REVIEW ARTICLE

Cholinergic Signaling in Myelination

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There is a long history of research on acetylcholine (ACh) function in myelinating glia, but a resurgence of interest recently as a result of the therapeutic potential of manipulating ACh signaling to promote remyelination, and the broader interest in neurotransmitter signaling in activity-dependent myelination. Myelinating glia express all the major types of muscarinic and nicotinic ACh receptors at different stages of development, and acetylcholinesterase and butyrylcholinesterase are highly expressed in white matter. This review traces the history of research on ACh signaling in Schwann cells, oligodendrocytes, and in the myelin sheath, and summarizes current knowledge on the intracellular signaling and functional consequences of ACh signaling in myelinating glia. Implications of ACh in diseases, such as Alzheimer's disease, multiple sclerosis, and white matter toxicity caused by pesticides are considered, together with an outline of major questions for future research.

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Key words: acetylcholine (ACh), acetylcholinesterase (AChE), muscarinic receptors, butyrylcholinesterase (BChE), nicotinic receptors, organophosphates

Introduction

Acetylcholine (ACh) has been implicated in myelination for decades, but the precise roles of ACh signaling in myelinating glia remain poorly understood. In part this is a consequence of the complexity of cholinergic signaling, mediated by a rich diversity of receptors and enzymes regulating ACh, compounded by the complexity of oligodendroglial development and myelination, but also because other signaling molecules regulating oligodendroglial development and myelination have received much more attention in recent years. In contrast, the function of cholinergic signaling in myelin was of intense interest between WWII into the 1960's; however, this was in the context of determining the mechanism of action potential propagation. The ionic basis of the action potential is so well established today, earlier theories of action potential generation are largely forgotten. Acetylcholine was the first neurotransmitter identified, and for many years acetylcholine signaling was the only known mechanism of electrogenesis. It was reasonable, therefore, to propose that excitation at nodes of Ranvier might well operate in a similar manner to the electrogenic neuromuscular junction or electric organ of *Torpedo* (Nachmansohn, 1959). Acetylcholinesterase (AChE) is the enzyme concentrated at cholinergic synapses, but also found elsewhere, that rapidly hydrolyzes acetylcholine to acetate and choline. In support of the chemical theory of action potential propagation, strong AChE activity at the node of Ranvier (Fig. 1) and in some cases less intense activity in internodal regions was evident by histochemical analysis at the light level (Adams et al., 1969; Brzin and Dettbarn, 1967). At the ultrastructural level, AChE staining is localized to axonal membrane of myelinated and unmyelinated sciatic nerve fibers (Schlaepfer and Torack, 1966). AChE activity at nodes of Ranvier was detected in myelinated frog nerve (Brzin and Dettbarn, 1967), axons of dorsal root ganglia and peripheral nerve (Novikoff et al., 1966), sciatic nerve (Schlaepfer and Torack, 1966), myelinating Schwann cells (Bogusch, 1991), and Schwann cells and axon of the squid giant nerve fiber (Villegas and Villegas, 1974). Pharmacological evidence for the chemical theory of action potentials was that AChE inhibitors, such as diisopropyl fluorophosphate (DFP), were found to block action potential generation in squid giant nerve (Bullock, 1947). Eccles argued against the chemical basis of the action potential in 1945 (Eccles, 1945) but the hypothesis remained viable into the mid 1960's.

Acetylcholine and Myelinating Glia

Despite the demise of the chemical basis of action potential propagation as a useful hypothesis, evidence for ACh signaling in myelinated axons remained strong. In the 1980's radioligand

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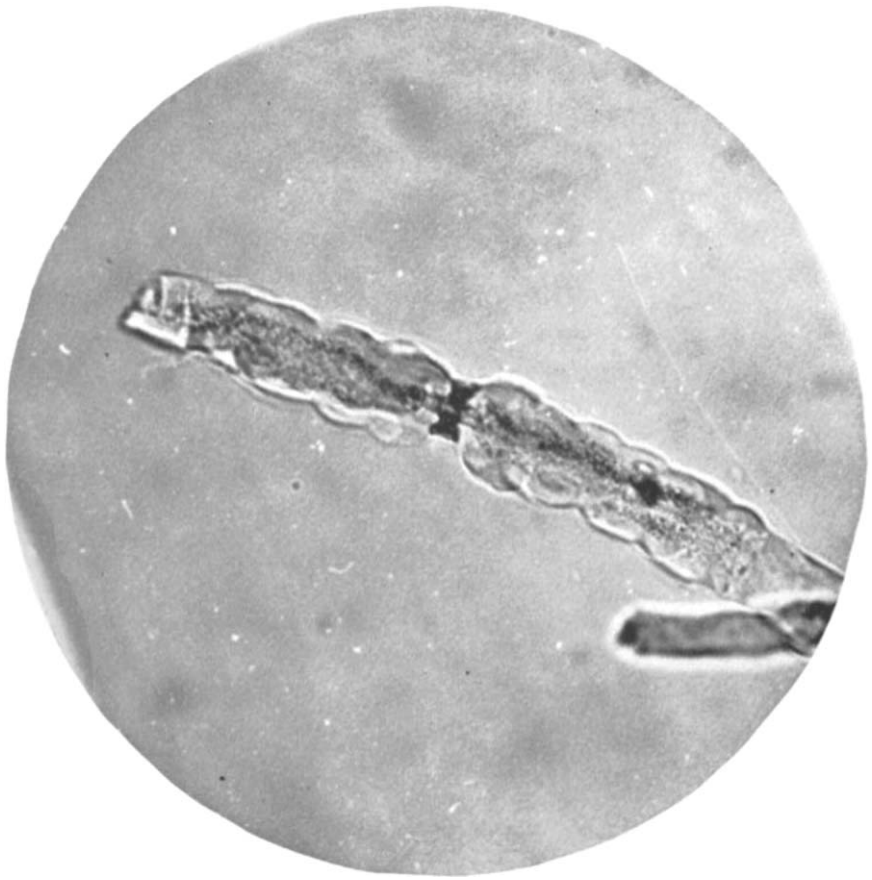

