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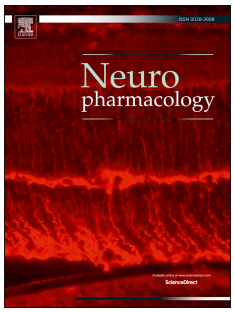

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Glutamate signalling: a multifaceted modulator of oligodendrocyte lineage cells in health and disease.

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Abstract:

Myelin is essential for the mammalian brain to function efficiently. Whilst many factors have been associated with regulating the differentiation of oligodendroglia and myelination, glutamate signalling might be particularly important for learning-dependent myelination. The majority of myelinated projection neurons are glutamatergic. Oligodendrocyte precursor cells receive glutamatergic synaptic inputs from unmyelinated axons and oligodendrocyte lineage cells express glutamate receptors which enable them to monitor and respond to changes in neuronal activity. Yet, what role glutamate plays for oligodendroglia is not fully understood. Here, we review glutamate signalling and its effects on oligodendrocyte lineage cells,and myelination in health and disease. Furthermore, we discuss whether glutamate signalling between neurons and oligodendroglia might lay the foundation to activity-dependent white matter plasticity.

Introduction

Myelin, produced by oligodendrocytes in the central nervous system (CNS), is essential for rapid information transmission and synchronisation of inputs between billions of neurons. Myelin plasticity, which has been largely neglected, is now increasingly invoked as a fundamental mechanism for learning (see Purger et al., 2016 and Tomlinson et al, 2016 in this issue). Myelination has been shown to be orchestrated by a plethora of cellular and molecular signals, including axonal diameter, growth factors, extracellular signalling factors, secreted molecules and adhesion proteins, neurotransmitter signalling and neuronal activity (reviewed by Emery, 2010). The latter two might be of particular importance for myelin plasticity, especially glutamate signalling as 90% of projection neurons, and thus the majority of myelinated axons in the CNS, are glutamatergic (Schmidt and Pierce, 2010) and oligodendrocyte lineage cells, at all stages, express glutamate receptors, enabling them to sense and respond to neuronal activity.

Here we will review how glutamate, a universal signalling molecule, can mediate seemingly unrelated processes such as migration, proliferation, differentiation and myelination, as well as cellular death, and regeneration.

Synaptic and non-synaptic glutamate signalling

Glutamate is the most common excitatory neurotransmitter in the CNS (see Box 1). This non-essential amino acid has a myriad of functions across species, both in and outside the CNS (see Box 2). In the CNS, glutamate signalling is commonly associated with synaptic transmission. The synapse, defined by a presynaptic and a postsynaptic site separated by the synaptic cleft (Palay, 1958), is the primary neuronal information hub. At glutamatergic synapses, the presynaptic machinery (which includes the canonical SNARE complex) releases glutamate through calcium- and activity-dependent vesicular release. The majority (90%) of synaptic transmission occurs in this fashion (Kaeser and Regehr, 2014), and thus can be named canonical glutamate release to distinguish it from other forms of vesicular release that also occur at the presynaptic terminal, such as spontaneous and asynchronous synaptic transmission (Kavalali, 2015; Kaeser and Regehr, 2014; see Figure 1), which can occur in activity-, calcium- and canonical SNARE independent manner.

Although widespread, glutamate release is not confined to the synapse. It can also occur outside the synapse by non-vesicular release of glutamate (occurring either in an activity-dependent or -independent manner) as well as by vesicular release (occurring either in an activity-dependent or -independent manner; Ziv and Garner, 2004; see Figure 1). The regulation and role of the varied forms of non-synaptic glutamate release are less known. Nonetheless, non-synaptic release of

neurotransmitter seems to play an important role early in development, particularly as the expression of functional glutamatergic (and GABAergic) receptors precedes synaptogenesis (Andreae and Burrone, 2015; Ziv and Garner, 2004; Demarque et al., 2002; Furuta et al., 1997). Possible sources of non-synaptic glutamate release are from the reversal of glutamate transporters (Káradóttir and Attwell, 2007) and the release from neuronal growth cones (Soeda et al., 1997), astrocytes (reviewed by Malarkey et al., 2008), microglia (Barger et al., 2007), or from damaged or dying cells (see Figure 1). Whether this non-synaptic form of communication serves a different purpose than synaptic transmission, remains to be investigated. However, as non-synaptic glutamate signalling is used by cells in many different tissues and throughout life, it might serve an equally important but lesser known function (see Box 2).

Throughout this review we use the term 'synaptic release' to refer to activity- and canonical SNARE-dependent vesicular release of glutamate, and 'non-synaptic release' for glutamate release that occurs outside the synapse (not differentiating between vesicular and non-vesicular non-synaptic release). We have omitted postulating the function of spontaneous, activity- and canonical SNARE-independent vesicular release at the axon-OPC synapse due to lack of information in the literature, although spontaneous TTX insensitive miniature events have been detected in OPCs (Káradóttir et al., 2005; 2008; Bergles et al., 2000; Ziskin et al, 2007; Kukley et al. 2007).

Oligodendroglia receive glutamatergic input

The presence of glutamate receptors in the oligodendroglial cell membrane throughout the lineage suggests that glutamate signalling is important for these cells both during their progenitor and their mature stage. Oligodendrocyte precursor cells (OPCs) in particular are well equipped to sense neuronal activity. They express the highest density of glutamate receptors compared to later lineage stages (De Biase et al., 2010) and receive synaptic input from neurons in both grey (Bergles et al., 2000; Jabs et al., 2005; Lin and Bergles, 2004) and white matter (Káradóttir et al., 2005, 2008; Kukley et al., 2007; Ziskin et al., 2007). These synaptic contacts seem to occur predominantly on entirely unmyelinated axons or unmyelinated segments of the axon (Kukley et al., 2007; Tomassy et al., 2014; Ziskin et al., 2007), which raises the question of whether synaptic input is involved in prompting OPC differentiation and myelination.

It is generally assumed that synaptic input is restricted to the progenitor state of oligodendroglia and disappears once differentiation begins (De Biase et al., 2010; Kukley et al., 2007), although oligodendrocytes express glutamate receptors throughout all lineage stages (Butt, 2006; Káradóttir et al., 2005; Lundgaard et al., 2013; Micu et al., 2006; Salter and Fern, 2005; but see Kukley et al., 2010;

see Table 1). Perhaps non-synaptic glutamate signalling becomes more important for differentiated oligodendrocytes (see Figure 1).

