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AGE-SPECIFIC LOCALIZATION OF NMDA RECEPTORS ON OLIGODENDROCYTES DICTATES AXON FUNCTION RECOVERY AFTER ISCHEMIA

Selva Baltan

Department of Neurosciences, Lerner Research Institute, Cleveland Clinic Foundation, Cleveland, Ohio, USA

Abstract

Oligodendrocytes and axons are the main targets of an ischemic white matter injury and the resultant loss of axon function underlies the clinical disability in patients who survive a stroke. The cellular mechanisms of ischemic injury change as a function of age in concordance with age-mediated structural changes in white matter. Shorter periods of injury cause rapid and robust loss of axon function together with widespread oligodendrocyte death. While blockade of NMDA receptors fails to benefit axon function, removal of extracellular Ca^{2+} during ischemia remarkably promotes axon function recovery in young white matter. However, these same approaches hinder axon function recovery and fail to protect oligodendrocytes in aging white matter. The obligatory GluN1 subunit of the NMDA receptor exhibits an age-specific expression pattern such that in young adult white matter, it is mostly localized on oligodendrocyte cell bodies, while in aging white matter, it is also observed on myelin processes. This age-dependent re-localization and redistribution pattern mimics GluN1 expression observed during development, but in reverse order. During development, GluN1 immunoreactivity traffics from astrocytes at postnatal day 4–11 (P4–11) to myelin processes at P12–18 and to oligodendrocytes cell bodies at P19–21. Although immature axons are more resistant to ischemia, blockade of NMDA receptors during ischemia at P4–11 and P12–18 worsens axon function recovery and fails to benefit axons at P19–21. Thus, age-specific expression patterns of NMDA receptor localization may seem to modulate the plasticity of oligodendrocytes and myelin in response to ischemia as a function of age in white matter.

Keywords

Compound action potential; myelin; Ca^{2+} ; development; axon function; electrophysiology; optic nerve

Corresponding Author Address: Department of Neurosciences, Lerner Research Institute, The Cleveland Clinic Foundation, 9500 Euclid Avenue, NC30, Cleveland, OH 44195, USA, baltans@ccf.org.

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1. Introduction

The human brain comprises equal percentages of gray and white matter by volume (Zhang and Sejnowski, 2000). Thus, the vast majority of injuries sustained after a stroke in human predictably involve both white and gray matter portions of the brain. Aging results in global changes, predominantly in white matter, such that the tissue becomes intrinsically more vulnerable to ischemic injury (Baltan et al., 2008), and the risk for ischemic stroke increases substantially with age (Ay et al., 2005). White matter architecture is distinct from gray matter and is unexpectedly complex. Axons with variable amount of myelin extend through oligodendrocytes, astrocytes, microglia, and precursor cells to establish a vital network between neurons. The cellular and molecular components of white matter are individually under attack during ischemia. Therefore, the structural composition of white matter, together with a concerted interaction among glial cells and axons, dictates the pathophysiological mechanisms and outcome of an ischemic attack (Baltan, 2009, 2014).

White matter components that are vulnerable to excitotoxicity and oxidative injury are the principle targets of an ischemic attack. After experimental induction of oxygen glucose deprivation (OGD), the earliest changes are observed on oligodendrocytes as swelling of the cell body, which extends to myelin processes and subsequently results in axonal beading (Baltan, 2014). These morphological changes are irreversible such that even after resuming normal oxygen and glucose for prolonged periods of time, structural disruption progresses. Meanwhile, axon function gradually diminishes and eventually results in complete loss of conduction (Baltan, 2014). Oligodendrocyte death and axon loss are the ultimate outcome following ischemia (60 min), which disables white matter function (Tekkok and Goldberg, 2001; Tekkok et al., 2005; Tekkok et al., 2007). Oligodendrocytes and myelin processes express a wealth of functional glutamate receptors such as AMPA/kainate (Sanchez-Gomez and Matute, 1999; Gallo and Ghiani, 2000; Li and Stys, 2000) and NMDA receptors (Karadottir et al., 2005; Micu et al., 2006). Although these receptors are critical for axon-glia signaling, they also set oligodendrocytes as a target for excitotoxic injury. Oligodendrocytes are the most abundant cells of white matter (~60% of all glial cells; (Tekkok and Goldberg, 2001; Nave and Trapp, 2008; Baltan, 2014), but they are also present in gray matter, mostly as satellites to neuronal cell bodies. Aging oligodendrocytes undergo a series of morphological changes characterized by bulbous enlargements along their processes and lumpy inclusions in their cytoplasm (Peters, 1966). Among the structural measures that both increase in frequency with age and correlate with cognitive decline are degenerating myelin sheaths and loss of nerve fibers. Despite these degenerative alterations, myelin repair and/or remyelination ensue. The internodal lengths become more numerous and often shorter (Gledhill and McDonald, 1977; Lasiene et al., 2009) and these nodal reorganizations are often associated with increased numbers of oligodendrocytes. Intrinsic properties of aged oligodendrocytes may functionally regulate these cells to external stimuli and dictate the response of white matter to ischemia.