FIGURE 1: Histochemical localization of acetylcholinesterase activity at the node of Ranvier. Single nerve fiber isolated from sciatic nerve bundle is shown. Note the dark precipitate at the node and weaker internodal staining. Histochemical analysis was performed by the Karnovky copper-ferrocynide procedure. Figure reprinted with permission by Brzin and Dettbarn (1967).

binding studies on purified myelin fractions isolated from brain identified acetylcholine receptors, notably M1 and M2 muscarinic receptor subtypes (described below) (Evans et al., 1985; Hammer et al., 1980; Larocca et al., 1987; Luthin and Wolfe, 1984; Watson et al., 1983, 1986a,b). These receptors were functional as shown by inhibited adenylate cyclase activity in myelin fractions treated with the acetylcholine receptor agonist carbachol (Larocca et al., 1987). The function of acetylcholine receptors in compact myelin remains unclear today, but as early as 1958, modifying cholinesterase activity was suggested as a possible treatment for multiple sclerosis (Zinnitz and Hammer, 1958).

In addition to AChE, pseudocholinesterase activity (also referred to as butyrylcholinesterase) (BChE) is found at high levels in white matter. Butyrylcholinesterase is a nonspecific cholinesterase enzyme that hydrolyzes many different choline-based esters. The substrate Butyrylcholine is a synthetic compound that is used to distinguish true AChE activity from pseudocholinesterase activity. Interestingly, both pseudocholinesterase and acetylcholinesterase activity increase in chick spinal cord culture between 8 and 16 days, the period of active myelination (Kim et al., 1972; State et al., 1977). AChE has been shown to contribute to apoptosis as a deoxyribonuclease implicating cholinergic signaling in early development (Du et al., 2015). BChE inhibition with antisense BChE transfection in the OLN-93 oligodendroglia cell line downregulates protein kinase A (PKA) and reduces cell proliferation (Robitzki et al., 2000).

In mature myelin, pseudocholinesterase replaces true cholinesterase activity, which predominates before and during myelination, suggesting that nonspecific cholinesterase enzymes may have a function in metabolism of myelin or biosynthesis of myelin (State et al., 1977). BChE can hydrolyze myelin proteolipid protein (PLP) and lead to myelin decompaction, for example (Pottie et al., 2011). A role of acetylcholinesterase in maintenance of the mature myelin sheath was suggested as early as 1952 by Ord and Thompson (1952). Although the function of cholinesterase activity in white matter was unknown at the time, developmental studies showing a correlation between acetylcholine receptor expression and that AChE activity peaked during periods of myelination, suggested ACh signaling as a mechanism for functional activity in the axon to stimulate myelination (Kim et al., 1972). The concept of activity-dependent myelination as a non-synaptic form of nervous system plasticity has gained broad experimental support recently (Fields, 2015).

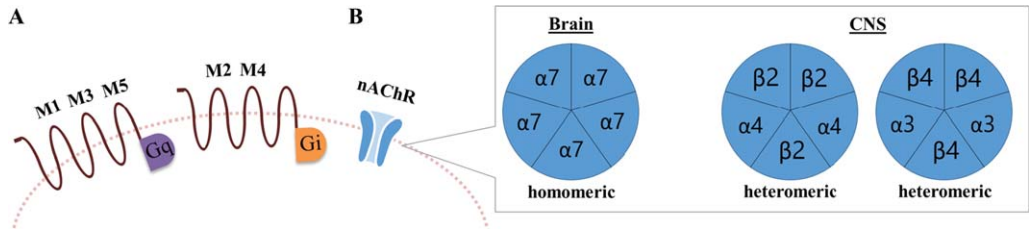

FIGURE 2: Structure of muscarinic and nicotinic acetylcholine receptors. (A) Muscarinic acetylcholine receptor is a transmembrane G-coupled protein. M1, M3, M5 are coupled to G_q (yellow). M2 and M4 receptors are coupled to G_i (green). (B) Nicotinic receptors (blue) are composed of alpha, beta, gamma subunits in homomeric and heteromeric combinations.

