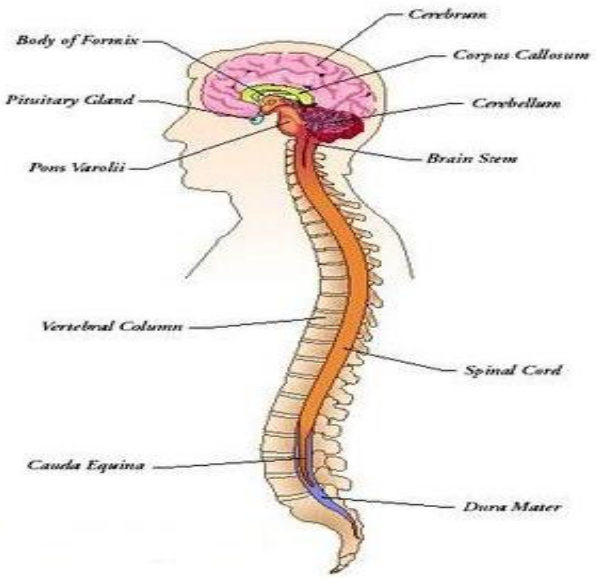
Chapter 1
Introduction

1.1: Introduction

The central nervous system (CNS) is the main part of the nervous system made up of brain and the spinal cord. The cerebrum is a part of the brain that controls voluntary actions, speech, thought and memory, while the cortex, also called gray matter, is the outer part of the cerebrum and is made of neurons and is said to be the brain's information processing center. The brain is divided into two halves: the right and left hemisphere; both of which are positioned on a central structure called the thalamus, which relays information between the peripheral input from the senses and the brain.

Other central structures include the hypothalamus, which regulates autonomic functions such as appetite and thirst, and the pituitary gland, which is partially responsible for growth, metabolism, and stress response (Tamraz *et al.*, 2004). The cerebellum is located posterior to the brainstem and plays a role in maintaining equilibrium and muscle tone. The spinal cord sends messages from the brain to different parts of the body and receives messages back by motor neurons of the CNS. The spinal cord is surrounded by the spinal column, which is made up of stacked bones called vertebrae (Fig.1.1).



St. John Fisher College, April 21, 2009

Figure 1.1: Central nervous system (CNS)

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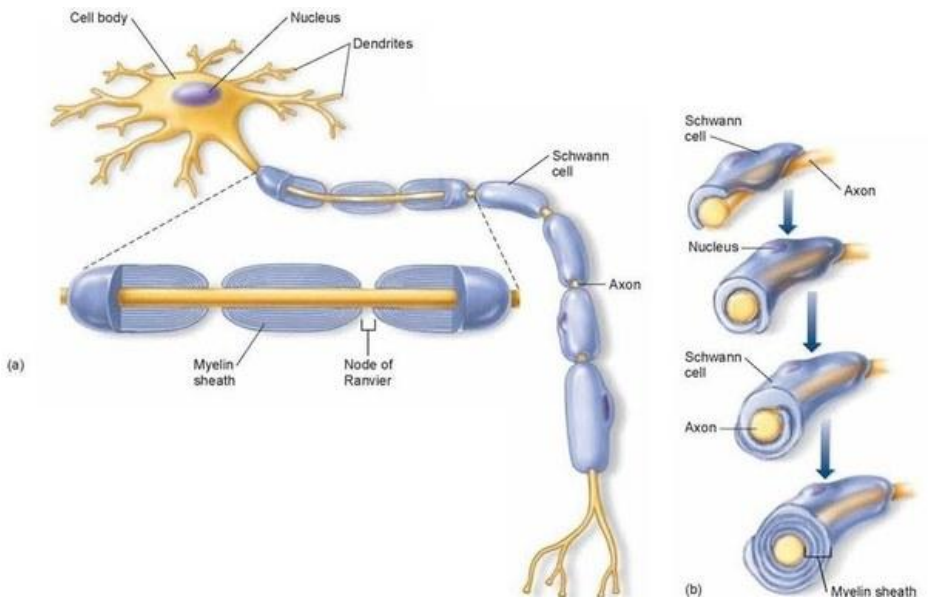
Neuronal signal propagation or action potential in vertebrates is accelerated by the electrical insulation of axons with an ensheathing, specialized glial plasma membrane: termed as myelin sheath (Morell *et al.*, 1999). Myelin is a fatty material that protects, and insulates nerves, enabling them to quickly conduct impulses between the brain and different parts of the body. Myelin also contains proteins that can be targeted by the immune system (Rumsby & Crang, 1977). Myelin wraps the nerves of both the central nervous system and the peripheral nervous system; the damage of the myelin in the central nervous system triggers many of the symptoms of multiple sclerosis (MS) and other demyelinating disorders. Nerve cells are encapsulated with sections of myelin, and the tiny spaces between the sections are called as nodes (Fig.1.2). As the brain sends messages through the nerves of the spinal cord, the impulses jump from node to node and the process is termed as salutatory signal transmission. The myelin sheath prevents these impulses from the nerve at that point (Morell *et al.*, 1999).



