker Pdgfra in the aging nerve, as well striking decreases in transcription factors that regulate OP differentiation - Nkx2.2, Sox10 and Olig2 (Figure 2A) - which are essential for initiating oligodendrocyte differentiation [49]. The microarray analysis also indicated increased glutamatergic and GABAergic signaling in the ageing optic nerve, whereas purinergic and adenosine signaling were proportionally decreased (Figure 2C). Purine receptors mediate both oligodendrocyte protection and destruction, and

dysregulation of purine signaling may be important in white matter shrinkage in the ageing brain [50]. GABA signalling was highest in the ageing nerve and inhibits OP proliferation [51], and increased glutamatergic signaling is consistent with oligodendrocyte pathology [23, 47]. Closer examination of glutamate receptors identified marked changes in vesicular glutamate transporters and NMDA receptors in the ageing optic nerve, with an 80-fold increase in the vesicular glutamate transporter VGLUT1 and a 25-fold increase in GluN2B and GluN2C (Figure 2C). OPs express GluN1, GluN2B and GluN2D, whereas oligodendrocyte NMDA receptors are likely to be formed from two GluN1, one GluN2 and one GluN3 subunit, which reduces their Mg^{2+} block and makes them active at resting membrane potential [52-54]. In addition, OPs express AMPA receptors that have significant Ca^{2+} permeability suggesting they lack the GluA2 subunit [35, 36]. There was an apparent increase in GluA2 and decrease in GluA4 in the ageing optic nerve, which may reflect a shift in AMPA receptor functionality. These results indicate that multiple aspects of glutamate signaling are deregulated in ageing white matter, which has implications for regulation of OP recruitment and myelination. Furthermore, there was a 3-fold decrease in the glial glutamate uptake transporter GLAST1 (EAAT1/Slc1a3), suggesting extracellular glutamate levels may be raised in the ageing nerve (Figure 2C). A concomitant decrease in glutamate uptake and increase in NMDA receptors would have major implications for oligodendrocyte/myelin pathology [55] and is consistent with changes in synaptic glutamate signalling being interwoven with disruption of OPs and myelin loss in AD, resulting in white matter shrinkage and cognitive decline.

Early changes in OPs in AD

Myelin defects are a prominent feature of human AD and animal models of AD. For example, in the 3xTg-AD model myelin defects coincide with Aβ plaques and impairment of synaptic activity [28, 46]. The 3xTg-AD mouse harbours three mutations: human presenilin-1 M146V ($PS1_{M146V}$), human amyloid precursor protein Swedish mutation (APP_{Swe}) and the P301L mutation of human tau (tau_{P301L}). This model develops both plaque and tangle pathology, in an age-related and progressive manner, in AD-relevant brain regions such as hippocampus, amygdala and cerebral cortex [46]. It is notable, therefore, that our examination of the 3xTg-AD mouse demonstrates marked changes in OPs throughout the hippocampus (Figure 3). OPs are widely distributed throughout the brain, including the hippocampus, a primary site of pathology in AD (Figure 3A). In age-matched non-Tg mice, OPs have a characteristic multi-processed morphology (Figure 3Bi) and often appear as duplets or triplets (Figure 3Ci, Cii), which indicates they were recently divided [56]. In the 3xTg-AD mouse, early changes in OPs are detected at 6-months, with apparent OP atrophy (Figure 3Bii, Biii) and a significant decrease in OP sister cells (Figure 3Ciii). At later stages, in the 24-month 3xTg-AD brain, OPs are intimately associated with Aβ plaques, which appear to be circumscribed by OPs and infiltrated by their processes (Figure 3D). This is consistent with changes in NG2 cells in human AD [57] and demonstrate disruption of OPs is an early feature of the disease and precedes overt myelin loss and synaptic dysfunction.

Summary and Conclusions

Studies by ourselves and others have demonstrated that neurotransmitter signalling is a prominent feature of white matter. As in grey matter, there is a predominance of glutamate-mediated synaptic signalling. Our microarray analysis identified that mechanisms for vesicular glutamate release are prominent in CNS white matter and physiological studies

