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CNS oligarchs; the rise of the oligodendrocyte in a neuron-centric culture

> If one desired to throw new light on the effect of disease, or injury, and of the process of healing in the brain, the best hope lay in the study of the non-nervous cells.
>
> *No Man Alone*, Wilder Penfield, 1977 (Gill and Binder, 2007)

NEUROGLIA

For the past 160 or so years the cells of the nervous system have been divided into two main categories: neurons and glia (Kettenmann and Verkhratsky, 2008). Prior to this, ever since the first image of a neuron was published in 1836 by Gabriel Valentin, the nerve cell had been in a class of its own (Lopez-Munoz *et al.*, 2006). Some 20 years later in 1856 the term neuroglia was introduced by the German physician Rudolph Virchow. Virchow, also known as the "Pope of pathology" (Kettenmann and Ransom, 2005; Magner, 2002), described a "connective substance ... in which nervous system elements are embedded" and referred to it as "nervenkitt" (or nerve putty). This description led to the use of the term "neuroglia," which derives from archaic Greek, meaning something sticky or clammy. The notion that neuroglia were there merely as neural putty was treated with the reverence usually reserved for a *bona fide* papal encyclical and as such neuroglia remained sidelined for decades to come. Even though Virchow was responsible for the term neuroglia coming into use, at this stage he did not recognize that it was made up of cells rather than an acellular connective tissue. In fact, it would be years until the cellular constituents of the neuroglia were fully defined in a drawn out, confusing and often controversial process (Somjen, 1988). At present glial cells of the central nervous system (CNS) are

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divided into two major categories: macroglia and microglia. The macroglia (also known as neuroglia) are made up of oligodendrocytes, astrocytes, $NG2^+$ cells and ependymal cells. The microglia are derived from myeloid-monocytic cells and are the resident macrophages of the CNS (Tambuyzer *et al.*, 2009).

THE THIRD ELEMENT – DISCOVERY OF THE OLIGODENDROCYTE

The cell that would eventually become known as the oligodendrocyte first appeared in the literature in 1900 when it was identified by the Scottish pathologist W. Ford Robertson. Robertson described a group of small cells with few processes that he believed to be mesodermal in origin and subsequently named them mesoglia. Despite this initial description, the incognito oligodendrocyte went largely unnoticed, or at least ill-defined, for the next two decades. The eminent neuropathologists Ramón y Cajal and del Rio-Hortega spent years debating the cell's very existence and nature. In 1911 Cajal defined a group of cells distinct both from neurons and astrocytes, the only glial cell thus far described. Cajal christened this group of cells with the inscrutable name of "the third element of Cajal." It was not until 1918 that Hortega was able to differentially stain the cell types of the third element of Cajal and introduced the terms microglia and oligodendroglia (Gill and Binder, 2007). However, due to difficulty with staining oligodendrocytes Hortega's mentor Ramón y Cajal could not reproduce the staining of his protégé and denied the existence of oligodendroglia, claiming that the third element was made up solely of microglia (Gill and Binder, 2007). Owing to Cajal's influence as a founding father of neurobiology and Nobel Prize winner, this pronouncement stymied further work on the oligodendrocyte for a number of years. It was not until the neurosurgeon Wilder Penfield visited Hortega's laboratory in Madrid 1924 that the controversy surrounding this enigmatic cell was resolved. In a somewhat avant-garde research proposal for the time, Penfield raised the funds to travel to Madrid where he adapted Hortega's staining methods to confirm the initial classification of glia and resolve the technical problems in specifically staining oligodendrocytes (Penfield, 1924). Hortega reported that these cells were present in large numbers in all regions of the CNS but predominantly in the white matter and that they were frequently located near neurons, blood vessels and in series along nerve fibers. Furthermore he suggested that the membrane

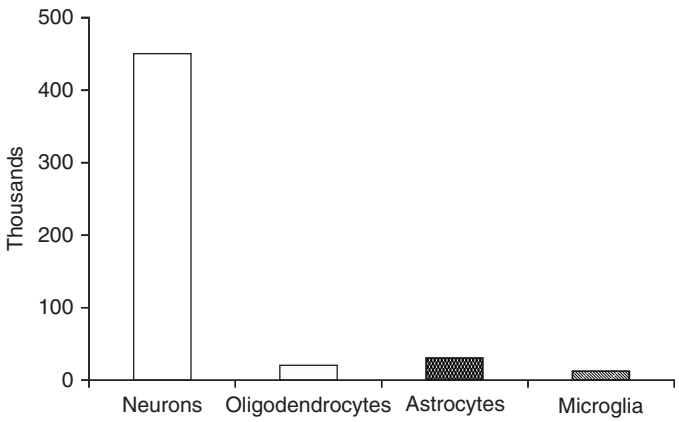

Figure 1.1 Number of publications in Pubmed after searching for each glial cell type.