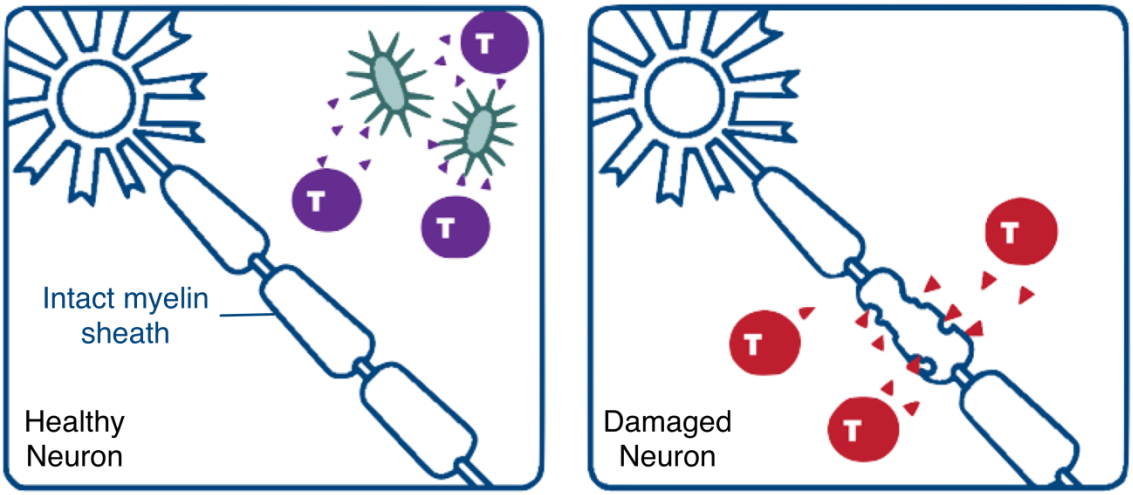
(Johnson, 2011)

Figure 1.2: Myelin sheath formation

Myelination is done by oligodendrocytes in the CNS and Schwann cells in Peripheral Nervous System (PNS) (Fig. 2). In the CNS myelination, early oligodendrocyte progenitors arise from neuroepithelium in the ventral spinal cord and migrate throughout

the brain parenchyma (Fu *et al.*, 2002, Ravanelli & Appel, 2015) and differentiate into myelinating cells (mature oligodendrocytes). Importance of CNS myelin for normal sensation, cognition, and motor function is significant and disruption of this homeostasis leads to an array of myelin-related disorders which often affect CNS physiology (Sherman & Brophy, 2005, Simons & Trotter, 2007). During inflammation and in chronic injury, myelination is affected and myelin proteins level goes down (Thomson *et al.*, 2008), these symptoms result in various demyelination diseases such as Multiple sclerosis.

In multiple sclerosis (MS), the body's immune system T cells attack the myelin sheath, which protects the nerve fibers. The T cells severely damage or destroy the myelin sheath from the nerves leaving them unprotected and uninsulated. (Ransohoff *et al.*, 2015) (Fig.1.3). Non-insulated nerves are then not able to transmit messages from the brain to the other body parts. The messages transmitted by the nerves are often delayed or distorted and the messages the brain receives may be misinterpreted.



(Dr. Jyoti Shankar 2 Jun 2017,BMC Blog)

Figure 1.3: Myelin sheath disruption

Myelin is lost in multiple areas, leaving scar tissue which due to its hardened characteristics is termed as sclerosis. The damaged areas of disrupted myelin sheath are

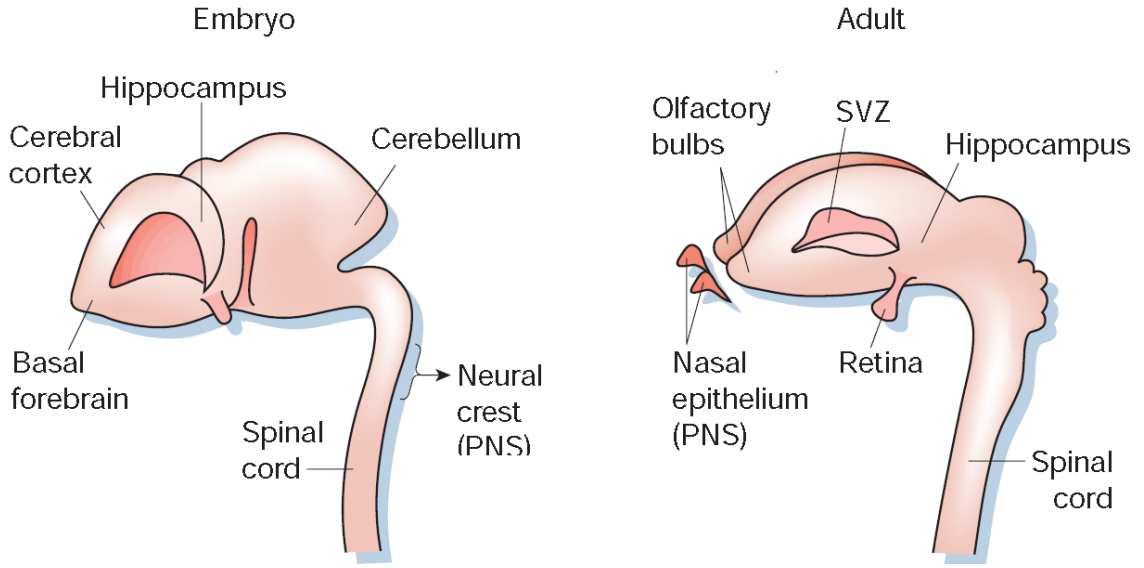
called plaques which can be identified by the technique of magnetic resonance imaging (MRI) thus assisting in assessing and monitoring the progression of multiple sclerosis.

Oligodendrocyte precursors can be identified in the ventral ventricular zone of the spinal cord (Noll & Miller, 1993) and brain regions (Kessaris *et al.*, 2006), by expression of growth factor receptors (Pringle & Richardson, 1993) and immunological profile (Ono *et al.*, 1995, Orentas & Miller, 1998). After neurogenesis is complete proliferating glial precursors are localized to a distinct region of the ventral ventricular zone dorsal to the floor plate (Noll & Miller, 1993). *In-vitro* condition approx 60% of these proliferating cells differentiate into oligodendrocytes (Noll & Miller, 1993). One of the major oligodendrocyte precursor mitogen is platelet-derived growth factor (PDGF) (Noble *et al.*, 1988, Richardson *et al.*, 1988) and oligodendrocyte precursors express the PDGF Receptor -α (PDGFR-α) (Pringle *et al.*, 1992) . Neural stem cells are also a potential resource of oligodendrocytes in the CNS.

