Novartis Foundation Symposium 276

PURINERGIC SIGNALLING IN NEURON–GLIA INTERACTIONS

2006

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Contents

Symposium on Purinergic signalling in neuron–glia interactions, held at the Novartis Foundation, London, 7–9 June 2005

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This symposium was based on a proposal made by R. Douglas Fields

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Chair's introduction

R. Douglas Fields

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This meeting comes at an exciting time. Neuroscientists are beginning to realize that we have overlooked half the brain! This is the part of the brain composed of non-neuronal cells: myelinating glia, astrocytes and microglia. For far too long neuroscientists had an artificially narrow conceptual view of the nervous system. I love the following quote from Robert Pirsig's book *Zen and the Art of Motorcycle Maintenance*: 'The truth comes knocking on the door. And you say "Go away. I'm looking for the truth". And so it goes away.' There is something missing in our text-book image of nerve endings on post-synaptic neurons. The black spaces between these structures are full of cells—astrocytes—but they are completely missing from most figures. We can use Ca^{2+}-sensitive dyes to see what these astrocytes are doing, and when we do, we find that these non-neuronal cells are communicating. We see now that there are two separate flows of information in the brain: a neuronal component of information processing propagated electrically and a glial component propagated through intercellular Ca^{2+} waves. And the two systems of cellular communication interact. The neurons communicate with glia, and glia can in turn communicate back to neurons and regulate the flow of information through the brain. How and why are these non-neuronal cells doing this (Fig. 1)?

We know something about how they do this. Stan Kater and colleagues looked at cultured astrocytes using a Ca^{2+}-sensitive dye and performed a clever experiment to determine if the intercellular communication between astrocytes required cell–cell contact, or whether astrocytes communicate by releasing signalling molecules (Hassinger et al 1996). They scratched the cells away to create a cell-free zone in a monolayer of astrocytes in culture to determine whether this communication is mediated only by a flow of ions through gap junctions between cells. This cell-free zone acted like a fire-break in a forest. Then they initiated a Ca^{2+} wave in the astrocytes to see whether the signal propagated across the cell free zone: it did. The results showed clearly that astrocytes are communicating by sending signals through the media, in a similar way to neurons communicating at synapses. One of the key signalling molecules, quickly identified, was ATP.

Early investigators in the field of neuron–glia interactions were not used to thinking about ATP as an intercellular messenger. But people quickly began to

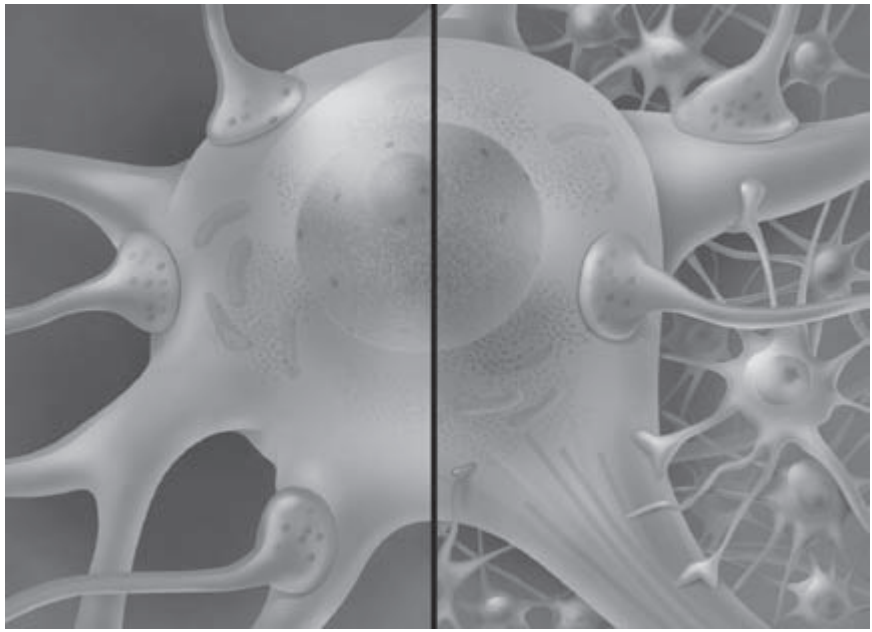

FIG. 1. The textbook view of the nervous system (left) typically excludes glia (right), which communicate among themselves using intercellular calcium waves and regulate synaptic transmission. Purinergic signalling is a major mechanism of intercellular communication between glia, and between neurons and glia.