Enhanced excitotoxicity is one of the essential pathways underlying the increased vulnerability of aging white matter to ischemia. The most prominent molecular structure leading to this enhanced excitotoxicity is the upregulation of GLT-1 (in human termed EAAT2) levels, which leads to an earlier and more robust glutamate release that remains

sustained after the end of OGD despite efficient control of baseline glutamate levels. Consequently, blockade of the reversal of the Na^+ -dependent glutamate transporters or blockade of AMPA/kainate receptors preserves aging axon function during OGD and promotes recovery comparable to young axons.

Accumulation of Ca^{2+} is an important step in the development of ischemic injury in young white matter. However, removal of extracellular Ca^{2+} or blockade of Ca^{2+} entry secondary to reverse operation of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger (NCX) worsens aging axon function recovery after OGD, a perplexing observation that implies Ca^{2+} entry during ischemia is a protective measure. The Ca^{2+} -independent nature of ischemic injury in aging white matter raises another important question as to how AMPA/kainate receptor activation mediates injury. Because blockade of AMPA/kainate receptors promotes axon function recovery across all age groups, it suggests that Na^+ entry, via AMPA/kainate receptors, rather than Ca^{2+} , mediates injury during ischemia in older white matter. Overload of Na^+ results in irreversible toxic swelling, even in the absence of extracellular Ca^{2+} (Rothman and Olney, 1995), and a rise in intracellular Na^+ promotes reversal of GLT-1, resulting in glutamate accumulation (Szatkowski et al., 1990). Together with the upregulation in GLT-1 expression, increases in intracellular Na^+ may be the leading cause of increased and early release of glutamate, overactivating AMPA/kainate receptors and creating a vicious cycle that underlies the vulnerability of aging white matter to ischemia. Furthermore, an increase in Na^+ concentration interferes with maintenance of the transmembrane ion gradient. This challenges the Na^+-K^+ ATPase pump and compromises the ability of aging axons to maintain membrane properties and axonal excitability, further contributing to an increased vulnerability to ischemia (Scavone et al., 2005). It is possible that Ca^{2+} release from intracellular Ca^{2+} stores (ICS), and the interplay between intracellular and extracellular Ca^{2+} , is more critical during ischemia in aging axons. Although a role for Ca^{2+} release from endoplasmic reticulum via IP_3 and ryanodine receptors is described in young white matter (Thorell et al., 2002), various Ca^{2+} -dependent neurophysiological (Landfield and Pitler, 1984; Campbell et al., 1996) and signaling pathways (Verkhratsky et al., 1998) remain unexplored in white matter, particularly as these relate to ischemia and aging.

Because blockade of NMDARs causes a similar worsening of axon function recovery, Ca^{2+} entry through NMDARs may also be an important element to protect aging axon function against ischemia. Alternatively, the role of NMDARs as axon–glia–metabolic couplers becomes more critical to support the increased energy burden of aging axons. It is also plausible that removal of Ca^{2+} may lead to glutamate release via hemichannels from aging astrocytes, thus exacerbating excitotoxic injury. These mechanisms remain to be explored. In this review, the focus is on the age-dependent expression pattern of NMDA receptors on oligodendrocytes and how activation of these receptors during ischemia may play a role in axon function recovery. We also compare and correlate these age-related changes of NMDA receptor distribution on oligodendrocytes with the developmental period, both structurally and functionally, to draw parallel conclusions. We propose that age-specific expression patterns of NMDA receptor localization in white matter may modulate the plasticity of oligodendrocytes and myelin in response to ischemia as a function of age.