Myelinating oligodendrocytes respond to neuronal activity (Fröhlich et al., 2014; Yamazaki et al., 2010), likely via an NMDA receptor-mediated calcium rise in the myelin sheath (Micu et al., 2016). Thus, glutamate signalling might in fact be ongoing between axons and oligodendrocytes throughout life. Micu and colleagues coined the concept of axo-myelinic synapses to describe activity-dependent vesicular release of glutamate from the axon to the oligodendrocyte (Micu et al., 2016).
Whether myelinating oligodendrocytes do receive synaptic input is still uncertain. It is possible that they have gone unnoticed due to the large membrane capacitance of mature oligodendrocytes (that could mask the detection of synaptic input during electrophysiological recordings).

Currently neither the role of synaptic input to OPCs nor the role of glutamate signalling to mature oligodendrocytes is fully understood. Below, we will review what is currently known about the effects of glutamate on oligodendroglial cells.

Glutamate and OPC migration

Before differentiating, OPCs form a dense network that spans the entire brain. Characteristically, OPCs are motile cells which can migrate long distances and continuously survey their environment via extended filopodia (Hughes et al., 2013). The activation of both AMPA/kainate and NMDA receptors in OPCs has been shown to increase motility through the modulation of the adhesion molecule PSA-NCAM (Wang et al., 1996) or the modification of Tiam1 signalling, which induces lamellipodium growth of leading OPC processes (Xiao et al., 2013). AMPA/kainate receptor activation also leads to the composition of an α_v-integrin and proteolipid protein (PLP) complex which associates with the AMPA receptor subunit GluA2 – a subunit which makes AMPA receptors impermeable to calcium (Gudz et al., 2006; see Figure 2). This leads to a reduction of GluA2 levels on the membrane, allowing for increased calcium conductance via AMPA receptors. This weakens OPC binding to the extracellular matrix and augments their migration rate (Gudz et al., 2006; Harlow et al., 2015). However, blocking axonal activity-dependent vesicular release *in vivo* has no effect on OPC migration (Hines et al., 2015; Mensch et al., 2015). These conflicting results might reflect differences between neonatal (OPCs *in vitro*) and postnatal OPCs (OPCs *in vivo*). Synaptic inputs could only be detected in OPCs after postnatal day 5 (P5) (Ziskin et al., 2007), although OPCs express glutamate receptors from birth (Káradóttir and Attwell, 2007). Hence, it is possible that neonatal OPC migration is guided by non-synaptic glutamate signalling, which might serve as a long-range cue to guide OPC migration towards an axonal target. Non-synaptic glutamate signalling is known to act as a long-range signal for other cell types. It leads migratory hippocampal pyramidal neurons and interneurons

towards their target location during early development (Manent et al., 2005, 2006) and helps shaping the dendritic arbor guiding post-synaptic terminals toward the presynapse (Andreae and Burrone, 2015; see also Box 2). Once an OPC associates with a target axon, synapses are presumably formed, which might function to fine-tune OPC localisation, adjust their surveying behaviour and guide their choice as to which axon to myelinate.

The source of the non-synaptic glutamate can currently only be speculated upon. In neurons, glutamate can be released non-synaptically through the reversal of glutamate transporters (Káradóttir and Attwell, 2007). The axons of retinal ganglion cells, for example, release glutamate in this way during early development - a mechanism that is lost once myelination is underway (Kriegler and Chiu, 1993). Glutamate can also be released from axons prior to synaptogenesis either by activity-dependent vesicular release or by spontaneous vesicular fusion, which is independent of neuronal activity and becomes almost undetectable once synapses are formed (Andreae and Burrone, 2015; see Figure 1).

Glutamate and OPC proliferation

Proliferation is an important feature of OPCs, which ensures the preservation of the progenitor pool and makes sure that demands can be met when cells differentiate in order to myelinate. The majority of studies using OPCs *in vitro* (Gallo et al., 1996) or cultured brain slices (Fannon et al., 2015; Yuan et al., 1998) have shown that activation of AMPA/kainate receptors inhibits OPC proliferation. However, glutamate enhances proliferation of OPCs derived from neuronal progenitors of the striatum or the SVZ (Brazel et al., 2005; Redondo et al., 2007), perhaps indicating that the effect of glutamate may be dependent on the developmental origins of the OPCs. Nevertheless, experiments using primary cortical OPC cultures demonstrate that sodium influx, through activated AMPA/kainate receptors, blocks delayed outward-rectifying potassium channels (Borges et al., 1994). Expression of these potassium channels is enhanced by the mitogen platelet-derived growth factor (PDGF)-Rα signalling and are exclusively expressed during the progenitor stage of oligodendrocyte lineage cells (Borges et al., 1994; Chittajallu et al., 2005; Hossain et al., 2014; Larson et al., 2015). Thus, glutamate can reversibly reduce the proliferative effects of PDGF by blocking outward potassium conductance (Borges et al., 1994; Chittajallu et al., 2005). It can therefore be hypothesised that glutamate may act as a stop-signal to pause proliferation as soon as OPCs reach their target neuron, where the axon-OPC synapse will be formed (see Figure 3A).

In vivo, the role that glutamate, or neuronal activity, plays in OPC proliferation is somewhat more obscure. When whiskers are removed in mice, presumably leading to a loss of sensory input to the barrel cortex and consequently to the loss of synaptic input to OPCs, OPC proliferation increases

(Mangin et al., 2012). On the other hand, increasing neuronal activity in the adult mouse brain, either through optogenetic stimulation of cortical neurons (Gibson et al., 2014) or through the acquisition of new motor skills (McKenzie et al., 2014), which arguably increases neuronal glutamate release, OPC proliferation is enhanced, not decreased. Matters are further complicated by studies which fail to see any correlation between glutamatergic input and OPC proliferation. Reducing either neuronal activity, or specifically neuronal activity- and canonical SNARE-dependent vesicular release of glutamate seems to have no effect on OPC proliferation (Hines et al., 2015; Mensch et al., 2015). A possible explanation may be that increased neuronal activity increases differentiation of OPCs (see below), and the increased proliferation seen *in vivo* reflects a secondary effect in which enhanced OPC proliferation is the result of more cells differentiating. Hughes et al (2013) elegantly demonstrated that within the OPC network, neighbouring OPCs are quick to proliferate and replace differentiating OPCs in order to maintain the network. It is difficult to determine whether glutamate *per se* increases OPC proliferation *in vivo* or whether this increase in proliferation is only a secondary effect of increased differentiation. Direct activation of AMPA/kainate receptors may decrease the response of OPCs to mitogens such as PDGF-α (Borges et al., 1994; Chittajallu et al., 2005; Gallo et al., 1996), thereby modulating proliferation and keeping the OPC population alert to alterations in activity that might initiate their differentiation. Another explanation could be that the inhibiting effects glutamate was shown to have *in vitro* on OPC proliferation reflect behaviours of prenatal and early postnatal OPCs *in vivo* since synaptic input is only detected from P5 in OPCs (Ziskin et al, 2007, see above). Perhaps, very localised glutamate release confined to the axon-OPC synapse has different effects on OPC proliferation than non-synaptic release of glutamate.