Neural stem cells have characteristics of self-renewal and give rise to neurons, astrocytes and oligodendrocytes. These cells hold a great promise for neural repair after injury or disease. NSCs can be obtained from the embryonic or adult CNS. At early developmental stages, NSCs are highly proliferative, giving rise to primordial of developing organs and producing multiple phenotypes that constitute organs and tissues (Weissman, 2000). In adulthood, Stem cells (SCs) are found in tissues as dormant cells that become activated only when differentiated cells in the tissue are lost and require a replacement. The neural stem cells (NSCs) throughout the developing CNS are capable of producing or generating oligodendrocytes progenitors (OP). Yet, in the embryonic spinal cord, the oligodendrocyte phenotype is induced by sonic hedgehog (Shh) in a restricted anterior region (Rogister *et al.*, 1999).

Neural stem cells (NSCs) have the capacity to self-renew and produce the three major cell types of the CNS: neurons, astrocytes, and oligodendrocytes and are therefore normally classified as being multipotent in nature. A great deal of attention has been given to precisely identifying the adult neural stem cell. Earlier work has suggested that ependymal cells were adult neural stem cells (Johansson *et al.*, 1999). Source of NSCs

are Blastocyst, fetal brain and Adult brain (Conti & Cattaneo, 2010, Temple, 2001) (Fig. 1.4). There are two established *in-vitro* systems for neural stem cells studies: one is neurosphere system and second is monolayer (Jensen & Parmar, 2006). GFAP expressing cell in the subventricular zone (SVZ) is capable of replenishing the SVZ after ablation and give rise to neurons (Doetsch *et al.*, 1999). NSCs express many surface markers such as Nestin, SOX2, GFAP, Tuj 1 (β-III-tubulin) and PDGFR-α for their characterization (Zhang & Jiao, 2015, Yuan *et al.*, 2011). GFAP containing cells can form neurospheres and give rise to neurons as well as glial cells (Laywell *et al.*, 1999). Additionally, reports also suggest that virtually all postnatally born neurons are ultimately derived from GFAP positive cells (Garcia *et al.*, 2004).



(Temple, 2001)

Figure 1.4: Source of Neural stem cells (NSC)

In the NSCs, *Hes1* and *Hes5* do not induce the development of astrocytes or oligodendrocytes but maintain undifferentiated cells in the ventricular zone. Therefore, it is unlikely that the Notch-Hes1/5 pathway determines the fate of astrocytes and oligodendrocytes in the developing telencephalon in CNS (Ohtsuka *et al.*, 2001). Inhibition of Notch-1 leads to neurogenesis and wnt & BMP pathway activation which is responsible for gliogenesis lineage of NSC differentiation (Tanigaki *et al.*, 2001).

Hirabayashi reported that BMP & wnt pathway inhibition in radial glial cells (Hirabayashi *et al.*, 2004) and Shh pathway in Motor restricted progenitor lead to activation of oligodendrocytes linage (Wang & Almazan, 2016) (Fig. 1.5)

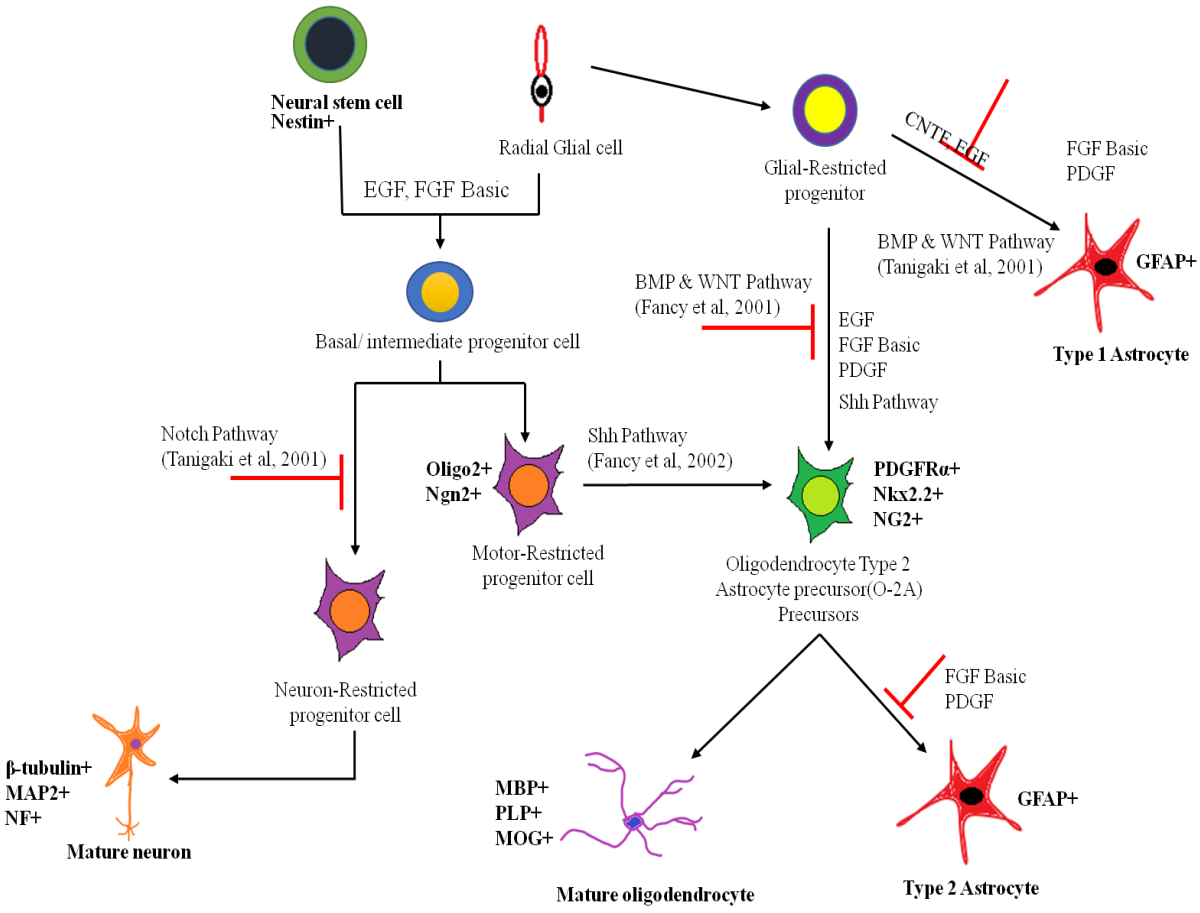
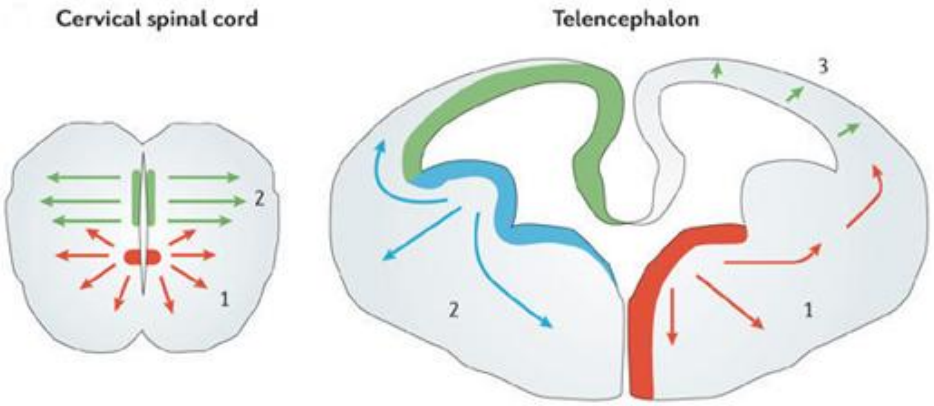