AChE is a diffusion limited enzyme, i.e., it catalyzes the hydrolysis of ACh more efficiently than ACh can diffuse into the extracellular milieu (Hasinoff, 1982). Such catalytic action ensures that cholinergic neurotransmission is precisely localized between the presynaptic and postsynaptic neuron, with little availability beyond the synapse. If ACh is essential for glial development and function, are there then extrasynaptic sources of ACh? Can ACh be released along axons like other neurotransmitters through vesicular (Kukley et al., 2007; Wake et al., 2015; Ziskin et al., 2007) and non-vesicular (Fields and Ni, 2010) release mechanisms? Another possibility is that glial cells may synthesize ACh to regulate their own biology via autocrine signaling. In support of this, oligodendrocytes have been shown by immunocytochemistry to express choline acetyltransferase (ChAT) (Lan, 1996; MacDonald et al., 2002), the enzyme responsible for the synthesis of ACh, and synthesis of ACh has been measured from Schwann cells of squid giant nerve (Heumann et al., 1981).

Acetylcholine Receptor Subtypes

Muscarinic acetylcholine receptors are regulated by G proteins, in contrast to nicotinic acetylcholine receptors, which are ligand-gated transmembrane channels (Fig. 2). Both acetylcholine receptor families are broadly expressed in the CNS, PNS, and other tissues, where they control a diverse range of functions (Fig. 3). Different muscarinic receptors vary in the G protein to which they are bound. M1, M3, and M5 receptors are coupled to G_q, and so their activation upregulates Phospholipase C (PLC), inositol triphosphate (IP3), and Ca^{2+}. The M2 and M4 receptors are regulated by G_i, which causes inhibition of cAMP production and inhibition of voltage-gated Ca^{2+} channels (Eglen, 2006).

Nicotinic acetylcholine receptors are broadly divided into four subfamilies based on different combinations of nAChR subunits from 17 nAChR genes. Each receptor has five subunits of homomeric or heteromeric combinations of proteins. In the brain, homomeric alpha 7 receptors are common, and these channels are permeable to sodium, potassium and calcium. Heteromeric receptors comprised of two alpha 4 and three beta 2 receptors are present in the CNS and they are mainly permeable to sodium and potassium. Heteromeric receptors comprised of two alpha 3 and three beta 4 subunits are also present in the CNS. The subunit composition of nicotinic receptors in the CNS differs from nicotinic receptors in muscle-type nicotinic receptors of the neuromuscular junction, and from the nicotinic receptors in autonomic neurons, but there is much less data on the types and heterogeneity of nicotinic receptors in oligodendroglia.

Intracellular Signaling in Oligodendrocytes from AChR Activation

Research in the 1990's identified AChR induced activation of intracellular signaling pathways in OPCs and related cell lines, and verified the presence of functional acetylcholine receptors in myelinating glia. Acetylcholine agonists were found to stimulate calcium signaling (Takeda et al., 1995), inositol phospholipid hydrolysis (Post and Dawson, 1992), inhibit PKC (Post and Dawson, 1992), activate mitogen-activated protein kinase in OPCs (Larocca and Almazan, 1997), phosphorylate CREB (Sato-Bigbee et al., 1999), and increase c-*fos* expression (Cohen et al., 1996; Larocca and Almazan, 1997) (Fig. 4). The intracellular signaling pathways activated by cholinergic stimulation in oligodendroglia and myelin fractions supported the possibility that transcriptional regulation by ACh signaling could modify oligodendrocyte development and function. Evidence for M1 and M2 receptor involvement in OPC development (Cohen and Almazan, 1994), and M3 receptors (Molina-Holgado et al., 2003) was clear, but with the development of genetic analysis, OPCs were found to express all five subtypes of muscarinic receptors in a complex pattern of expression that displayed regional, developmental, and cell-specific heterogeneity (De Angelis et al., 2012; Ragheb et al., 2001).

The functional consequences of ACh receptor activation in cell culture studies indicated that differentiation, proliferation, and survival of OPCs are influenced by acetylcholine receptors in a complex manner that depends on developmental stage and receptor subtype (Belachew et al., 1998; He and McCarthy, 1994). Acetylcholinesterase inhibitor added to developing mouse cerebellar cultures was found in 1974 to