around central myelinated fibers, identified by Cajal, is actually a derivative of oligodendroglia cells with functions similar to those of Schwann cells of peripheral myelinated fibers. This assertion could not be confirmed until the advent of electron microscopy in the 1960s. Hortega later went on to define perineuronal or interfascicular oligodendrocytes of four classes according to their number of processes and morphology, a classification which remains largely unchanged.

Despite the eventual acceptance of the oligodendrocyte as a distinct cell, the twentieth century was akin to the dark ages for glia. It would be a long time before any of the glia, least of all oligodendrocytes, would be acknowledged as key players in the nervous system in their own right. This can be simply illustrated by a Pubmed search using each cell type as a keyword (Figure 1.1).

MORPHOLOGY OF OLIGODENDROCYTES

Oligodendrocytes far outnumber neurons in the mammalian nervous system (Simons and Trajkovic, 2006) and their organization and structure are integral to a functional nervous system. The name oligodendrocyte, meaning "few branches," was coined at a time when methods for staining the cell were suboptimal and investigators had difficulty visualizing it in its entirety. The name oligodendrocyte is, therefore, something of a misnomer considering the number of its octopus-like processes each of which enwraps a length of axon.

In contrast to Schwann cells, the myelinating cells of the peripheral nervous system (PNS), oligodendrocytes can myelinate axonal lengths or internodes of more than one axon. During development oligodendrocyte progenitor cells (OPCs) migrate from the neural tube into what will become the white matter of the CNS, so called because of its high lipid content and therefore "fatty" or white appearance macroscopically (Simons and Trotter, 2007) (discussed further in Chapter 3). During this migration the OPCs continually extend and retract their processes in an attempt to sense other OPCs in the vicinity so that the cells become uniformly distributed (Kirby *et al.*, 2006). This highly organized distribution of OPCs helps to ensure periodically spaced internodes along the entire length of an axon (Kirby *et al.*, 2006), from where each adjacent internode originates and is myelinated by a different oligodendrocyte (Baumann and Pham-Dinh, 2001). The number of processes that each oligodendrocyte extends out to myelinate individual axonal internodes varies according to the area of the brain and the species of the animal. However, it has been estimated that an individual oligodendrocyte can maintain up to 60 internodal segments (Remahl and Hildebrand, 1990) obliging it to sustain a vast surface area of cell membrane. For example, an average oligodendrocyte with a somal diameter of 12 μm myelinates around 20 axonal segments, each 3 μm in diameter (Siegel *et al.*, 1999). If each of these internodes is 500 μm long with six myelin lamellae the oligodendroglial soma maintains up to 640 times its own surface area in membrane (Siegel *et al.*, 1999). The initiation and maintenance of these vast amounts of plasma membrane during myelination requires the extraordinary coordination of a number of cellular processes and is also discussed further in Chapter 3.

ORGANIZATION OF MYELINATED AXONS

While most references to myelin relate to the compacted, specialized plasma membranes of oligodendrocytes of the CNS or Schwann cells of the PNS, there is now an important understanding of the molecular organization of myelinated axons in regions of the membrane that remain uncompacted. As a result, myelin is now more specifically defined as: (1) compact myelin, those spirals of specialized plasma membrane and (2) non-compact myelin. The use of the term "myelin sheath" throughout the book refers to this complex of compact and non-compact myelin.

Compact myelin

Compact myelin is formed when the oligodendrocyte wraps spirals of plasma membrane around a segment of axon. The cytoplasm is extruded from between the layers so that the lipid-rich oligodendrocyte plasma membranes fuse together. This compaction of the oligodendroglial membrane creates the distinctive, lined, appearance of myelin when viewed by transmission electron microscopy. This lined appearance is the result of the apposing extracellular surfaces of the plasma membrane compacting to form the intraperiod line, while the cytoplasmic surfaces form the major dense line. Myelination of an axon allows the action potential to travel up to 100 times faster than it would along a smaller unmyelinated axon: 430 km/h compared to 3.6 km/h (Karadottir and Attwell, 2007). This specialized compact myelin alone makes the oligodendrocyte indispensible for normal CNS function including all motor, sensory and higher functions of the brain (Nave, 2008). The mechanisms of myelination resulting in the spiraling lamellae of the compact myelin and the signals that regulate this complex process are discussed in greater detail throughout the book.