Figure 1.5: Neural stem cell differentiation pathway and linage specific markers

Oligodendrocyte is a main key player of CNS myelination. Oligodendrocytes generate from various sources of spinal cord and brain in different subclasses. Initially motor neuron precursors are formed in the ventral ventricular zone in the spinal cord region which further leads to neurogenic or gliogenic switch and which at the end forms oligodendrocytes precursor cells (OPCs) (Lu *et al.*, 2002, Sun *et al.*, 1998, Takebayashi *et al.*, 2002, Zhou & Anderson, 2002). These OPCs then migrate through the spinal cord and differentiate into mature myelin forming oligodendrocytes. Later, an additional

source of OPCs arises in the dorsal spinal cord, contributing to 10–15% of the final oligodendrocyte population in the spinal cord (Cai *et al.*, 2005, Fogarty *et al.*, 2005, Vallstedt *et al.*, 2005). In the brain, the first wave of OPCs originates in the medial ganglionic eminence and anterior entopeduncular area of the ventral forebrain which populate the entire embryonic telencephalon and cerebral cortex. The second wave arises from the lateral and/or caudal ganglionc eminences followed by a third wave of OPCs from the postnatal cortex (Kessaris *et al.*, 2006) (fig.1.6).



(Richardson et al., 2006)

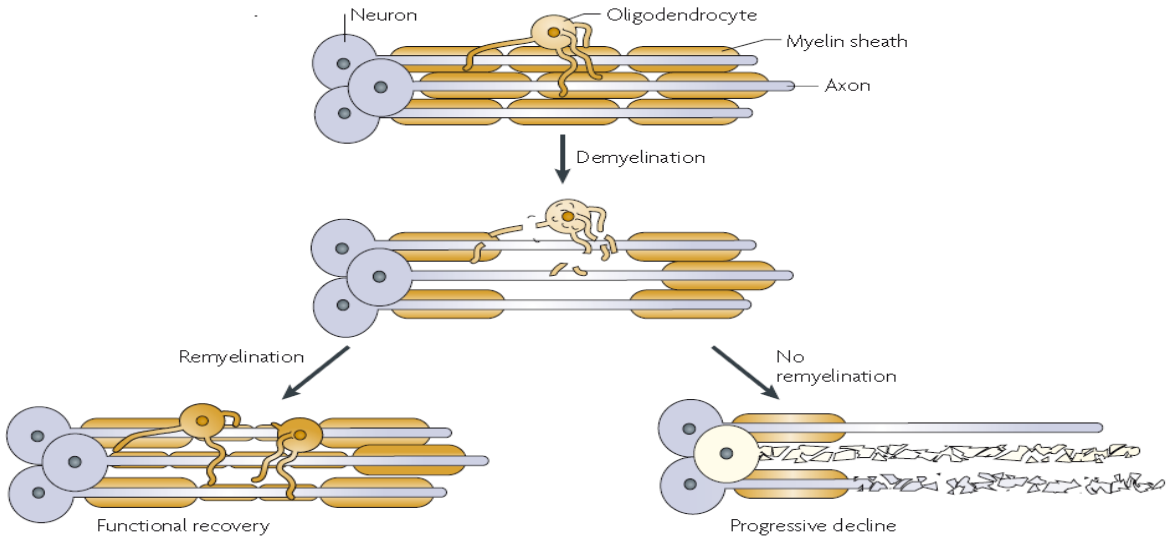
Figure 1.6: Oligodendrocyte generation

Reports have revealed that when any one of them is destroyed at source by the targeted expression of a toxin gene in mice, the remaining cells spread into the vacant territory and restore the normal distribution of OPCs so that animal can be able to produce myelin and develop, survive and behave normally itself (Kessaris *et al.*, 2006).