Glutamate and OPC differentiation

The role neuronal activity may play in OPC differentiation, myelination and myelin plasticity in adulthood is one of the most exciting and rapidly developing research areas of myelin biology. Glutamate might be an important mediator of neuronal activity, but its effects on oligodendrocyte differentiation and myelination are poorly understood. Before glutamate was known to be the major neurotransmitter in the CNS (see Box 1), ultrastructural studies showed that myelin thickness correlates with the axonal calibre (Duncan, 1934). Axonal diameter exceeding a certain threshold seems to be sufficient to induce myelination, which can occur independently of axonal activity and glutamate signalling, as both nanofibres and fixed axons, when co-cultured with OPCs, become myelinated (Bechler et al., 2015; Lee et al., 2012a; Li et al., 2014). However, an increasing body of evidence shows that neuronal activity, presumably mediated by glutamate, can regulate the differentiation of oligodendroglia. These seemingly contradictory observations may not be incompatible, but merely reflect that myelination can be accomplished in two complementary ways: dependent and independent of axonal activity (Lundgaard et al., 2013). One of the key factors for

activity-dependent myelination, it seems, is glutamate. However, the role of glutamate receptors is complex and it is possible that metabotropic glutamate receptors (mGluRs), AMPA/kainate and NMDA receptors serve different functions in regulating differentiation and myelination (as well as in the homeostasis of oligodendrocytes in general).

Activating OPC mGluRs prompts differentiation (Spampinato et al., 2014; Wake et al., 2011) and regulates AMPA receptor expression (Zonouzi et al., 2011) and calcium influx, mediated by ionotropic glutamate receptors (iGluRs). Calcium influx via iGluRs up-regulates the expression of immediate early genes (Gallo et al., 1996; Lundgaard et al., 2013; Pende et al., 1994), which are associated with the regulation of cell growth and differentiation (Herschman, 1991; McMahon and Monroe, 1992). Yet, glutamate, predominantly via AMPA/kainate receptors, inhibits OPC differentiation *in vitro* (Gallo et al., 1996) and similarly, in cerebellar slices, AMPA/kainate agonists reduce the number of mature oligodendrocytes in a concentration dependent manner (Yuan et al., 1998). *Vice versa*, blocking either AMPA/kainate receptors or neuronal activity increases the number of $CC1^+$ immature oligodendrocytes (Fannon et al., 2015), suggesting that glutamate, acting via AMPA/kainate receptors, curbs differentiation. Despite the increase of $CC1^+$ cells, these cells failed to fully differentiate into myelin basic protein (MBP)-expressing mature oligodendrocytes (Fannon et al., 2015). Perhaps, AMPA/kainate receptor activation is needed to guide OPCs to their target axons, and prime them for 'the differentiation signal' thus, blocking AMPA/kainate receptors releases them to differentiate unspecifically as it does not seem to be sufficient to initiate myelination (see Figure 3B).

The final differentiation step of oligodendrocytes has been linked to NMDA receptors, acting through the Akt/mTOR pathway (Li et al., 2013; Lundgaard et al., 2013). NMDA receptors are found on OPC processes (Káradóttir et al., 2005; Salter and Fern, 2005) and inside compact myelin sheaths (Káradóttir et al., 2005; Micu et al., 2006). In neurons, NMDA receptors serve as coincidence detectors which, due to their voltage-sensitive magnesium block, detect separate glutamatergic inputs converging on the cell. Once activated, NMDA receptors are far more sensitive to glutamate and more permeable to calcium than AMPA/kainate receptors (see Figure 2C). In a similar manner, NMDA receptors might be important activity-detectors in OPCs. In myelinating co-cultures, the growth factors neuregulin (NRG) and brain-derived neurotrophic factor (BDNF) have been shown to switch myelination to become regulated by neuronal activity. In this mode, differentiation and myelination depend on OPC NMDA receptor activation (Lundgaard et al., 2013; see Figure 3C). Inhibiting either neuronal activity or NMDA receptors leads to a significant reduction of myelination (Li et al., 2013; Lundgaard et al., 2013). Activating NMDA receptors, on the other hand, promotes myelination and the local production of both MBP and myelin oligodendrocyte glycoprotein (MOG) *in vitro* (Cavaliere et al., 2012; Li et al., 2013; Lundgaard et al., 2013; Wake et al., 2011). In stem cells

derived from the subventricular zone (SVZ), NMDA receptor activation leads to commitment to the oligodendrocyte lineage. As their differentiation progressed, NMDA subunits GluN1 and GluN2A increased while GluN2B and GluN3 decreased suggesting that maturation is matched by a differential expression profile of NMDA receptor composition (Cavaliere et al., 2012; see Figure 2C and Table 1).

In vivo, the role of NMDA receptors in the context of myelination is less clear. Concomitantly deleting the NMDA receptor subunits GluN2D and GluN3A in mice resulted in subtle myelin defects in myelin ultra-structure (periodicity), whereas independent deletion showed no phenotype (Micu et al., 2016). While this seemingly supports the hypothesis that NMDA receptors are involved in the terminal oligodendroglial differentiation steps, it has to be noted that in this study the knockout of the NMDA receptor subunits was global, thus affecting multiple cell types. On the other hand, deleting the compulsory subunit GluN1 exclusively in oligodendroglia (driven by PDGF-R-α, Olig2 or PLP promoters) had no obvious effect on myelination (De Biase et al., 2011; Guo et al., 2012), as detected by g-ratio (De Biase et al., 2011) and immunohistochemistry analysis (De Biase et al., 2011; Guo et al., 2012), albeit myelin ultrastructure was not analysed. However, in these mice an upregulation of calcium-permeable AMPA receptors was detected in OPCs (De Biase et al., 2011), possibly to compensate for the lack of NMDA receptors. Whether the mode of myelination independent of neuronal activity, which does not depend on NMDA receptor activation (Lundgaard et al., 2013), is sufficient to myelinate axons in development, or whether calcium-permeable AMPA receptors can compensate for the lack of NMDA receptors, is still unclear. Additionally, NMDA receptor activation might also be important for regulating myelin architecture or fine tuning myelin structure.