Non-compact myelin

Although the term myelin is usually used in reference to regions of compact myelin, some of the oligodendrocyte plasma membrane remains uncompacted – the non-compact myelin. This arrangement is better visualized by a conceptual unwrapping of the oligodendrocyte (Figure 1.2). If the myelin were unraveled and stretched out it would be a flat, trapezoid sheet of compacted plasma membrane lined by a tube of cytoplasm. This tube of cytoplasm contains the usual cell organelles and cytoskeletal components and is known as the non-compact myelin. When each trapezoid process is rolled around an axonal length, the non-compact myelin rim forms loops by attaching to the lateral extremities of the myelinated internode. These regions of non-compacted cytoplasm are called the paranodal loops and have a crucial relationship with the axon, forming the axo–glial junction – the largest of all mammalian adhesion complexes (Brophy, 2001). The process of myelination directs the polarization of both the oligodendrocyte and the axon to form distinct domains at the axo–glial junction; the paranode and the adjacent juxtaparanode,

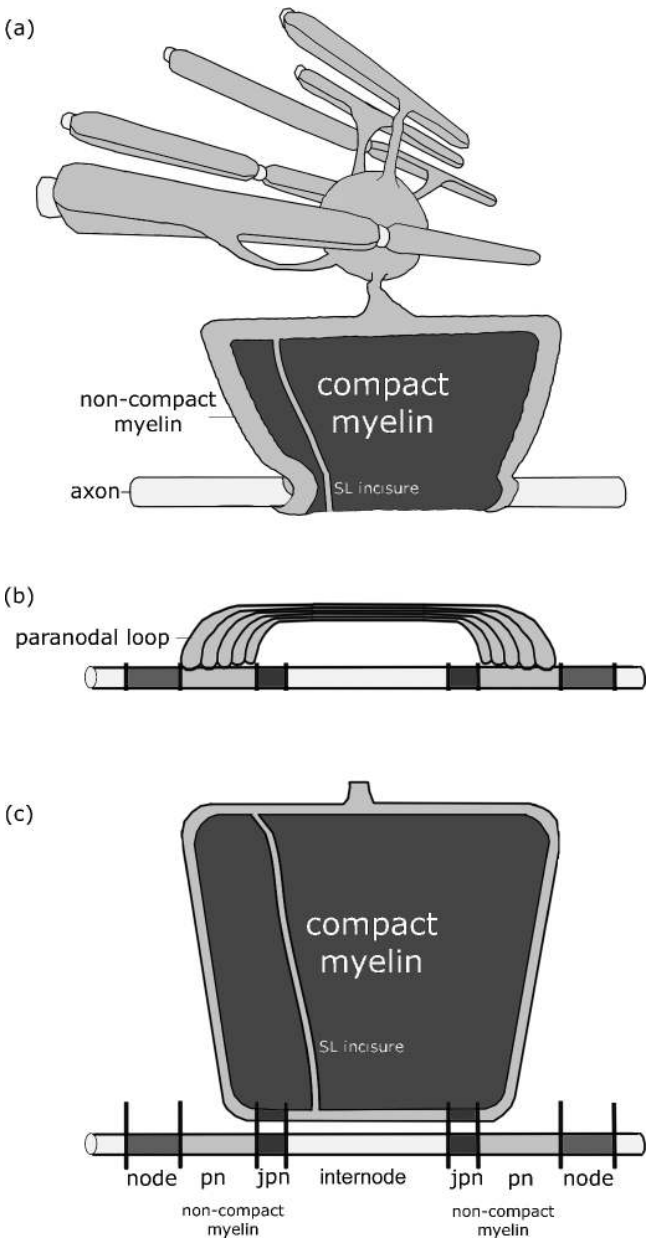

Figure 1.2a–c The conceptual unwrapping of an oligodendrocyte process reveals the regions of the cell that are made up of compact and non-compact myelin. (a) If the myelin were unraveled and stretched out it would be a flat, trapezoid sheet of plasma membrane with its uncompacted and compacted regions. Occasionally there are also