Myelination in Vertebrate is an evolutionary advancement essential for motor, sensory, and cognitive function. In CNS, Myelin, a multi lamellar differentiation of the oligodendrocyte plasma membrane, ensheaths axons to facilitate electrical conduction. Myelination is one of the most pivotal cell-cell interactions process for normal brain development. It involves extensive information exchange between differentiating oligodendrocytes and axons (Bercury & Macklin, 2015). During inflammation or any other unhealthy condition in brain, CNS myelination is affected. Disruption of this

myelin sheath is called as demyelination. Multiple growth factors are potential candidates which affect the demyelination processes (VonDran *et al.*, 2011). Many cytokines and chemokines have been identified for having a direct effect on oligodendrocytes lineage cells followed by myelination (Armstrong, 2007). Demyelinating disorders of the central nervous system are among the most crippling neurological diseases affecting at various stages of life. Most prominent demyelinating disease is multiple sclerosis (Kotter *et al.*, 2011), a chronic inflammatory demyelinating disease of the CNS. Remyelination is the progression by which new myelin sheaths are restored to demyelinated axons of neurons, enabling them to regain the ability to carry action potentials by saltatory conduction and to recover lost function (Smith *et al.*, 1979, Jeffery & Blakemore, 1997).

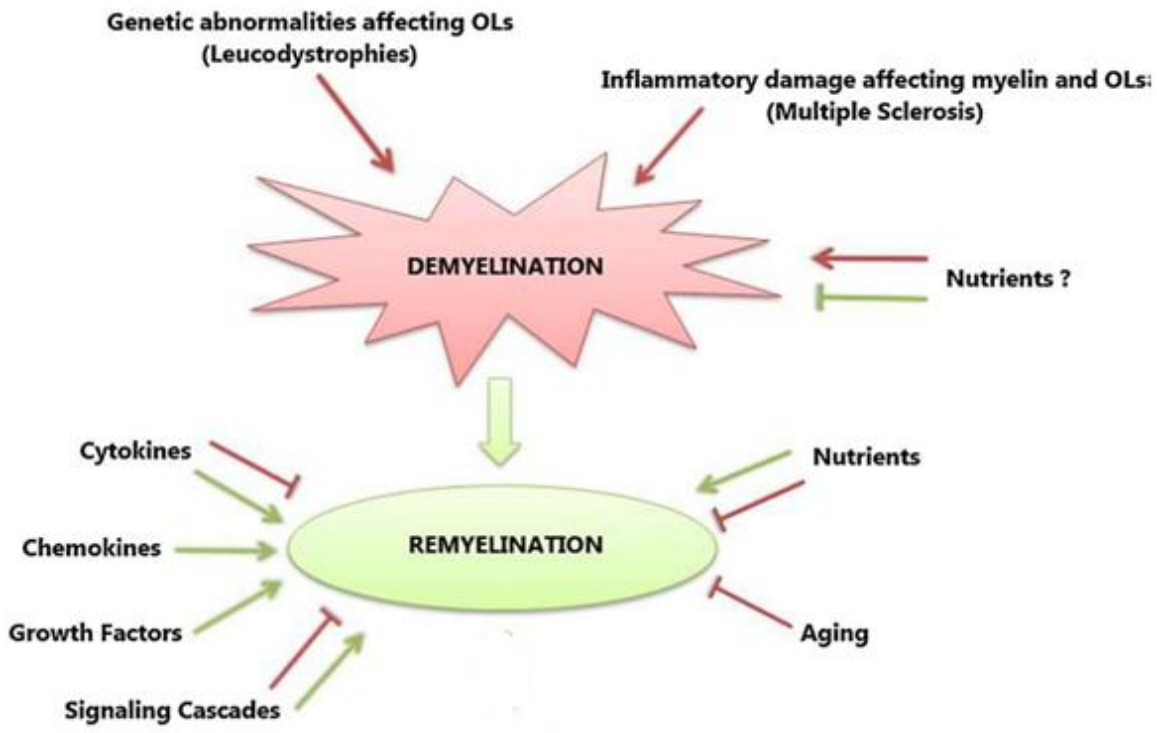
Remyelination in multiple sclerosis lesions varies noticeably, indicating ability for repair that is not pleased in lesions with demyelination. Remyelination is limited to chronic disease. In experimental models of demyelinating disease, depletion of OPC population inhibits OPC differentiation into remyelinating oligodendrocytes in the lesion environment (Armstrong, 2007) (Fig. 1.7)



(Franklin, 2008)

Figure 1.7: Remyelination in the central nervous system (CNS)

Multiple growth factors are the potential candidates which affect the remyelination/demyelination processes (VonDran *et al.*, 2011) (Fig.1.8). Demyelination is classified in two categories either genetic abnormalities affecting Oligodendrocytes (OLs) or inflammatory damage affecting myelin and OLs (Adamo, 2014). Extensive remyelination can be achieved by modulating the activity of growth factor signalling pathways of endogenous OP cells. Several growth factors regulating OP proliferation, differentiation and survival have been identified and well-studied. Therefore, growth factors can be the key signalling molecules for development of strategies to improve conditions with poor remyelination. Signaling pathways possibly involved in the remyelination process include those mediated by leucine-rich repeat- and Ig domain-containing NOGO receptor-interacting protein 1 (LINGO-1), Wnt, Sonic hedgehog (Shh), and Notch1. LINGO-1 is a known negative regulator of OL differentiation (Mi *et al.*, 2005). Growth factors are biologically active polypeptides controlling target cell growth, differentiation and are important during the remyelination process. Platelet derived growth factor (PDGF), insulin-like growth factor-1 (IGF-1), and epidermal growth factor have been shown to enhance remyelination (Mason *et al.*, 2000, Mason *et al.*, 2003, Murtie *et al.*, 2005, Vana *et al.*, 2007). Report demonstrated that epidermal growth factor receptor signaling is involved in both the repopulation by OPCs and the remyelination of lysolecithin-induced corpus callosum demyelination (Aguirre *et al.*, 2007). Other studies suggest that the neurotrophins may also impact recovery from demyelination. Following a lysolecithin induced lesion, neurotrophin-3 (NT-3) decreases the demyelinated volume and increases myelin basic protein (MBP) OLGs in the lesion site (Jean *et al.*, 2003). *In-vitro* studies characterized the potential of defined growth factors to induce a specific cellular response. In this context, several cytokines and chemokines have been identified for having direct effects on oligodendrocytes lineage cells (Armstrong, 2007).