Myelin and glutamate signalling

AMPA/kainate and NMDA receptors might regulate different steps in the differentiation phase, but once myelination is achieved, it seems their orchestrated efforts are needed. Mature oligodendrocytes respond to neuronal stimulation by a calcium rise in myelin only when both AMPA and NMDA receptors work in concert (Micu et al., 2016). Moreover, NMDA receptor activation regulates mitochondria movement within the myelin sheath (Rinholm et al., 2016), presumably to provide energy to myelin sheaths wrapped around the most active axons and to coordinate myelin synthesis (Rinholm et al., 2011). This suggests that glutamate signalling may not only be important for the initiation and completion of differentiation, but also for myelin maintenance and plasticity. This is in line with studies showing that OPCs preferentially myelinate active, vesicle-releasing neurons *in vitro* (Wake et al., 2015) and *in vivo* (Hines et al., 2015) and that myelin sheaths wrapped around active neurons are more likely to be preserved (Hines et al., 2015). Myelin, therefore, may be involved in activity-dependent plasticity of the neuronal circuit. Myelination patterns vary significantly between

axons (Tomassy et al., 2014), which might reflect a myelinic way of synchronising electrical signals. Even small changes in myelin thickness, internodal spacing or the length of the nodes of Ranvier or the axonal hillock (determined by the placement of the first myelin segment) will affect the propagation speed of electrical signals (Pajevic et al., 2014; Ford et al., 2015).

Taken together, these studies show that, depending on the microenvironment, glutamate could be one of the key factors regulating differentiation and myelination of oligodendroglia and might be involved in circuit plasticity by inducing myelin adjustments. However, the effects of glutamate are complex and further experiments are needed to elucidate its role in differentiation and myelination.

The destructive and instructive roles of glutamate in white matter disease

Glutamate as a regulator of myelination might not only be important for development and white matter plasticity, but also in the context of myelin regeneration in disease. Both beneficial and harmful effects of glutamate in white matter have been reported, suggesting a dual role for glutamate in white matter disorders. There is a variety of diseases affecting the white matter, ranging from genetic leukodystrophies, multiple sclerosis (MS), periventricular leukomalacia (PVL), stroke and traumatic injuries (reviewed by Almad et al., 2011; Goldberg and Ransom, 2003; Baltan, 2015 in this issue) to psychiatric disorders like schizophrenia (Flynn et al., 2003) and substance abuse (reviewed by Feng, 2008). Emerging hypotheses also implicate early white matter changes as a contributing component in neurodegenerative diseases such as dementia (including Alzheimer's disease; Prins et al., 2015; O'Dwyer et al., 2011a, 2011b; Van Rooden et al., 2014; reviewed by Brickman et al., 2015) and associate oligodendrocyte degeneration with amyotrophic lateral sclerosis (Kang et al., 2013). Therefore, a better understanding of the role of glutamate in myelin disorders could have wide implications.

Examples of the harmful side of glutamate can be seen in ischemia and inflammation in which the extracellular glutamate concentration can rise to excitotoxic levels (reviewed by Choi, 1994; Káradóttir and Attwell, 2007). As the white matter is located at the distal end of the brain vasculature (Duvernoy et al., 1981) it is very susceptible to changes in blood flow and oligodendrocytes themselves are very sensitive to oxygen and glucose deprivation, the hallmarks of stroke, hypoxia or vascular dementia. There are many possible sources of the excess glutamate under such conditions. Activated microglia can release glutamate during inflammation (Barger et al., 2007; Noda et al., 1999; Piani et al., 1991; Takaki et al., 2012), whilst injured neurons can release abnormal levels of glutamate (Mark et al., 2001) due to their high intracellular glutamate concentrations (Danbolt, 2001). Furthermore, astrocytes, oligodendrocytes and neurons all express glutamate transporters (Domercq et al., 1999; Kanai and Hediger, 1992; Kugler and Schmitt, 1999; Pitt et al., 2003; Danbolt et al., 1992;

reviewed by Allen et al., 2004), which can be down-regulated and therefore fail to clear extracellular glutamate. Glutamate transporters can also reverse, for example due to a deterioration of ionic gradients, often caused by a lack of energy supply, as occurs in ischemia. This leads to glutamate being released instead of cleared by the transporters (Kriegler and Chiu, 1993; Rossi et al., 2000; reviewed by Danbolt, 2001; Káradóttir and Attwell, 2007). The down-regulation of these transporters was observed in MS lesions concomitantly with high concentrations of extracellular glutamate (Lehmann et al., 2009; Pitt et al., 2003). Another source of glutamate could be the blood, especially in MS and other diseases where the blood-brain barrier is compromised (Minagar and Alexander, 2003), as the glutamate concentration in plasma is much higher than in the extracellular space of the CNS. Depending on the location and method used for measuring glutamate levels, the reported values vary considerably, ranging from 0.02 µM to 20 µM for the extracellular concentration, and from 30 µM to 100 µM in the plasma (Hawkins, 2009; Moussawi et al., 2011; Smith, 2000).

The increase in extracellular glutamate levels due to non-synaptic release from the sources described above can then induce cytokine production and activate immune cells promoting inflammation (see also Box 2). In addition, glutamate can cause excitotoxic cell death mediated by an 'overactivation' of glutamate receptors. Such an 'overactivation' leads to extensive calcium influx toxic to the cells and to sodium influx resulting in swelling and lysis of cells, which can in turn release even more glutamate (Choi, 1987; Káradóttir and Attwell, 2007; Trump and Berezesky, 1995). This escalating cycle of glutamate excitotoxicity can be blocked by using glutamate receptor antagonists, which have been shown to prevent ischemia-induced cell death *in vivo* (Mattson, 2003; Mark et al., 2001).