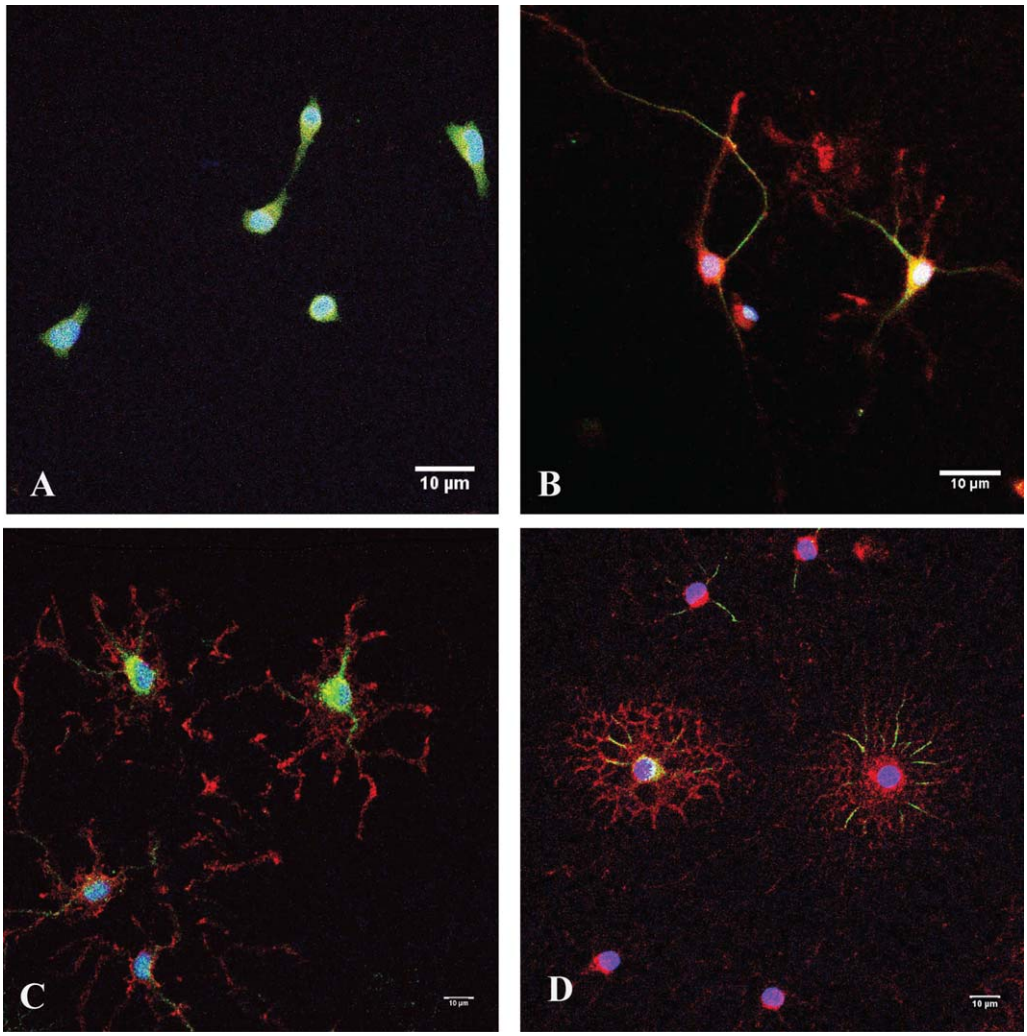

FIGURE 3: OPCs and premyelinating oligodendrocytes express muscarinic acetylcholine receptors. mAChR M1 receptor identified by immunocytochemistry on rat primary cell cultures stained for (A-D) NG2 (red) and M1 receptor (green). Cells were fixed 4 hrs (A), 24 hrs (B), 96 hrs (C) and 1 week (D) after isolation. Bar = 10 μm. NG2 chondroitin sulfate proteoglycan (1:500, Cat. MAB5384, Chemicon International, Billerica, MA, USA), Olig2 (1:500, Cat. 18953, IBL Co., LTD, Japan), NG2 chondroitin sulfate proteoglycan (1:500, Cat. AB5320, Millipore), Olig2 (1:500, MABN50, Millipore), mAChR M1 (1:500, Abcam, Cat. Ab111100).

inhibit myelination (Toran-Allerand, 1974). Research in cell culture also showed that voltage-gated calcium entry promotes OPC maturation (Cheli et al., 2015), and muscarinic acetylcholine receptors mediate oligodendrocyte progenitor survival through Src-like tyrosine kinases and PI3K/Akt pathways (Cui et al., 2006). Oligodendrocytes derived from multipotent neural precursors express cholinergic phenotype, the ACh synthesizing enzyme choline acetyltransferase (Lan et al., 1996), and the ACh transporter in GalC positive oligodendroglia (MacDonald et al., 2002). Culturing neurospheres in cholinergic receptor antagonist atropine decreases the number of GalC spheres, providing another line of evidence for muscarinic AChR function in oligodendrocyte development (MacDonald et al., 2002).

More recent research confirms that antagonists of acetylcholinesterase (benztropine/clemastine) promote oligodendrocyte differentiation (Deshmukh et al., 2013; Mei et al., 2014). In these studies, M1, M3, and M4 receptors were the main subtypes of AChRs expressed in OPCs, whereas all muscarinic receptor subtypes were found to be expressed at low levels in mature OLs. These receptor expression data support the suggestion that ACh may contribute to the maintenance of an immature proliferating progenitor pool and impede progression toward a mature state (De Angelis et al., 2012). Studies providing evidence for AChRs in oligodendrocytes are summarized in Table 1, and the associated functional effects of these receptors reported in the literature are provided in Table 2.