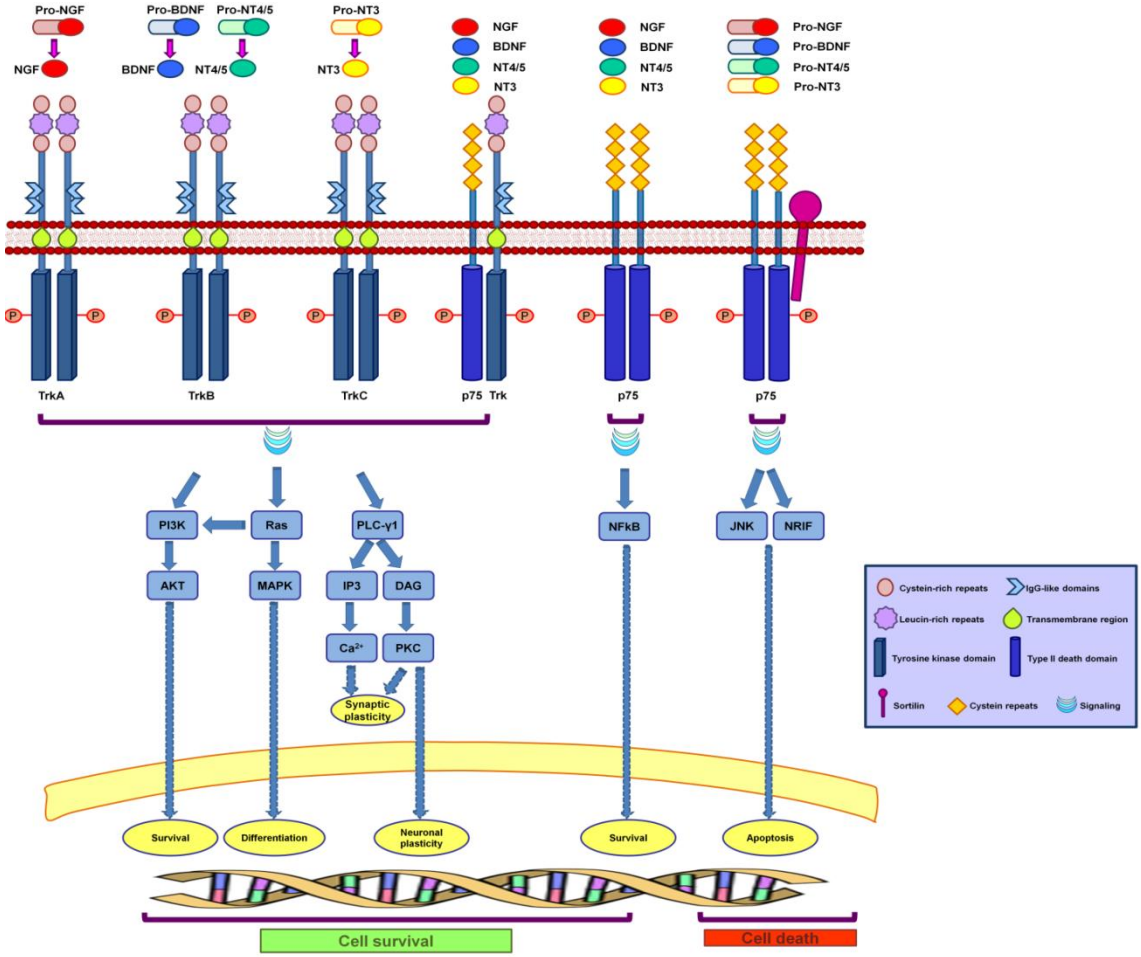


(Adamo, 2014)

Figure 1.8: Multiple growth factors affect the remyelination/demyelination processes

Neurotrophins are one of the internally or externally factors responsible for affecting and regulating myelination (Xiao *et al.*, 2009). Neurotrophins belongs to a class of secreted growth factors that are capable of inducing cell survival, differentiation, or growth (Allen & Dawbarn, 2006). They are classified in four types: Nerve growth factor (NGF), Brain derived neurotrophic factor (BDNF), Neurotrophin-3 (NT-3), and Neurotrophin-4/5 (NT-4/5). Neurotrophins are initially synthesized as precursor proteins, known as proneurotrophins, and are subsequently cleaved to release the mature neurotrophins proteins. Proneurotrophins are biologically active and signal through a receptor complex consisting of the p75 neurotrophin receptor (p75NTR) and sortilin. Once cleaved, the

mature neurotrophins are no longer able to interact with this complex, but rather interact with two distinct classes of transmembrane receptors: the receptor tyrosine kinase Tropomyosin-related kinase (Trk), and the structurally unrelated p75NTR, a member of the tumor necrosis factor receptor super family. Interactions of neurotrophins with the Trk receptors are selective in nature; NGF binds selectively to TrkA, BDNF and NT-4/5 to TrkB, and NT-3 to TrkC (Lu *et al.*, 2005). In addition, all the neurotrophins can bind non-selectively to p75NTR (Reichardt, 2006, Hempstead, 2006, Arevalo & Wu, 2006). Many pathways are activated through NTs such as ERK, PLC-γ, PI3K which are involved in several important functions of the nervous system (Bucci *et al.*, 2014), such as cell migration, proliferation, differentiation as well as in myelination events (fig. 1.9).