In contrast to neurons lost due to ischemia or glutamate excitotoxicity, myelin can be regenerated. We have recently demonstrated that remyelination, like myelination, is regulated by neuronal activity and depends on glutamate release from the demyelinated axon (Gautier et al., 2015; Lundgaard et al., 2013). This demonstrates that despite being toxic at high concentrations, glutamate also has an instructive role in regeneration. Remyelination is carried out by OPCs that migrate to the site of damage, proliferate, differentiate and wrap their processes around the demyelinated axons to form a new myelin sheath (see reviews in this issue by Chamberlain et al., 2016; Tognatta & Miller 2016). It has been shown recently that demyelinated axons upregulate presynaptic proteins in the ethidium bromide lesion model in rats (Gautier et al., 2015), in lysolecithin lesions in mice (Etxeberria et al., 2010; Sahel et al., 2015) and in MS patients, and are able to form synapses with recruited OPCs (Gautier et al., 2015; Sahel et al., 2015). These OPCs express AMPA/kainate receptors and, at later stages, also NMDA receptors (Gautier et al., 2015). Blocking either neuronal activity, vesicular release of glutamate, AMPA/kainate or NMDA receptors in the lesion inhibits remyelination (Gautier et al., 2015; Li et al., 2013; Lundgaard et al., 2013). The time course of glutamate receptor expression in OPCs in demyelinated lesions is in line with the described roles of AMPA and NMDA receptors

during myelination: AMPA receptors are important in the early phase of OPC proliferation and initial differentiation, whereas NMDA receptors are expressed at later time points and are needed for myelin sheath formation (Gautier et al., 2015; Li et al., 2013; Lundgaard et al., 2013). Similar to myelination, the effects of NMDA receptor activation on remyelination seem to be mediated via the Akt/mTOR pathway (Li et al., 2013; Lundgaard et al., 2013) and as in development (Mensch et al., 2015) it is predominantly the (re)myelination of small diameter axons that depends on neuronal activity and glutamate. Larger calibre axons still become myelinated when vesicular release of neurotransmitters is blocked (Mensch et al., 2015) and are also remyelinated even when activity or synaptic signalling are inhibited (Gautier et al., 2015).

The contradictory effects of glutamate in white matter disease, with excitotoxicity on the one hand and the promotion of remyelination on the other, become apparent in MS. In this autoimmune disease spontaneous remyelination can occur in patients. However, with ageing and after cycles of demyelination and remyelination, symptomatically mirrored by phases of relapse and remission, remyelination will ultimately decline resulting in chronically demyelinated axons (Franklin, 2002). The failure of remyelination is a major contributing factor to the accumulation of neuronal loss and disability (reviewed by Bjartmar et al., 2003). Therefore, as glutamate signalling regulates remyelination, enhancing this communication might be a promising therapeutic approach in these cases.

However, the pathogenesis of MS is complex and influenced by many factors (see reviews in this issue Chamberlain et al., 2016; Sedel et al., 2016; Tognatta and Miller, 2016). Inflammation, for example, has been linked to the initial attack on the myelin sheath and to neurodegeneration seen in MS patients. Inflammation is high in the initial phase of MS and since cell death occurs predominantly in highly inflamed areas, it has been suggested that excess inflammation causes neuronal loss (Trapp et al., 1998). Inflammation might be the primary cause of neurodegeneration occurring independent of oligodendrocyte damage, as neuronal loss can be observed in normal-appearing white matter in MS patients (Evangelou et al., 2000). This inflammation-induced neurodegeneration might be due to disruptions of glutamate release and clearance that are documented in MS tissue (reviewed by Frigo et al., 2012; Tilleux and Hermans, 2007). Glutamate concentrations are significantly increased in the cerebrospinal fluid, in acute brain lesions and in normal appearing white matter of MS patients (Srinivasan et al., 2005; Stover et al., 1997). In addition to neuronal degeneration due to pathological increases in glutamate, there might also be direct detrimental effects on oligodendrocytes, as perfusion of kainate into the optic nerve was shown to generate MS like demyelinated lesions (Matute et al., 1997).

Based on these observations, reducing glutamate levels seems a sensible therapeutic strategy. Indeed, the FDA-approved antibiotic ceftriaxone increased the expression of the glutamate transporter GLT-1 (EAAT2) *in vitro*, provided neuroprotection *in vivo* in disease models encompassing excessive glutamate levels (Rothstein et al., 2005; but see Melzer et al., 2008) and alleviated clinical symptoms in experimental autoimmune encephalomyelitis (EAE) (Melzer et al., 2008; Ramos et al., 2010). Similarly, blocking AMPA signalling with NBQX reduced disease severity in EAE (Ohgoh et al., 2002; Pitt et al., 2000; reviewed by Smith, 2000). Remarkably, NBQX administration prevented EAE-related changes in glutamate transporter expression, indicating that AMPA receptor activation regulates transporter expression. It is not clear, however, if the observed beneficial effects are due to a protection of oligodendrocytes or neurons, or both. The primary neuronal loss in EAE might be too rapid and substantial to reveal any secondary neurodegeneration that would occur in MS due to impaired oligodendrocyte survival. In addition to AMPA receptor inhibitors, also NMDA receptor antagonists have been tested in EAE and MS patients: Memantine and amantadine improved neurological symptoms in EAE and memantine and MK801 mediated neuroprotection in experimental autoimmune optic neuritis (Sühs et al., 2014; Sulkowski et al., 2013; Wallström et al., 1996). Amantadine is prescribed against fatigue, a clinical hallmark of MS, but whether its effect is mediated via NMDA receptor inhibition is unclear as the drug can affect numerous other pathways, such as dopamine signalling (Scatton et al., 1970). Memantine trials for MS were terminated because of reversible neurological side effects (https://clinicaltrials.gov/ct2/show/NCT00638833), but it is approved for Alzheimer's disease where it is thought to protect neurons by preventing glutamate excitotoxicity (Lipton, 2006).

The seemingly contradicting findings on glutamate being potentially toxic, but also essential for instructing myelin regeneration, could be explained by the need for a localised and timed communication between axons and OPCs, rather than a general sustained glutamate release that increases the extracellular glutamate concentration to toxic levels. Such a pathological increase in overall glutamate levels might disturb the synaptic signalling required for myelin regeneration and instead stimulate non-synaptic glutamate signalling pathways, thus promoting, for example, the activation of immune cells and inflammation – which could exacerbate the damage.