Schwann cells in the peripheral nervous system also participate in cholinergic signaling. A summary of the literature on AChR expression in Schwann cells is provided in Table 3, and evidence for the functional effects of AChRs in Schwann cells is provided in Table 4. Schwann cells express muscarinic

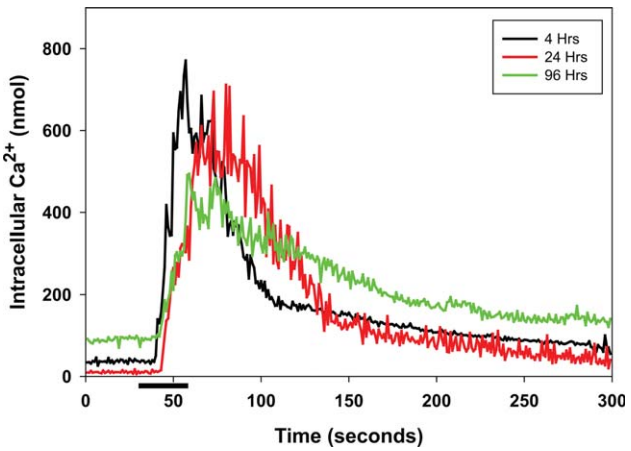

FIGURE 4: Intracellular calcium concentration in OPCs and pre-myelinating oligodendrocytes increases in response to application of 50 μM acetylcholine (horizontal bar). Each trace represents the concentration of intracellular calcium averaged from 17-29 responding cells at 4 hrs (black), 24 hrs (red) and 96 hrs (green) in cell culture. Calcium concentration (nanomolar) was determined from the ratio of average fluorescence at 340:380 nm excitation after loading cells with the calcium indicator Fura-2, and calibrated using the Grynkiewicz et al. (1985) equation.

receptors M1, M2, M3, and M4 (Loreti et al., 2006). Evidence for ACh signaling in axon-Schwann cell communication is strengthened by the finding that acetylcholine receptors on Schwann cells localize to the axon-Schwann cell boundary (Rawlins and Villegas, 1978). During development, Schwann cell precursors highly upregulate expression of Argin, a proteoglycin responsible for the clustering of AChR on the membrane (D'Antonio et al., 2006).

Additionally, sciatic nerves of M2/M4 knock-out mice show degenerating axons and alterations in myelin organization (Uggenti et al., 2014). Activation of M2 receptor arrests cell cycle and promotes Schwann cell differentiation via upregulation of *Sox10* and *Krox 20* and downregulation of *c-jun*, *Notch-1* and *Jagged-1*, and increased expression of myelin proteins (Uggenti et al., 2014). Moreover, activation of AChR on Schwann cells modifies the viscosity of the myelin membrane (Verdiyan et al., 2016).

Nicotinic Acetylcholine Receptors

In addition to muscarinic AChRs, nicotinic acetylcholine receptors are also expressed in oligodendroglia where they influence oligodendrocyte differentiation. Patch-clamp recordings, immunostaining, calcium imaging, and intracellular signaling studies show the presence of ionotropic nicotinic acetylcholine receptors in NG2 cells (OPCs) in hippocampal slices of mice (Velez-Fort et al., 2009). In these studies, functional nAChRs are found during the second postnatal week, a period in which OPCs become the most abundant proliferative cell type in CA1 stratum radiatum. Pharmacological evidence indicates that OPCs express alpha7 containing nAChRs (Velez-Fort et al., 2009), but PCR and immunocytochemical studies of OPCs from rat corpus callosum show expression of several nicotinic AChR subunits, including alpha 3, alpha 4, alpha 5, alpha 8, beta 2 and beta 4 (Rogers et al., 2001). In these studies, 65% of OPCs increased intracellular calcium in response to nicotine application and the response was sensitive to the nAChR alpha 4/beta 2 antagonist DHbetaE (dihydro-beta-erythroidine) and sensitive to the voltage-sensitive calcium channel antagonist nifedipine. A third of these cells exhibited calcium oscillations that continued in the presence of nicotine at intervals of 20–30 seconds, which diminished in amplitude over a period of 2–3 minutes. Together these studies suggest that nicotinic receptors could influence myelination. In support of this hypothesis, donepezil, an AChE inhibitor developed for treatment of Alzheimer's disease, was found to stimulate differentiation and maturation of OPCs without affecting proliferation or cell viability (Imamura et al., 2015). Increased myelin associated glycoprotein (MAG) and myelin basic protein (MBP) promoter activity accompanies donepezil-induced oligodendrocyte differentiation as shown by Luciferase assay, indicating that donepezil increases myelin gene expression through enhanced gene transcription (Imamura et al., 2015).