each with different and very specific structural and molecular characteristics. Whether oligodendrocytes also contain channels of uncompacted myelin analogous to the transverse processes and Schmidt–Lanterman incisures of the PNS Schwann cell is somewhat controversial (Ghabriel and Allt, 1981). These regions of non-compact myelin are continuous with the paranodal region and traverse the compacted myelin lamellae – the transverse processes running parallel to the axon and the Schmidt–Lanterman incisures running through the spirals of compact myelin. However, Schmidt–Lanterman incisures, although rare in the CNS, can be frequently seen in large fibers where the axon is at least 2 μm in diameter and whose myelin is at least 0.5 μm thick (Blakemore, 1969). It has been suggested that these regions play a role in the maintenance and metabolism of myelin by allowing the exchange of compounds between the membranous and cytoplasmic compartments of the oligodendrocyte. Further analysis of their function and frequency has been hindered by the lack of specific markers available for their identification in the CNS (Trapp and Kidd, 2004). Nonetheless, domains of non-compact myelin are now recognized as critically important in dissecting disease mechanisms affecting both oligodendrocytes and Schwann cells and are discussed further in Chapter 2. The contributions to saltatory conduction made by compact myelin as an insulator and by non-compact myelin as a diffusion barrier to ion channels vastly increase the speed of nerve conduction such that the acquisition of myelin by vertebrates some 425 million years ago made a significant impact on the course of evolutionary history.

Caption for Figure 1.2 (cont.)
longitudinal channels within the compacted region containing cytoplasm, Schmidt–Lanterman (SL) incisures, that course through the spirally compact myelin. (b) When each trapezoid process is spiraled around an axonal length, the innermost non-compact myelin rim forms the paranode at each end of the myelinated internode and in contact with the axon. (c) These regions of non-compact myelin organize the oligodendrocyte/axon complex into distinct molecular and functional domains vital for saltatory conduction, known as the node of Ranvier, the paranode (pn), juxtaparanode (jpn) and internode and are discussed further in Chapters 2 and 4. Adapted from (Salzer, 2003). See color plate section for color version of this figure.

EVOLUTION OF MYELINATION: MYELIN MAKES THE WORLD GO AROUND

Animals have developed two different strategies in an attempt to increase the speed of nerve conduction – a distinct evolutionary advantage. The first, and the more primitive, is to send neuronal signals using large or even giant axons (Hartline and Colman, 2007). By increasing the diameter of the axon small invertebrates have been able to acquire an increased conduction velocity. However, this strategy is only effective for relatively small animals ranging from 0.1 to 30 cm in size (Zalc, 2006). The second strategy is to increase conduction velocity by insulating the axons with a myelin or myelin-like covering. Ensheathing neuroglial cells form intimate associations with neurons in all animals except for two phyla, the Ctenophora and the Cnidaria, which include jellyfish, anemone and corals (Schweigreiter *et al.*, 2006). The glial cell–axon pairing is seen in invertebrates ranging from the nematode to the echinoderms (Hartline and Colman, 2007) but only some of these organisms elaborate a myelin-like covering. Indeed, the invertebrates generate an assortment of multilayered glial membranes that act as rudimentary forms of myelin. These structures range from loosely wound to tightly packed membranes ensheathing axons in either a spiral or a concentric arrangement (Schweigreiter *et al.*, 2006). An example of concentric layering of the glial membrane is seen in the copepods, small ocean crustaceans, and is an adaptation that gives these creatures a distinct evolutionary advantage (Davis *et al.*, 1999; Zalc *et al.*, 2008). Myelin allows copepods to move up to 200 body lengths in milliseconds (Davis *et al.*, 1999) providing them with the acceleration needed to escape predators in dangerous ocean environments. This non-compact myelin in invertebrates is sufficient for the needs of small creatures but not for larger vertebrates. In contrast to the diverse myelin organization seen in invertebrates, vertebrate myelin structure is highly conserved from the most ancient myelin present in sharks through to that in humans (Hartline and Colman, 2007). In evolutionary terms, increased conduction velocity bestowed by myelination allows rapid responses, in the sense not only of making a quick getaway in the avoidance of predation but also in attacking prey. The requirement for rapid and more efficient nerve conduction may be related to increasing complexity within the vertebrate CNS. Owing to the restrictions on brain size imposed by the skull, individual oligodendrocytes gained the ability to myelinate multiple axonal

lengths, which provided a mechanism by which neuronal numbers could increase without a concomitant increase in oligodendrocyte cell number. This process allowed the brain to increase its complexity with minimal volume increase, enabling a greater capacity for information processing, response to stimuli and more efficient feedback loops (Hartline and Colman, 2007). In this way the acquisition of myelin made vertebrates faster and smarter, setting them on the path to becoming highly evolved. However, hand in hand with this increased complexity comes the potential for dysfunction and the resultant pathological consequences.