Conclusions

It is becoming evident that the source of glutamate or the mechanism of its release, whether it is activity-dependent vesicular release (i.e. fast), or non-vesicular release (i.e. slow) may convey different regulatory functions for oligodendrocyte proliferation, differentiation and myelination, both during development and in disease. This combined with the fact that oligodendrocytes express different glutamate receptor types and subunits at different stages during development (Table 1),

suggests that glutamate might have different effects depending on the signalling mechanisms at different timepoints. Thus, the seemingly conflicting roles that have been identified may reflect different mechanisms of 'release' (or administration of glutamate in *in vitro* studies) as well as differential timepoints of oligodendrocyte lineage cell development or the age of the animal in use. Hence, to fully understand the role of glutamate in oligodendrocyte biology, it needs to be studied in the context of release and the age and developmental stage of the oligodendrocyte lineage cell.

Given the increasing body of evidence found for white matter plasticity and *de novo* myelination in adulthood, it is important to expand our knowledge of glutamate signalling in the context of myelination. This will also deepen our understanding of its role in disease and help to develop new therapies that prevent glutamate excitotoxicity, without curtailing the beneficial effects of glutamate signalling needed for myelin regeneration.

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Figure legends:

Figure 1: Multiple ways of glutamate release

(A-D) Vesicular glutamate can be released in an activity- and canonical SNARE-dependent (A, B; canonical) or -independent way (C, D; non-canonical). Both types of vesicular release can occur at synapses in the mature CNS. However, they can also be observed in absence of a postsynaptic density for example prior to synapse formation in development when growing axons release neurotransmitters. This vesicular release is thought to guide the postsynaptic dendrite to form the final synaptic contact, a mechanism possibly copied by OPCs to form the axon-OPC synapse. Although depicted here as axonal release, vesicular release of glutamate can also be observed in non-neuronal cells, such as astrocytes. Furthermore, there is non-vesicular release of glutamate (E) from various sources e.g. microglia, astrocytes or injured cells, from the blood stream or from reversal of glutamate transporters for example in cells that suffer from energy shortage in ischemia.

The mode of glutamate release might differentially affect the downstream signalling in oligodendroglia as the glutamate concentration and the location and timing of glutamate receptor

activation differ. To what extent these different modes of glutamate release play a role for OPC migration, proliferation, differentiation and myelination in vivo is not yet fully understood.

Figure 3: Effects of glutamate on OPC proliferation, differentiation and myelination

Generally, glutamate seems to inhibit proliferation in OPCs (see text for exceptions) (A). mGluR activation promotes cell maturation and differentiation, whilst activation of AMPA/kainate receptors inhibits differentiation (B). When myelination is carried out in the activity-dependent mode, glutamate was shown to promote the expression of myelin proteins and myelination through NMDA receptors (C).

Table legend:

Tab. 1: Ionotropic glutamate receptor expression in oligodendrocytes and OPCs

Overview of RNA and protein expression of the different ionotropic glutamate receptor subunits in oligodendrocytes and OPCs. Data obtained from cell lines were not included in the table. References and the species from which the data were obtained are listed below.

[1] Patneau *et al*., 1994 (rat); [2] Li and Stys, 2000 (rat); [3] Matute *et al*., 1997 (rat); [4] Itoh *et al*., 2002 (rat); [5] Cahoy *et al*., 2008 (mouse); [6] Zhang *et al*., 2014 (mouse); [7] Salter and Fern, 2005 (mouse); [8] Garcia-Barcina and Matute, 1996 (bovine); [9] Micu *et al*., 2006 (rat); [10] Káradóttir *et al*., 2005 (rat); [11] Burzomato *et al*., 2010 (rat); [12] Lundgaard *et al*., 2013 (rat); [13] Piña-Crespo *et al*., 2010 (rat); [14] Kukley and Dietrich, 2009 (mouse); [15] Alix and Fern, 2009 (rat); [16] Wang *et al*., 1996 (rat); [17] Hamilton *et al*., 2010 (mouse); [18] Liu *et al*., 2002 (rat); [19] Guo *et al*., 2012 (mouse); [20] Hossain *et al*., 2014 (rat); [21] Bakiri *et al*., 2008 (rat); [22] Newcombe *et al*., 2008 (human); [23] Rosenberg *et al*., 2003 (rat); [24] Harlow *et al*., 2015 (mouse); [25] Yoshioka *et al*., 1995 (rat); [26] Brand-Schieber and Werner, *Neurosci Lett*, 2003 (GluK5 in mouse, not in rat; GluA4 in rat, not in mouse); [27] Fogarty *et al*., 2000 (rat); [28] Chew *et al*., 1997 (rat, GluA1 and GluK3 in presence of bFGF/PDGF); [29] Gallo *et al*, 1994 (rat); [30] Deng *et al*., 2006 (rat); [31] Ong et al., 1996 (rat); [32] Li *et al*., 2013 (rat); [33] Gudz *et al*., 2006 (rat); [34] Xiao *et al*., 2013 (rat)

Tab. 1: Ionotropic glutamate receptor expression in oligodendrocytes and OPCs

AMPA Receptor Subunits			
GluA1	RNA	OPC	Low [1][4][5][6][28]/Yes [25]
		Oligo.	Low [4][5][6]/Yes [25] /No [3]
	Protein	OPC	Yes [18][20][28][30] /No/Low [4][24]
		Oligo.	No [2][3][4][20] /Yes [22]
GluA2	RNA	OPC	Yes/High [1][4][5][6][25][28]
		Oligo.	Yes/High [4][5][6][25] /No [3]
	Protein	OPC	Yes [4][18][20][23][24][30][33]
		Oligo.	Yes [4][22] /No [2] /Low [20]
GluA3	RNA	OPC	Yes/High [1][4][5][6][25][28]
		Oligo.	Yes/High [3][4][5][6][25]
	Protein	OPC	Yes [4][24][30]
		Oligo.	Yes [2][4]
GluA4	RNA	OPC	High [1][4][5][6][28][29] /No [25]
		Oligo.	High [3][4][5][6] /No [25]
	Protein	OPC	Yes [4][17][18][20][23][24][28][30][31][33]
		Oligo.	Yes [2][3][4][7][15][26] /Low [20]