2. Aging increases the vulnerability of oligodendrocytes to ischemia

The structural composition and function of white matter are integrated; therefore, age-dependent modification of white matter underlies pathophysiological mechanisms of ischemic white matter injury. Age-dependent adjustments in white matter architecture presumably allow for better adaptation to the compromised energy status of the tissue (Baltan et al., 2008). Among these adaptive changes, glutamate content, upregulation of GLT-1 levels, and impaired mitochondrial dynamics render white matter more vulnerable to ischemia (Baltan et al., 2008; Baltan, 2014). These changes in the excitotoxic pathway initiating and merging onto the oxidative pathway (Baltan 2009) is an essential step in ensuing irreversible injury; therefore, age-dependent changes in the excitotoxic injury pathway determine the extent and outcome of injury. Exposing mouse (Swiss Webster or BL6 males) optic nerves (MONs) obtained from animals at 1, 6, 12, 18, and 24 months of age to OGD at 37°C confirmed that aging axon function is more vulnerable to ischemia (rapid loss of axon function) and recovers less in a duration-dependent manner (Baltan et al., 2008). Axon function is quantified as the area under the characteristic three peaked compound potential (CAP), which reflects the combined response of all axons stimulated using constant current pulses to evoke maximal CAPs (Stys et al 1992; Cummings et al 1972). On the other hand, if OGD duration is constant, axon function recovers less (Baltan et al., 2008) and is associated with more extensive oligodendrocyte death as age increases (Figure 1). These age-dependent functional and structural responses to injury are not simply a reflection of earlier establishment of injury, but rather are a result of changes in ischemic injury mechanisms with age. It is plausible that oligodendrocytes undergo some structural and molecular changes during the aging process and that these changes contribute to the increased vulnerability of aging white matter to ischemia. It is now well-established that oligodendrocyte-axon interactions are important for supporting axon function under physiological and pathological conditions (Nave and Trapp, 2008; Trapp and Nave, 2008). Consequently, protection of oligodendrocytes by blockade of AMPA/kainate receptors during an ischemic attack protects axon structure and promotes functional recovery (Tekkok and Goldberg, 2001; Tekkok et al., 2007; Baltan et al., 2008). On the other hand, interventions that do not preserve oligodendrocytes are not beneficial to axon function. For instance, blockade of NMDA receptors during OGD fails to protect oligodendrocytes and axon function (Figure 2A) (Tekkok and Goldberg, 2001; Tekkok et al., 2007; Baltan et al., 2008). Due to conflicting reports on the effect of different NMDAR blockers on white matter protection (Tekkok et al., 2007; Bakiri et al., 2008; Manning et al., 2008), we tested three different NMDAR antagonists: a pore blocking agent, MK-801 (10 μ mol/L (n=15) or 20 μ mol/L (n=2)), a glycine binding site blocker, 7-CKA (50 μ mol/L, n=5), and an NMDA binding site blocker, DL-APV (100 μ mol/L, n=5), which collectively confirmed our findings (Tekkok et al 2007). Furthermore, combining AMPA/kainate and NMDA receptor antagonists does not provide additional protection to axon function (Figure 2C). These results do not negate the fact that NMDA receptors are activated on oligodendrocytes, but suggest that activation of these receptors does not mediate white matter ischemic injury. On the contrary, the combination of NMDA and AMPA/kainate blockers suppresses axon function recovery, suggesting that activation of NMDA receptors during ischemia exerts protection to axons (Figure 2C- see NBQX vs NBQX + 7CKA). Experiments on aging white