ACh Signaling in Myelinating Glia in Disease

George Bartzokis suggested acetylcholinesterase inhibitors may improve myelin integrity in Alzheimer's disease (AD), based on MRI studies of AD patients who had been treated with AChE inhibitors to promote cholinergic synaptic function (Bartzokis, 2007). BChE has also been implicated in AD, in treatment for AD (Greig et al., 2002), and BChE genotype is a factor in AD (Lane and He, 2013). With respect to demyelinating diseases, cholinesterase inhibitors have been shown to improve remyelination in multiple sclerosis (MS) and in animal models of MS. AChE inhibitor treatment improved cognitive deficits present in rats with experimental auto-immune encephalomyelitis (EAE) (D'Intino et al., 2005), and chronic hypofusion (Wang et al., 2010). Abiraman et al., (2015) reported that solifenacin, a mAChR antagonist, induced oligodendrocyte differentiation of human OPCs transplanted into a mouse model of hypomyelination. The human OPCs isolated from fetal human brain were found to have high levels of M3 receptors (Abiraman et al., 2015). Non-selective muscarinic receptor antagonists promote myelin repair in rodents (Deshmukh et al., 2013; Mei et al., 2014). Clemastine, an antimuscarinic compound, rescues behavioral changes and enhances remyelination in a cuprizone mouse model of demyelination (Li et al., 2015). In these studies, the behaviors analyzed were open field tests and Y-maze learning, which are considered assays of schizophrenia-like behaviors. Clemastine also enhances myelination in the prefrontal cortex and rescues behavioral changes

TABLE 1: Expression of Acetylcholine Receptors in Oligodendrocyte Lineage Cells

AChR Subtype	Evidence of Expression	Additonal Reported OL Stage Markers	Paper
M1	PCR	A2B5+, GalcC+, LB1+, MBP+	Abiraman et al. (2015), Ragheb et al. (2001), Cohen and Almazan (1994), De Angelis et al. (2012)
	Immuno	LB1+, MBP+	De Angelis et al. (2012)
	Northern Blot		Larocca and Almazan (1997)
M2	PCR	A2B5+, GalcC+, LB1+, MBP+	De Angelis (2012), Ragheb et al. (2001), Cohen and Almazan (1994)
	Immuno	LB1+, MBP+	De Angelis et al. (2012)
	Northern Blot		Larocca and Almazan (1997)
M3	PCR	LB1+, A2B5+, Cd140α+, O2A+, A2B5+, GalcC+	De Angelis et al. (2012), Ragheb et al. (2001)
	Immuno	LB1+, MBP+	De Angelis et al. (2012)
	Northern Blot		Larocca and Almazan (1997)
	Radioligand competitive binding	A2B5+, GalcC+	Ragheb et al. (2001)
M4	PCR	A2B5+, GalcC+, LB1+, MBP+	De Angelis et al. (2012), Ragheb et al. (2001)
	Immuno	LB1+, MBP+	De Angelis et al. (2012)
M5	PCR	LB1+, MBP+	De Angelis et al. (2012), Ragheb et al. (2001)
nAChR α3, α4, α5, α7, β2, β4	PCR, Immuno	O2A+ A2B5+	Rogers et al. (2001)

observed in socially isolated mice (Li et al., 2015; Liu et al., 2016). Sexual dimorphism is evident in oligodendrocyte development, myelination, and risk for multiple sclerosis (Swamydas, et al, 2009). Both hormonal and non-hormonal factors are involved in the sexually dimorphic aspects of myelination and remyelination, but progesterone is known to promote myelin regeneration (El-Etr et al., 2015). Interestingly, neurosteroids can act directly on some AChRs. Inhibition of nicotinic receptors by progesterone promotes MBP and 2′,3′-Cyclic-nucleotide 3′-phosphodiesterase (CNPase) expression in oligodendrocytes isolated from neonatal rats (Baulieu and Schumacher, 1997).

Together these studies suggest the involvement of cholinergic signaling in white matter disorders that are associated with cognitive decline, demyelination, and neuropsychiatric disorders, and that potential therapeutic measures to mitigate these white matter disorders may be possible by modifying cholinergic signaling.

Toxins Disrupting ACh Signaling in Myelinating Glia

Nerve gas and many pesticides are acetylcholinesterase inhibitors. Developmental effects of low level exposure to AChE inhibitors are well established by epidemiological data (Bouchard et al., 2010; Engel et al., 2007; Garry et al., 2002; Rauh et al., 2006, 2012; Young et al., 2005). Traditionally, cholinergic synaptic transmission has been considered the pathophysiological basis for developmental impairments associated with low-level organophosphate exposure, but recent research shows that synaptic (Kukley et al., 2007) and non-synaptic vesicular neurotransmitter signaling (Wake et al., 2011, 2015) occurs between axons and OPCs, and is important in activity-dependent regulation of OPC development and myelination. Notably, vesicular release of glutamate from electrically active axons stimulates the initial events in myelination by promoting local synthesis of myelin basic protein (Wake et al., 2011), resulting in preferential myelination of electrically active axons (Wake et al., 2015). This axo-glial neurotransmitter signaling suggests the possibility that some of the toxic effects of agents disrupting ACh signaling could disrupt myelination. Since myelination proceeds through development and postnatally, and impairments at critical periods can have long-term consequences, interference of cholinergic regulation of myelination at critical periods could result