Kainate Receptor Subunits			
GluK1	RNA	OPC	Yes [5][6][25] /No [1][28]
		Oligo.	Yes/Low [5][6][25] /No [3]
	Protein	OPC	No [14]
		Oligo.	N/A
GluK2	RNA	OPC	Yes [1][5][6][25][28]
		Oligo.	Yes [3][5][6][25]
	Protein	OPC	N/A
		Oligo.	N/A
GluK3	RNA	OPC	Yes [1][6][25][28]
		Oligo.	Yes [3][6][25]
	Protein	OPC	N/A
		Oligo.	N/A
GluK4	RNA	OPC	Yes [1][5][6][25][28]
		Oligo.	Yes/Low [3][5][6][25]
	Protein	OPC	N/A
		Oligo.	Yes [27]
GluK5	RNA	OPC	Yes/High [1][5][6][25][28]
		Oligo.	Yes/High [3][5][6][25]
	Protein	OPC	Yes [23]
		Oligo.	Yes [3][8][15][26]

NMDA Receptor Subunits			
GluN1	RNA	OPC	Yes [5][6][16]
		Oligo.	Low [5][6][19]
	Protein	OPC	Yes [10][16][32][34]
		Oligo.	Yes [9][10][11][13][15][21][32] /Low [19]
GluN2A	RNA	OPC	Low [5] /No [6][16]
		Oligo.	Low [5] /No [6]
	Protein	OPC	Yes [32] /No [12]
		Oligo.	Yes [19][32] /No [12]
GluN2B	RNA	OPC	Low [5] /No [6][16]
		Oligo.	Low [5] /No [6]
	Protein	OPC	Yes [32]
		Oligo.	Yes [32]
GluN2C	RNA	OPC	Low [6] /No [5][16]
		Oligo.	Low/No [5][6]
	Protein	OPC	N/A
		Oligo.	Yes [10][11]
GluN2D	RNA	OPC	Yes [5] /Low [6] /No [16]
		Oligo.	Low [5][6]
	Protein	OPC	No [12]
		Oligo.	No [12]
GluN3A	RNA	OPC	Yes [6]
		Oligo.	Low [6]
	Protein	OPC	Yes [12]
		Oligo.	Yes [11]
GluN3B	RNA	OPC	Low/No [5][6]
		Oligo.	Low/No [5][6]
	Protein	OPC	N/A
		Oligo.	N/A

Glutamate - too common to be exciting!

Early in the 20th century, it was found that glutamate (also known as L-glutamate or glutamic acid) occurred in high concentrations in the brain. At the time, outside its role as a building block for proteins, glutamate was thought to be predominantly involved in energy metabolism – it is a metabolic intermediate of the citric acid cycle and involved in diverse metabolic processes such as protein synthesis, glycolysis and gluconeogenesis and forms part of the nitrogen-urea cycle. It is enriched in protein containing food, but can also be synthesised in the body.

In the 1950s, a series of findings argued that glutamate was a neurotransmitter in the CNS, but this concept was faced with great scepticism. As glutamate is such an abundant amino acid it did not fit the description of a neurotransmitter at the time. It was not until several decades later that the role of glutamate for synaptic transmission was established (Watkins, 1972; Watkins and Jane, 2006). Today, glutamate is known to be the major excitatory neurotransmitter in the central and enteric nervous system of mammals (Kirchgessner, 2001).

Box 1

Canonical and non-canonical glutamate signalling

Glutamate signalling in the CNS is generally thought to be mediated by activity- and canonical SNARE-dependent vesicular release at the pre-synapse (i.e canonical glutamate signalling). However, glutamate as a signalling molecule and the expression of glutamate receptors are neither limited to the nervous system nor to organisms which have a nervous system. Non-canonical glutamate signalling is characterised by activity- and canonical SNARE-independent release of glutamate and can occur both at the synapse as well as outside synapses (Manent et al., 2005).

Non-canonical glutamate signalling in the CNS regulates:

- Migration patterns of immature hippocampal pyramidal neurons (Manent et al., 2005)
- Proliferation of stem cells of the SVZ via metabotropic glutamate receptor (mGluR5) signalling (mGluR5 expression precedes synaptogenesis) (Di Giorgi-Gerevini et al., 2005)
- Retinal progenitor cell proliferation during a time in development when synapses have not yet formed (Martins et al., 2006)
- Motility (Rzeski et al., 2001) and proliferation (Kalariti et al., 2005) of tumour cells
- Dendritic arbour formation and directs dendrites to presynaptic terminals prior to synapse formation (Andreae and Burrone, 2015)
- Commitment to the neuronal fate and increases the differentiation rate into GABAergic neurons through mGluR activation in embryonic stem cells (Sarichelou et al., 2008)
- Synapse stabilisation (Kaeser and Regehr, 2014)

Non-canonical neurotransmitter signalling in non-neuronal tissue

(see also reviews by Gill and Pulido, 2001; Hinoi et al., 2004; Julio-Pieper et al., 2011)

- Bone: Non-synaptic glutamate signalling promotes differentiation of osteoblast precursors into osteoblasts, boosts osteoblast survival and leads to cell maturation (Genever and Skerry, 2001; Lin et al., 2008)
- Blood: Differentiation of megakaryocytes is regulated by non-synaptic activation of NMDA receptors expressed by megakaryocytes (Genever et al., 1999)
- Hormonal release: Non-canonical glutamate signalling regulates hormonal release in the pineal gland and the pancreas (Storto et al., 2006)
- Immune system: Glutamate signalling activates T-cells as well as lymphocyte cytokine production and survival in the mammalian immune response (Boldyrev et al., 2004; Chiocchetti et al., 2006; Pacheco et al., 2004)
- Cancer: Glutamate signalling, via NMDA receptors, promotes tumour growth and invasion (Li and Hanahan, 2013)

Non mammalian non-canonical glutamate signalling

Glutamate signalling and glutamate receptor expression can be found in all living organisms, even in primitive ones lacking a nervous system and in earliest marine metazoans (Forde and Lea, 2007). In prokaryotes and eukaryotes, glutamate can serve as a chemotactic signal and trigger feeding-related locomotion (Bakker et al., 2007; Bellis et al., 1991; see review by Forde and Lea, 2007). In Arabidopsis plants, glutamate receptors are expressed in a higher density in the roots (Chiu et al., 2002) which respond to glutamate with rapid membrane depolarisation (Dennison and Spalding, 2000), leading to root growth, altered root tip morphology and branching (similar perhaps to axonal growth cone and dendritic spine growth) (Sivaguru et al., 2003; Walch-Liu et al., 2006). Glutamate may also be involved in the defence response as it regulates the expression of defence genes in plants (see review by Forde and Lea, 2007).