matter further verify that NMDA receptors are functional and are activated during ischemia. Exposing MONs from 12-month-old mice to OGD in the presence of the NMDA receptor blocker 7CKA hinders recovery, even after a shorter period of OGD (Baltan et al., 2008) (Figure 2B), suggesting that NMDA receptors located on oligodendrocytes are activated during an ischemic attack. These data support the concept that NMDA receptor antagonism does not provide effective protection to young or aging white matter (Baltan et al., 2008) (Figure 2A). Likewise, complementary experiments show that oligodendrocytes are only preserved when AMPA/kainate receptors are blocked; NMDA receptor blockade fails to prevent oligodendrocyte death in young (Tekkok and Goldberg, 2001) or aging white matter, even after 30 min of OGD (Figure 1B). How aging affects oligodendrocytes and whether the role of NMDA receptors on oligodendrocytes changes with aging are intriguing questions that we are actively pursuing. Curiously, Bakiri et al. (Bakiri et al., 2008) reported that AMPA/kainate receptor blockade alone or memantine alone (NMDAR blocker) do not promote axon function recovery after OGD, but MK-801 alone or memantine together with AMPA/kainate receptor blockade benefit axons function. On the other hand, an *in vivo* study reported memantine protected immature oligodendrocytes and myelin against hypoxic/anoxic injury (Manning et al., 2008). The discrepancy may stem from methodological differences between the studies such that Bakiri et al. (2008) used P28 rat optic nerves where single peaked action potentials were elicited by voltage pulses at 33°C and Manning et al. used P6 rat pups (2008), while our studies utilized 4-8-week-old MONs where maximal compound action potentials (CAPs) with three characteristic peaks at 37°C were evoked by constant current (Tekkok et al., 2007).

3. Age-specific expression patterns of NMDA receptors on oligodendrocytes may determine their function

GluN1 (Collingridge et al., 2009), the functional subunit of NMDA receptors (Kutsuwada et al., 1992; Monyer et al., 1992; Ishii et al., 1993), is expressed in optic nerves at every age (Baltan et al., 2008) and shows an age-specific distribution (Figures 3). Mature oligodendrocytes express a majority of GluN1 subunits on their cell bodies, while more GluN1 expression is observed on myelin processes on aging oligodendrocytes (Figure 3) as previously reported (Karadottir et al., 2005; Salter and Fern, 2005; Micu et al., 2006). Blockade of NMDA receptors during OGD at younger ages, when they are located predominantly on oligodendrocyte cell bodies, has no effect on axon function recovery (Figure 2A) and does not protect oligodendrocytes; however, when these receptors are expressed on myelin processes during aging, blockade impedes axon function recovery after ischemia (Figure 2B) and causes widespread oligodendrocyte death (Figure 1B). Upon activation of NMDA receptors during ischemia, a distinct pathway of Ca^{2+} influx coupled to a specific cellular compartment- cell body vs. cell body and myelin processes of oligodendrocytes- seems to dictate oligodendrocyte-axon interactions and determine axon function recovery following an ischemic episode. Aging specifically seems to regulate the distribution and function of NMDA receptors, as opposed to AMPA/kainate receptors (data not shown); therefore, blockade of AMPA/kainate receptors protects and promotes axon function recovery equally in every age group of optic nerves (Baltan et al 2008) (Figure 1B). Further support that Ca^{2+} influx during OGD is important to support aging axon function

recovery comes from experiments when extracellular Ca^{2+} is removed (replaced with equimolar Mg^{2+}) (Baltan et al., 2008). This approach almost entirely avoids axon ischemic injury and spares oligodendrocytes in young MONs, while it completely impairs axon function recovery and disrupts oligodendrocytes in aging MONs (Baltan et al., 2008). Ca^{2+} acts as a second messenger and mediates a wide range of cellular responses involved in almost all of cellular physiology, ranging from optimal functioning to excitotoxicity and cell death. The Ca^{2+} hypothesis in aging brain suggests that the degree and duration of Ca^{2+} dysregulation predicts the degree of cognitive decline (Foster and Kumar, 2002). The loss of axon excitability and axon function when Ca^{2+} influx or NMDA receptors are blocked may provide further support for age-related cognitive decline (see below).