(Bucci et al., 2014)

Figure 1.9: Neurotrophins interaction with its receptor and signalling

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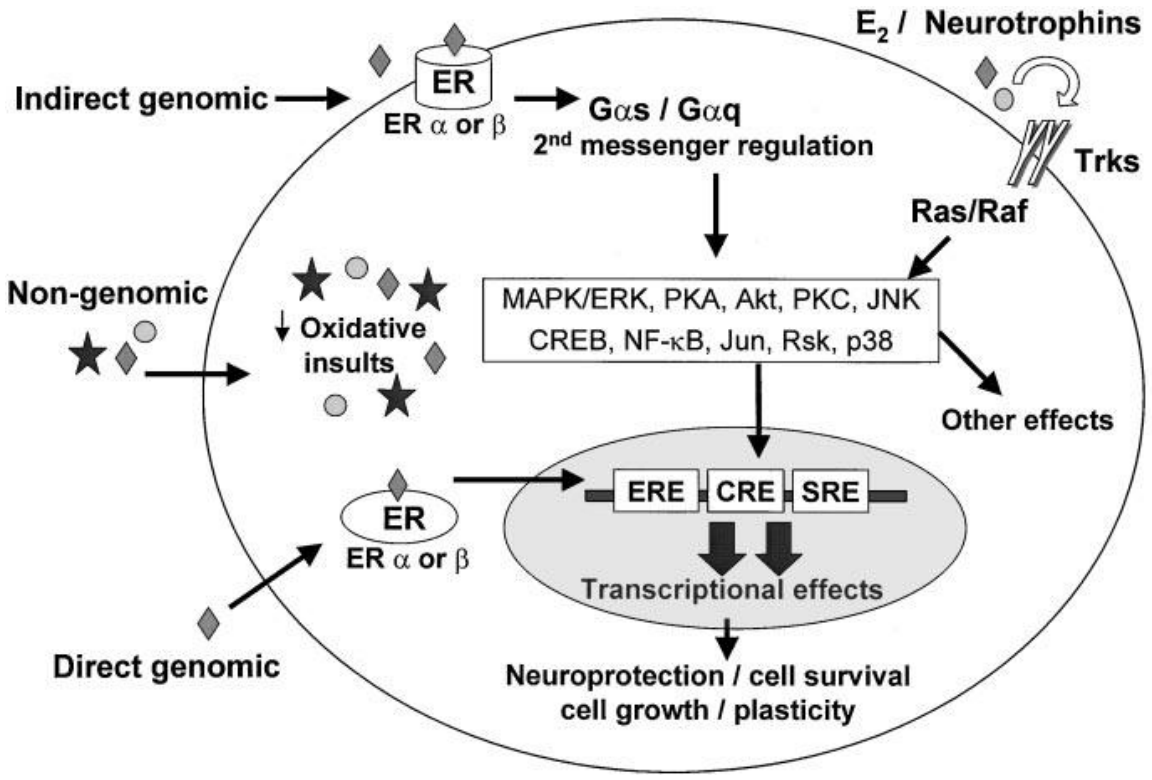
Neural stem cells proliferation and differentiation regulated by the endogenous genes (Artavanis-Tsakonas *et al.*, 1999, Osawa *et al.*, 2005), as well as by various factors including neurotrophic factors (Benoit *et al.*, 2001). Neurotrophic factors exist in the local microenvironment of the brain which plays an important role in protecting neural functions and repairing brain injuries by supporting the survival of neurons, promoting their growth and differentiation, and maintaining their functions (Joannides *et al.*, 2007, Wang *et al.*, 2006). Moreover, the effects of BDNF and NGF have been reported to exert a marked impact on the proliferation and differentiation of NSCs (Liu *et al.*, 2014, Chen *et al.*, 2014). Involvement of neurotrophins in neural stem cells differentiation has been a major focus since last one decade.

These growth factors are also involved in the regulation of OPC migration. Most extensively studied growth factor is PDGF (Frost *et al.*, 2009). Reports reveled that neurotrophins are also involved in migration of other cells; BDNF stimulates migration of cerebellar granule cells and promote the migration of cortical neurons (Borghesani *et al.*, 2002) and NGF enhances migration of Schwann cells in the brain (Anton *et al.*, 1994). In addition, neurotrophins receptor (Trk receptors) are also present in oligodendrocytes and they are involved in most of the essential functions of oligodendrocytes such as oligodendrocytes lineage, survival of immature oligodendrocytes and potentiation of mitogenic effect of other growth factors (Cohen *et al.*, 1996, Grinspan, 2002, Coulibaly *et al.*, 2014). Reports have suggested that many factors are responsible for OPC proliferation (Vela *et al.*, 2002, Levine & Reynolds, 1999, Barres & Raff, 1993). Very few studies have revealed role of BDNF and NGF in OPC proliferation. NGF enhances the survival of differentiated oligodendrocytes (Cohen *et al.*, 1996) whereas BDNF affects its development via TrkB receptor (Van't Veer *et al.*, 2009) and promotes CNS myelination via a direct effect on oligodendrocytes (Xiao *et al.*, 2010). However, the regulatory mechanism by which Trk receptors' affect OPC proliferation and differentiation is still unclear. Overall, scattered information is available regarding neurotrophins and its Trks receptors in context of oligodendrocytes lineage and this calls

for a detailed investigation. Recent studies identify neurotrophins as important regulators of both peripheral and central myelination; NGF controls axonal receptivity to myelination by Schwann cells in the PNS (Chan *et al.*, 2004) whereas BDNF is a well-studied astrocyte secreted molecule known to promote CNS myelination (Xiao *et al.*, 2010, Fulmer *et al.*, 2014). During inflammation and in chronic injury, myelination is affected and myelin proteins level goes down (Thomson *et al.*, 2008). At this particular time exogenous molecules like growth factors try to recover the normal condition for survival of CNS myelinating cells mainly oligodendrocytes. However, our understanding on role of neurotrophins and their receptors in myelination recovery in cytokine affected (inflammation condition) myelination is still vague.