Box 2

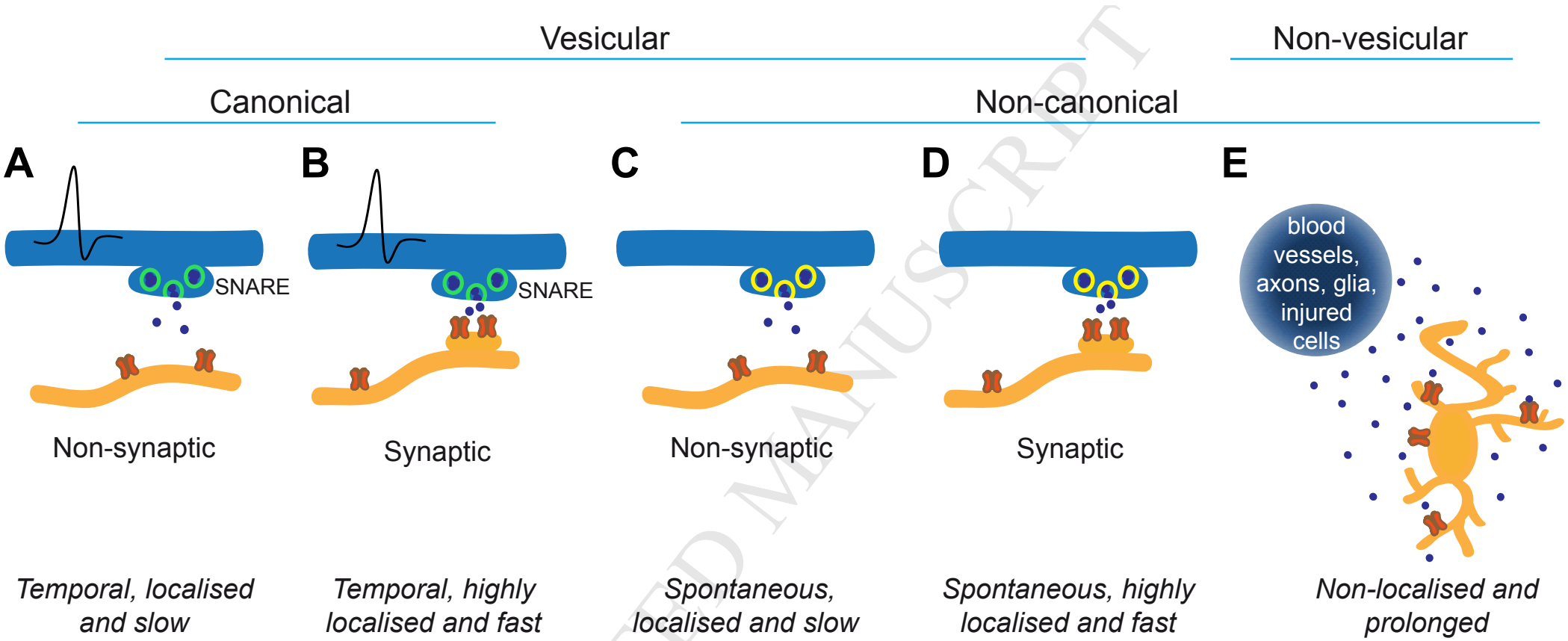

Figure 1

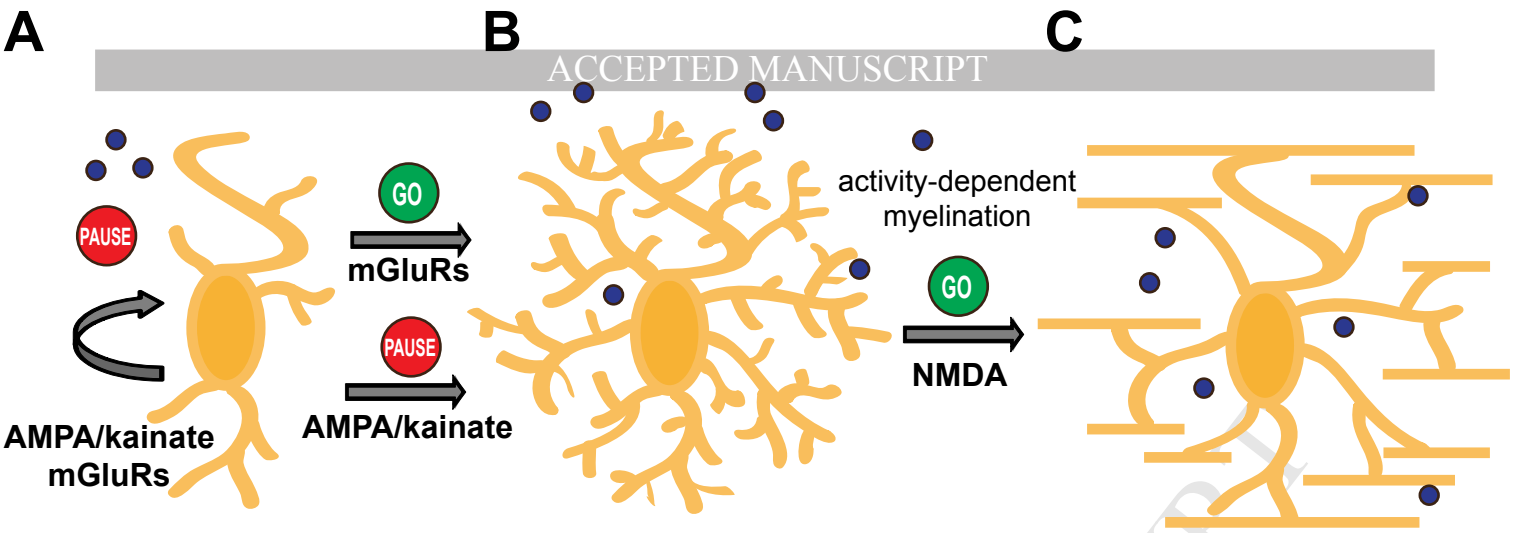

Figure 3

Highlights

Glutamate is a universal signalling molecule across cell types and the phylogenetic tree

Glutamate modulates OPC migration, proliferation, differentiation and (re)myelination

Glutamate regulates activity-dependent myelination and potentially myelin plasticity

CNS glutamate release occurs in multiple forms at the synapse and outside synapses

The information conveyed by glutamate signalling depends on the release mechanism