Brain development and aging show overlapping and unique patterns of change (Tamnes et al., 2013). It has been suggested that common mechanisms may be implicated in brain maturation and degenerative changes in aging (Wines-Samuelson et al., 2005; Wines-Samuelson and Shen, 2005). Characterizing GluN1 subunit localization during optic nerve development revealed a maturation-dependent and cell-specific NMDA receptor expression pattern (Figure 3). Immature MONs at postnatal day 4-11 (P4-11, yellow) and P8-12 (purple) show diffuse GluN1 labeling, while labeling (red) becomes more cell-specific at P19-21 (orange). Note the prominent drop in MON cellularity and cellular organization apparent with the number of glial cell nuclei. On closer inspection, at P4-11, GluN1 subunit expression mostly co-localizes onto GFAP (+) astrocytes (Figure 3C, yellow); at P12-18, GluN1 immunoreactivity is most prominent on myelin associated glycoprotein (MAG) myelin processes (Figure 3C, purple); and at P18-21 (orange), GluN1 labeling co-localizes predominantly onto adenomatous polyposis coli (APC) (+) mature oligodendrocyte cell bodies (Figure 3C). If the hypothesis that GluN1 localization determines axon function recovery following OGD is correct, then blockade of NMDA receptors earlier than P18-21 should impair axon function recovery (Figures 2D,E,F). Indeed, immature axons at P4-11 and P12-18 recover less when NMDA receptors are blocked (Figures 2D and E), while MONs obtained at P18-21 showed comparable recovery whether or not NMDA receptors were blocked following OGD (Figure 2F). Note that immature axons are relatively more resistant to OGD (60 min), which rapidly changes over the course of development (Figures 2D, E and F), presumably due to axon nodal architectural formation as a function of oligodendrocyte maturation. It is important to note that at an earlier stage of development (P2), premyelinated axon cylinders express GluN1 subunits at focal regions and the NMDA receptor antagonists MK-801 or memantine improve axon recovery after OGD (90 min) (Huria et al., 2014). Interestingly, at this age, NMDA receptors do not mediate Ca^{2+} influx or cell death, as almost all of these receptors are expressed by astrocytes (Huria et al., 2014). On the other hand, contradictory to our findings at P4-11, Salter and Fern (Salter and Fern, 2005) reported that ischemia causes a Ca^{2+} -dependent detachment and disintegration of myelin sheaths assessed by loss of GFP (+) oligodendrocyte fluorescence at P10 (Salter and Fern 2005), which was attenuated by NMDAR blockade. However, this study did not monitor axon function and was carried out in optic nerves obtained from FVB/N female mice.

Taken together, these findings support the novel concept that NMDA receptors on oligodendrocytes exhibit an age-specific localization, which presumably dictates their role in

normal physiological and pathological conditions. It is also interesting that developmental changes, to a certain extent, are reversed with aging at a much slower pace, at least as it applies to NMDA receptor distribution and their role in ischemia. NMDA receptors are selectively more vulnerable to aging than other glutamate receptors in mice and in multiple other mammalian species (Magnusson et al., 2010), including non-human primates (Hof et al., 2002). The subunit composition of NMDA receptors on oligodendrocytes may show age-specific variations in addition to their expression levels and distribution, as has been reported in gray matter (see review by Magnusson et al 2010).

4. Conclusions

The primary novel finding of our studies is that blockade of NMDA receptors worsens functional outcome in aging white matter. This seminal finding has important clinical implications to explain why NMDA receptor antagonists that conferred protection to neuronal cell bodies in experimental stroke settings failed to provide any benefit in clinical stroke trials. It is essential to preserve the functional integrity of both white matter and gray matter, since neuronal populations whose axonal connections have been destroyed or disabled are functionally useless. Furthermore, therapeutic options that have proven successful in gray matter, in this case NMDA receptor blockade, may be useless or even harmful in white matter. In this way, our studies challenge the existing convention in stroke research that assumes a common mechanism of injury in the brain.