have demonstrated glutamate release is triggered by axonal electrical activity. Glutamate released by axons activates AMPA- and NMDA-type glutamate receptors on OPs and oligodendrocytes to regulate OP recruitment and myelination throughout life. Notably, there is myelin loss and white matter shrinkage in the ageing brain, which is accelerated in dementia. We provide evidence that glutamate signalling is deregulated in ageing white matter, which provides a potential mechanism for glutamate-mediated damage of oligodendrocytes/myelin. In addition, we show that OPs are disrupted at an early stage in the 3xTg-AD model, consistent with a potential disruption of glutamate-mediated recruitment of OPs. Several studies have described altered glutamate receptors in the hippocampus in the 3xTg-AD model and in human AD [58], and a study in the 3xTg-AD mouse brain has described that increased synaptic spontaneous vesicular glutamate release is an early feature of the disease [59]. These studies paint a picture in AD of a vicious cycle of disruption of synaptic glutamate signalling and impaired OP regenerative potential, coupled with loss of oligodendrocytes and myelin, resulting in further impairment of synaptic signaling and OP recruitment. In this respect, NMDA receptors are central to both neuronal and oligodendrocyte/myelin pathology. Memantine blocks excessive NMDA receptor activation and is an effective treatment for both mild and moderate-to-severe AD [60]. The increase in NMDA receptors indicated in our analysis of ageing white mater suggests that oligodendroglial NMDA receptors may be a target of memantine in AD, rescuing white matter and breaking the cycle of synaptic disruption and oligodendrocyte/myelin loss.

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Figure Legends

Figure 1. Changes in vesicular apparatus in ageing white matter. Axons release glutamate by vesicular mechanisms to regulate OPs and myelination in CNS white matter, which is devoid of neuronal cell bodies and conventional neuronal synapses. Microarray analysis of optic nerves aged 6-weeks and 18-months indicate there is a major shift in vesicular release machinery that may have important implications for myelin loss in ageing white matter.

Figure 2. Glutamate signaling and oligodendrocyte/myelin genes are dysregulated in ageing white matter. Microarray analysis of optic nerves aged 6-weeks and 18-months, indicating a summary of the relative expression levels of the main oligodendrocyte and OP genes (**A**), the main neurotransmitter receptors (**B**), and fold-changes in the levels og transcripts associated with glutamate signaling(**C**). There was marked increase in vesicular glutamate transporter VGLUT1 and NMDA-receptor subunits GluN2A and GluN2B, in addition to a 3-fold decrease in the glial glutamate uptake transporter GLAST1. These changes in multiple aspects of glutamate signaling correlate with dysregulation of genes involved in OP differentiation and myelination in ageing white matter. In addition, an increase in glutamate release and decrease in glutamate uptake, coupled with and increase in oligodendroglial NMDA receptors, would have potential pathological consequences for oligodendrocytes/myelin, resulting in white matter shrinkage and cognitive decline. Hence, blocking excessive activation of oligodendroglial NMDA receptors with memantine, which is an effective treatment for AD, would potentially rescue white matter loss in AD and slow down cognitive decline. Glutamate released vesicularly by axons acts on AMPA- and NMDA-type receptors on OPs and oligodendrocytes to regulate myelination and mediate myelin pathology in CNS white matter.

Figure 3. Oligodendrocyte progenitors (OPs) are altered in AD. Immunolabelling for the NG2 was used to identify OPs. (**A**) Collage of confocal micrographs illustrating the overall distribution of OPs in the hippocampus. (**B**) Representative images of OPs in the hippocampus of 6-month old non-Tg (**Bi**) and 3xTg-AD (**Bii**), illustrating OP atrophy at an early stage of the disease, which was confirmed by quantification of OP total cell volume (**Biii**); data are mean ± SEM from 25 cells from 3 sections from *n*=3 mice, ****p<0.001,* ANOVA with Newman–Keuls multiple comparison *post-hoc* analysis. (**C**) NG2 immunostaining (green) and nuclear labeling with Hoechst (blue) illustrates the presence of OP duplets, an indication of recently divided 'sister cells'; maximum intensity projection of *z*-stack (**Ci**), together with a single z-section and orthogonal section through the *y-y* plane showing juxtaposed OP duplets (**Cii**), and quantification of sister cells per constant field of view (FOV) in the hippocampus (**Ciii**), indicating a significant decrease in OP cell division at 6-months in the 3xTG-AD model compared to age-matched non-Tg (data are mean ± SEM from 3 sections per animal, *n*=3 or 4 animals; ***p<0.01,* ANOVA with Newman–Keuls multiple comparison *post-hoc* analysis). (**D**) Confocal images of hippocampus from 24-month 3xTg-AD mouse double immunostained for NG2 (green) and Aβ (red), illustrating the

intimate association of OPs with Aβ plaques in a maximum intensity *z*-stack projection (**Di**) and single z-section (**Dii**). Scale bars = 100 μm in A and 20 μm in B-E.