TABLE 2: Functional Effects of Cholinergic Signaling in Oligodendrocyte Lineage Cells

AChR Subtype	Evidence	Agonist	Antagonist	Paper
M1	Antagonism reduced OPC proliferation induced by muscarinic agonist	Muscarine, Carbachol	Pirenzepine	De Angelis et al. (2012)
	Antagonism promoted immature OPC differentiation in vitro		Benztropine	Deshmukh et al. (2013)
	Antagonism inhibited calcium influx		Pirenzepine	Cohen and Almazan (1994)
M2	Antagonism had no effect on OPC proliferation	Arecaidine, Muscarine	Gallamine	De Angelis et al. (2012)
	Antagonism had no effect on calcium influx		Metoctramine	Cohen and Almazan (1994)
M3	Most abundantly expressed subtype	Muscarine,	4-DAMP	Ragheb et al. (2001), De Angelis et al. (2012)
	Antagonism reduces phosphorylation of p42, CREB and mRNA levels of *c-fos* in vitro		4-DAMP, Benztropine	Ragheb et al. (2001), Deshmukh et al. (2013)
	Antagonism increased MBP expression in immature OPCs in vitro and in vivo.		Darfenicin, Solifenacin, Benztropine	Deshmukh et al. (2013), Abiraman et al. (2015)
M4	Antagonism reduced OPC proliferation induced by muscarinic agonist	Muscarine, Carbachol	Tropicamide	De Angelis et al. (2012)
General mAChR	Antagonism reduced OPC proliferation induced by muscarinic agonist	Muscarine, Carbachol	Atropine	De Angelis et al. (2012)
	Agonism increases cytosolic calcium	Carbachol, Methacholine		Takeda et al. (1995), Belachew et al. (1998), He and McCarthy (1994), Simpson and Russell (1996)
	Agonism increased OPC proliferation via PKC, MEK and MAPK activation	Carbachol	Atropine	De Angelis et al. (2012), Larocca and Almazan (1997)
	Agonism reduced apoptosis and increased Fyn phosphorylation	Carbachol	Atropine	Cui et al. (2006)
	Agonism increased CREB phosphorylation in immature OPCs	Carbachol		Sato-Bigbee et al. (1999)
	Antagomism promoted remyelination (MBP and CNP expression) in vivo		Clemastine	Mei et al. (2014), Li et al. (2015)
	Antagonism reversed social avoidance behavior of socially isolated mice		Clemastine	Liu et al. (2016)

TABLE 2: Continued

AChR Subtype	Evidence	Agonist	Antagonist	Paper
	Agonism blocks OPC commitment	Oxotremorine-M, Chlorpyrifos (AChEi)		Abiraman et al. (2015)
a7 nAChR	a7 Specific agonism produced calcium influx	PNU-120596	Methyl-lycaconitine (MLA)	Velez-Fort et al. (2009)
General nAChR	Sex specific differential myelin gene expression after prenatal nicotine treatment	Nicotine		Cao et al. (2013b)
	Agonism increases cytosolic calcium		Dihydro-β-erythroidine	Rogers et al. (2001)
	ACHEi increased myelin related gene mRNA and protein levels	Donepezil	Mecamylamine	Imamura et al. (2015)

in the persistent effects on I.Q. and cognitive impairments that are seen in epidemiological studies. Microarray analysis of neonatal rats exposed to daily doses of chlorpyrifos or diazinon on postnatal days 1–4 have altered expression of genes involved in development of glia and myelin, as well as genes involved in neural cell growth, neural differentiation, oxidative stress, excitotoxicity, and neurotransmitter synthesis and receptors (Slotkin and Seidler, 2007).

Given the wide-ranging effects on OPC proliferation, differentiation, and survival, the effects of exposure to ACh inhibitors would vary greatly depending on the stage of development that is impacted. Developmental exposure to chlorpyrifos in rats decreases mRNA for MAG (Betancourt et al., 2006), but the effects differ depending on the developmental period of exposure. Organophosphate pesticide administered to developing rats prenatally (E17-20) causes enhancement of MBP immediately (gestational day 21), but postnatal treatment (P11-14) reduces MBP at P15-20. Adding to this complexity, males were found to be preferentially sensitive to this postnatal exposure (Garcia et al., 2003), a result that may reflect sex-specific differences in the rates of development or effects of sex hormones on white matter.

Nicotine exposure also affects oligodendroglial development and myelination. Maternal smoking during and even before pregnancy can affect myelin development (Zhao et al., 2014). A study in which female zebrafish were exposed to nicotine and then bred with drug-naïve males and maintained in a nicotine-free environment throughout embryonic development found mRNA expression of many genes encoding myelin proteins became downregulated in 4 dpf larvae, but these same genes were upregulated in 14dpf larvae. Treatment of Sprague-Dawley rats with nicotine through an osmotic minipump from gestational days 4-18 results in altered gene expression in offspring that persists postnatally and into adulthood. The genes affected are consistent with those involved in psychiatric disorders during adolescence (Cao et al., 2013a). At one month of age following nicotine exposure, alterations in oligodendrocyte cell number were seen in the prefrontal cortex of treated males, but not in other areas (Cao et al., 2013b).