Another interesting finding is that aging leads to structural and functional changes in the glutamate signaling system of oligodendrocytes by reorganizing NMDA receptor expression patterns on cell bodies and the myelin processes that they manufacture. The expression of functional NMDA receptors in myelin sheath and their ability to mediate Ca^{2+} accumulation does not necessitate that they mediate disruption to myelin, but may underlie an important signaling cascade in myelin to support axon function. Moreover, during development, reciprocal oligodendrocyte-axon signaling plays a key role in the initiation and completion of myelination – a delicately regulated process by Neuroregulin, which switches oligodendrocytes from the activity-independent to the activity-dependent mode of myelination by increasing NMDA receptor currents in oligodendrocytes (Lundgaard et al., 2013). Because blockade of these receptors when they are populated on myelin processes fails to protect axons against ischemia, age-specific, NMDA receptor-dependent axo-myelinic communication seems to be essential for guiding axon function recovery after ischemia. The age-specific expression pattern of oligodendrocyte NMDA receptor complexes may be one of the factors underlying the increased vulnerability of aging white matter to ischemia.

Aging specifically alters NMDA receptors and the relationships observed between NMDA receptor expression levels and their distribution in gray matter are associated with cognitive decline and memory and learning impairments during aging (Newcomer et al., 2000). NMDA receptor subunit expression also changes with aging. For instance, GluN1 subunit expression levels decrease concomitant with a progressive hypo-functioning of NMDA receptors in dentate, cortex, and hippocampus (Morrison and Gazzaley, 1996; Magnusson, 1998) due to reduced binding to the NMDA site. In aging white matter, NMDA receptors

redistribute and expand onto myelin processes in addition to oligodendrocyte cell bodies in a similar but reverse order to the developmental period, during which NMDA receptors condense onto oligodendrocyte cell bodies from MAG(+) myelin compartments. Unexpectedly, GluN1 subunit protein levels increase in aging white matter (data not shown), as opposed to reported reductions in aging gray matter (Newcomer et al., 2000). There are different mechanisms of aging that affect the NMDA receptor complex within gray matter and obviously in comparison between gray and white matter. If NMDA receptors assume a function depending on their location, the question remains as to what the driving force is that regulates NMDA receptor expression patterns.

Development and aging are high energy demand periods that are characterized by increased demand and/or switches in preferred energy substrates. Emerging evidence implies a role for myelin and oligodendrocytes in axonal energy metabolism (Hirrlinger and Nave, 2014; Baltan, 2015). Lactate efficiently supports axon function in the absence of glucose and becomes a preferred energy metabolite when axons discharge at high frequencies (Brown et al., 2003; Tekkok et al., 2005). A similar lactate transport system between oligodendrocytes and the axons they myelinate would suggest a novel metabolic coupling pathway in white matter (Hirrlinger and Nave, 2014; Baltan, 2015). Conditions that activate this lactate shuttle system and the signaling mechanisms that mediate activation of this system are currently of great interest. Whether NMDA receptors contribute to and reorganize their distribution and function with respect to evolving metabolic demands of white matter during development and aging to support axon function remains to be explored. Future studies are expected to unravel the details of oligodendrocyte-myelin-axon metabolic coupling and the role of glutamate signaling in sustaining CNS function.

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HIGHLIGHTS

- 1)** Blockade of NMDA receptors worsens functional outcome in aging white matter.
- 2)** NMDA receptors on oligodendrocytes exhibit an age-specific localization
- 3)** NMDARs may play a role as axon–glia-metabolic couplers during aging and development.

Figure Baltan 1.

(A) Aging oligodendrocytes are more vulnerable to ischemia. Numerous APC (+) oligodendrocytes (green) are observed among GFAP (+) astrocytes (red) and their nuclei stained with Sytox (blue) in young (1 month old, upper left) and aging (12 months old, lower left) mouse optic nerves (MONs) kept under control conditions. Oxygen glucose deprivation (OGD) for 45 min resulted in widespread oligodendrocyte death in aging MONs ($91 \pm 11\%$ APC (+) cells lost, $n=4$) (right lower panel), while the same insult caused a more modest loss of oligodendrocytes in young MONs ($35 \pm 9\%$ APC (+) cells lost, $n=4$). Scale bar = 20 μm . (B) Blockade of NMDA receptors during ischemia causes widespread oligodendrocyte death in aging MONs. Blockade of AMPA/kainate receptors with NBQX (30 μM , AMPA/kainate receptor antagonist) prevented oligodendrocyte death after OGD (60 min) in aging MONs (12 months old) (upper panel). Blockade of NMDA receptors with 7CKA (50 μM) failed to protect aging oligodendrocytes ($4 \pm 1\%$ APC (+) oligodendrocytes left, $n=9$) against even a shorter period of OGD (30 min) (lower panel). Scale bar = 10 μm .