In the brain astrocytes are the most abundant glial cells and play a variety of complex and essential functions in the healthy CNS including house-keeping functions, providing nutrients and serve as markers for diseased conditions. Astrocytes are the main production house for growth factor and neurotrophins (Sofroniew & Vinters, 2010). NGF and BDNF are the most widely distributed in Astrocytes and engaged in many of the essential functions of the brain such as neural protection (Allen *et al.*, 2013, Cheng & Mattson, 1994), neuron outgrowth (Hannan *et al.*, 2015, Labelle & Leclerc, 2000), glial cell proliferation (Tsiperson *et al.*, 2015, Douglas-Escobar *et al.*, 2012) and myelination (Xiao *et al.*, 2010, Chan *et al.*, 2004). Many instructive and extractive factors are responsible for secretion function of astrocytes, hormones are one of them. 17-β estradiol, 17β-estradiol (E2), a steroid hormone plays a diverse role at cellular and molecular levels in brain cells. Secretion of this hormone is mainly by the ovaries, brain and fat tissue through aromatization of testosterones. E2 regulates cell proliferation, differentiation and survival in the developing brain (TORAN-ALLERAND, 2005, MacLusky *et al.*, 1987). E2 also regulates neural plasticity and dendritic spine density in the brain region (Matsumoto & Arai, 1981, Gould *et al.*, 1990, Woolley *et al.*, 1990). Two distinct estrogen receptors (ERs), ER alpha (ERα) and ER beta (ERβ) are distributed in the brain regions (Mitra *et al.*, 2003, McEwen *et al.*, 1997, Shughrue *et al.*, 1997, Register *et al.*, 1998) and located in nucleus as well as in cytoplasm (fig. 1.10). Mode of action of these receptors is as per its localization (Lee & McEwen, 2001). Nucleus-initiated receptor

signaling activate new gene transcription by association with estrogen response elements (EREs) in the DNA(Nelson & Bulun, 2001), also called direct genomic action while membrane associated receptors cooperate with growth factor receptors or G protein-coupled receptors to activate kinase cascades through indirect genomic action (Hammes & Levin, 2007, Levin, 2005, Thomas *et al.*, 2005, Vasudevan & Pfaff, 2007). However, role of E2 in the modulation of NGF and BDNF in cortical astrocytes and the mechanism driving this modulation is still not well understood.



(Lee & McEwen, 2001)

Figure 1.10: Mode of actions of estradiol and their receptors

In summary, however, very little is known in regards to the modulation of cell fate decision of endogenous NSCs and OPCs via appropriate signaling mechanism involving these neurotrophins action in producing large number of oligodendrocytes and enhancement of remyelination which is a hallmark feature of many demyelinating

diseases. Outcome of this study will provide insight into the role of BDNF and NGF for oligodendrocytes lineage and valuable information on the molecular mechanisms governing remyelination involving neurotrophins. Some relevant potential therapeutic strategies can be developed based on understanding of neurotrophin mediated remyelination in developing brain and can be used further as a target for treatment of diseases caused by oligodendrocytes dysfunction. Study also demonstrates the neurotrophins modulatory effect of E2 with assessing the effect in astrocytes cell survival. This study may lead to further understanding of neuroprotective role of E2 in central nervous system disorders such as demyelination.

1.2: Rationale of the study

CNS myelination is most fundamental phenomenon of the brain. This complex process is regulated by many internal as well as exogenous factors. Demyelination / loss of myelin sheath is caused due to a number of factors among which elevated cytokine levels and involvement of growth factors are most crucial. Many studies have revealed the importance of growth factors specially Neurotrophins in cure of demyelination conditions. Neurotrophins were primarily identified as factors which influenced brain cell survival, differentiation and growth. However, till now very few reports suggest role of NGF and BDNF in neural stem cells differentiation pattern as well as glial cells migration and maturation.Stem cells differentiation particularly into oligodendrocytes is one of the limiting factors in demyelinating diseases such as MS, Alzheimer's and Leukodystrophies. Thus, present study focuses on potential of NGF and BDNF in driving the NSCs differentiation lineage towards oligodendrogenesis. In addition, understanding the neurotrophin mediated regulatory mechanisms of Oligodendrocyte progenitor migration and proliferation is crucial to being able to dissect out the subsequent processes that culminate in differentiating mature oligodendrocytes capable of myelination. Inflammation mediated demyelination, through cytokines, is a hallmark feature of many demyelinating diseases. The role of exogenous NGF and BDNF in remyelination, by their ability to protect myelin and re-bounce the myelin protein levels, needs detailed studies. Apart from this direct action of these neurotrophins, study is further extended towards glial cell, specifically astrocytes, secreted NGF and BDNF role in neuroprotection which

ultimately lead to regulation of CNS myelination. Current study was extended to understand the influence of steroid hormone, 17β-estradiol (E2) in regulating NGF and BDNF levels in astrocytes which play vital role for neuroprotection. Thus, overall study focuses on the role of neurotrophins (NGF and BDNF) and their receptors along with the underlining signaling mechanism behind the NSCs differentiation into oligodendrocytes, OPC migration, remyelination and neuroprotective effect of E2 affected neurotrophins in CNS myelination.

1.3: Objectives of the study

Based on the above understanding, the following objectives were defined for present study:

1. To determine the role of BDNF, NGF and their receptors in the regulation of Neural Stem Cell differentiation into Oligodendrocytes

2. To study the signaling mechanism of BDNF and NGF in Oligodendrocyte Progenitor Cells (OPCs) proliferation, migration and differentiation

3. To identify the involvement of BDNF and NGF in remyelination in cytokine affected myelination

4. To study the mechanism underlying estradiol mediated neurotrophins expression in cortical astrocytes