Inflammation plays an integral role in both the etiology and consequent pathology of most neurodegenerative disorders (Amor et al., 2010, 2014). On one end of the spectrum, demyelinating disorders like multiple sclerosis are believed to be primarily driven by an autoimmune response against myelin antigens. On the other end, chronic low-grade inflammation due to hypoxia is attendant in cerebral hypoperfusion, which results in cognitive decline and white matter damage (Cho et al., 2006; Farkas et al., 2004; Otori et al., 2003). Acetylcholine has been shown to play a critical role in ameliorating inflammation via stimulation of nicotinic receptors (Wang et al., 2003). As such, administration of Huperzine A, a natural acetylcholinesterase inhibitor, reduced chronic inflammation in a rat model of cerebral hypoperfusion (hypoxia), accompanied by recovery in memory deficits and myelin damage. However, preincubation with a nicotinic Acetylcholine receptor antagonist before hypoxia abolished all gains made by acetylcholinesterase inhibition (Wang et al., 2010). Thus anti-inflammatory role of ACh via stimulation of nicotinic receptors can contribute to the protective effects of ACh in neuropathology.

Disruption of anti-inflammatory effects of ACh signaling through nAChR can also contribute to persistent inflammation in demyelinating disorders like MS. BChE activity

TABLE 3: Expression of Acetylcholine Receptors on Schwann Cells

nAChR Subtype	Evidence of Expression	Paper
M1	PCR, Immunopercipitation	Loreti et al. (2006)
M2	PCR, Immunopercipitation	Loreti et al. (2006)
M3	PCR, Immunopercipitation	Loreti et al. (2006)
M4	PCR	Loreti et al. (2006)
nAChR	Radioligand competition binding	Villegas (1974), Rawlins and Villegas (1978), Verdiyan et al. (2016)
nAChR	Electron Microscope Autoradiography	Rawlins and Villegas (1978)

was found to be higher in MS lesions (Darvesh et al., 2010) which can potentially lead to increased hydrolysis and thus reduced availability of ACh to ameliorate inflammation.

Summary and Future Directions

A renaissance of interest in cholinergic signaling in oligodendroglial development and myelination seems possible considering the relative neglect of this neurotransmitter in comparison to other neurotransmitters and signaling molecules that have been the focus of attention in myelin research more recently. This renewed interest is fueled by another renaissance of interest in activity-dependent myelination, as well as by the therapeutic potential to promote remyelination through pharmacological manipulation of ACh signaling.

Research ahead on cholinergic signaling in myelinating glia confronts many challenges. The most prominent of which is understanding the heterogeneity of different ACh receptors among oligodendroglia, the changes in their expression during development, disease, and repair, and determining the functional actions of each of these different receptors for the same neurotransmitter that are expressed on these cells. How do muscarinic and nicotinic receptors operate to influence oligodendroglial development and function differently? What are the selective and overlapping functions of the five different muscarinic AChRs on oligodendroglia, and the differences in intracellular signaling associated with each one? Likewise, what are the functions of AChE and BChE in white matter? What is the source of ACh in white matter? Is ACh signaling only relevant to axon-glial signaling in cholinergic axons or does ACh act as a signal for myelinating glia on axons that use other neurotransmitters as well? Is ACh signaling involved in activity-dependent myelination? Can drugs be identified that will selectively activate the appropriate AChR to promote remyelination without influencing the many other processes in neurons and glia that utilize ACh signaling? Do environmental toxins and exposure to low-levels of nerve agents cause long-term alterations in white matter, and are these changes part of the pathophysiology of

TABLE 4: Functional Effects of Cholinergic Signaling in Schwann Cells

AChR Subtype	Evidence	Agonist	Antagonist	Paper
M2	Agonism decreases proliferation, causes cell cycle arrest, and induces differentiation via upregulation of Sox10 and Krox20	Acrecaidine		Uggenti et al. (2014)
General mAChR	Agonism resulted in decreased cAMP	Muscarine		Loreti et al. (2006)
	Agonism results in increased intracellular calcium	Acetylcholine		Jahromi et al. (1992)
General nAChR	Agonsim results in Schwann cell hyperpolarization	Nicotine		Villegas (1978), Verdiyan et al. (2016)
AChR	Agonism decreases lipid bond vibration and produces a change in viscosity of myelin lipids	Acetylcholine		Verdiyan et al. (2016)

developmental delay in children exposed to pesticides or in soldiers suffering chronic debilitation from Gulf War illness?

All of these questions are critical to understanding how myelination is regulated, how it becomes dysregulated in disease, and in finding new treatments for demyelinating disease and possibly neuropsychiatric disorders. A vast amount of new information on ACh function in myelinating glia must be obtained. This will require years of research, but all of these important questions can be addressed and answered experimentally using currently available techniques.

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