OLIGODENDROCYTES MOVE TO THE FORE IN GRAY MATTER DISEASES

Oligodendrocyte involvement in psychiatric disorders

For decades neuroscientists have paid negligible attention to how the study of white matter could contribute to answering the big questions in learning, memory and psychiatric disorders. Instead intense focus was directed towards the inner workings of neurons and their transmission. However, it is apparent that the role of the non-neuronal cells of the CNS in both health and disease has been significantly underrated. As will be discussed throughout this book, study of the "non-nervous" cells of the brain is providing important new leads in the quest for both causes and treatments of neurological diseases. Rapid advances in the understanding of the complexity of the glia/neuron relationship are challenging traditional views. The oligodendrocyte is no longer regarded as an inert axonal comfort blanket but is emerging as a dynamic player in brain function. This paradigm shift is particularly evident with regard to neurodegenerative, psychiatric and mood disorders and pain (Watkins *et al.* 2005) where it once would have been heretical to suggest that oligodendrocytes play an important role. While oligodendrocyte pathology has long been recognized in diseases such as multiple sclerosis where the breakdown of compact myelin is obvious histopathologically, powerful laboratory techniques are now able to define more subtle changes/abnormalities in oligodendrocyte function or specific attack and damage (Antony *et al.*, 2004; Barnett and Prineas, 2004). The use of unbiased genomic and proteomic screening to identify genes or proteins associated with neurodegenerative and psychiatric

disorders has revealed unexpected associations with oligodendrocyte and myelin genes. This shift can be exemplified by the spate of reports detailing the dysregulation of myelin-related genes in schizophrenia (Hakak *et al.*, 2001; Karoutzou *et al.*, 2008; Roy *et al.*, 2007; Sugai *et al.*, 2004; Tkachev *et al.*, 2003), bipolar disorder (Adler *et al.*, 2006; Kato *et al.*, 2007) and other psychiatric disorders.

Even mechanisms typically associated with neuronal dysfunction such as deregulation of dopaminergic signaling can be influenced by the oligodendrocyte (Roy *et al.*, 2007). For example, neuregulin-1 (NRG1) and its receptor erb4 are vital for brain development and have previously been genetically linked to schizophrenia (Mei and Xiong, 2008; Stefansson *et al.*, 2002). Although the NRG pathway is important for the development and function of neurons, astrocytes and oligodendrocytes the selective blocking of erb signaling exclusively in oligodendrocytes has significant effects on not only oligodendrocytes but also neuronal functioning (Roy *et al.*, 2007). In transgenic mice, selective blockade of erb signaling in oligodendrocytes resulted in irregularities in oligodendroglial structure such as fewer myelin lamellae ("thinner myelin") as well as changes in neuronal dendritic arborization. Although oligodendrocyte abnormalities could have been predicted, the targeted oligodendroglial disruption of erb signaling also resulted in an unexpected dysregulation of the dopaminergic system. In fact, inactivation of erb4b in oligodendrocytes interfered with dopaminergic neurotransmission to such an extent that behavior was affected in a manner relevant to psychiatric disorders: increased anxiety, enhanced sensitization to amphetamine and hypoactivity (Roy *et al.*, 2007). Such symptoms as these would traditionally have been ascribed to dysfunction of neural transmission; however, in this case they can be directly attributed to oligodendrocyte pathology. This example highlights the often subtle but critical role the oligodendrocyte plays in total brain function.

Perturbation of oligodendrocyte function in transgenic models

Improved technologies have not only been paramount in dissecting the role of oligodendrocytes in disease states but have also exposed previously unknown functions in the normal CNS. Transgenic mouse models for example have become useful in highlighting the importance of oligodendrocyte–axon interaction in the healthy CNS where