Figure Baltan 2.

Blockade of NMDA receptors during OGD hinders recovery in aging axons. The NMDA receptor glycine binding site antagonist 7CKA (50 μM) does not improve compound action potential (CAP) recovery (A) after 60 min of OGD in young (1 month old) MONs. (B) A similar approach worsens the recovery of aging axons, followed by a delayed loss of CAP area, even after 30 min of OGD in aging (12 months old) MONs. *** : $p < 0.001$, one-way ANOVA. (Reproduced and Modified from Tekkok et al 2007 and Baltan et al 2008). (C) Combined blockade of NMDA and AMPA/kainate receptors does not provide additional protection to axon function. Histograms summarize CAP area recovery when young MONs were exposed to 60 min OGD (White), OGD + MK-801 (yellow, 10 μM, NMDA receptor ionic pore blocker), OGD + NBQX (red, 30 μM, AMPA/kainate receptor blocker), OGD + MK-801 + NBQX (green), OGD + 7-CKA (blue, 50 μM), OGD + NBQX (cyan), MK-801 + 7-CKA + NBQX (olive). Note that CAP area recovered less with 7CKA + NBQX and MK-801 + 7CKA + NBQX. *** $p < 0.0006$, one-way ANOVA. (D) Blockade of NMDA receptors during development hinders axon function recovery after ischemia. Blockade of NMDA receptors during OGD (blue, 60 min) using MK-801 (10 μM, ionic pore antagonist) (A) at postnatal day 4 to 11 (P4-11, yellow) and (E) at P12-18 (maroon) hinders axon function recovery. (F) At P18-21 (orange), NMDA receptor blockade failed to benefit axon function recovery. Note that the extent of axon function suppression during OGD combined with MK-801 was more prominent at P4-11 and P12-18 (red arrows) compared to OGD only. *** $p < 0.001$, ** $p < 0.009$, NS; $p = 0.35$, one-way ANOVA.

Figure Baltan 3.

(A) NMDA receptors are expressed by young and aging oligodendrocytes. GluN1 functional subunits (red) were detected on oligodendrocyte cell bodies in young MONs (1 month old, upper panels) and were detected on and around oligodendrocyte cell bodies in aging MONs (12 months old, lower panels). Cell nuclei were labeled with Sytox (blue, right upper and lower panels). Scale bar = 10 μ m. (Reproduced and modified from Baltan et al 2008). (B) GluN1 labeling for NMDA receptors shows developmental specificity. In MONs from postnatal day 4-11 (P4-11, yellow), GluN1 labeling (red) was diffuse, while in P8-12 MONs (purple), GluN1 labeling (red) was diffuse but began to co-localize with some nuclei. In the P19-21 group (orange), GluN1 labeling (red) became more cell-specific. Glial nuclei are labeled in blue (Sytox). Scale bar = 30 μ m. Note the prominent drop in MON cellularity and cellular organization of cells observed with Sytox (+) glial nuclei number (blue) and organization. (C) NMDA receptors are expressed in a cell-specific manner during development. At postnatal day 4 (P4, yellow), functional subunits of the NMDA receptor (GluN1; red) were primarily co-localized onto GFAP (+) astrocyte (green) cell bodies and proximal processes (yellow, merged). At P12 (purple), GluN1 (red) was almost completely localized onto MAG (+) compartments of myelin processes (yellow, merged) while also beginning to outline some cell bodies (white arrows). At P18 (orange), there was a considerable reduction in GluN1 (red) expression levels localized principally onto APC (+) oligodendrocytes cell bodies (green). Glial nuclei are shown in blue (Sytox). Scale bar = 10 μ m.