address questions such as how ATP could be released from an astrocyte (Eduardo Lazarowski will cover this in the book), and which receptors on astrocytes can be activated by ATP (Geoff Burnstock and Maria Abbracchio will address this subject). Once these receptors are activated, it is important to determine how they signal intracellularly (Joe Neary and Beth Stevens will address this in their papers). Glial biologists immediately wanted to know how they could begin studying this system of purinergic communication (Ken Jacobson will discuss the pharmacology that allows us to activate and inactivate certain purinergic receptors). Then the field began to appreciate that as ATP breaks down to adenosine it activates different types of purinergic receptors, and that there is an extracellular set of enzymes that regulate this degradation and synthesis (Herb Zimmerman will talk about this important aspect of purinergic signalling).

At the Novartis Foundation meeting on P2 purinoceptors 10 years ago, many of the people in this room were grappling with the nature of P2 receptors, how the various types were distinct from each other, how they signalled and which drugs should be used to selectively activate or inactivate these receptors. The tools

weren't really ready to launch into functional studies at that time, but now we are able to begin doing this and explore the functional consequences of ATP signalling between neurons and glia.

ATP is key in regulating glial interactions with neurons and glial regulation of synaptic transmission. ATP is released with neurotransmitter and it acts upon purinergic receptors in perisynaptic glia. The glial cells in turn release any number of neuromodulatory substances to regulate postsynaptic or presynaptic function. The astrocytes can then communicate among themselves by sending ATP signals through astrocytic networks to perhaps affect another synapse to modulate neurotransmission at a distant site. We have at this meeting Richard Robitaille who will be talking about his work on perisynaptic glia at the neuromuscular junction, and then we will move to the retina where Eric Newman will talk about purinergic receptors regulating neuronal firing patterns in the retina. We will then move into the brain with Phil Haydon's work on adenosine and ATP regulating synaptic function by interactions with perisynaptic astrocytes in the hippocampus.

There is more to nervous system function than just the millisecond to millisecond interactions at synapses. In my lab, we are interested in how the brain develops and modifies its structure and function through experience and learning. These are slow processes, and neuron–glia interactions may be particularly well suited to participate in slower nervous system phenomena.

In the field of nervous system development, ATP and purinergic receptors have not really entered into our thinking, but I think this is something that will soon change. Let me give an example of a developmental process that is regulated by impulse activity, and may involve neuron–glia interactions. Jeff Lichtman has data showing that early in development all muscle fibres are innervated by multiple axons, but shortly after birth all but one are eliminated, leaving one muscle fibre innervated by only one axon. Jeff's lab is able to visualize, in living animals over several days, the withdrawal of these synapses by using fluorescently labelled neurons. In some of the images they have noticed ghost-like fingers pulling these withdrawing axons away. Working with a colleague, Wes Thompson, they did the opposite experiment, engineering a mouse with fluorescent glia (Schwann cells), so now the axons appear as ghosts. As the axon withdraws, it follows the path dictated by the glial cell (W. Thompson, personal communication and T. Misgeld and J.W. Lichtman, personal communication). It is now becoming clear that we will never understand synapse formation and remodelling if we fail to consider the interactions between neurons and glia. Understanding this process of activity-dependent regulation of nervous system development comes down to a question of cell–cell communication: what are the molecules that mediate these kinds of communication. The kinds of molecules people in the development field traditionally think of are growth factors, peptides and cell adhesion molecules. Moses Chao will expand this view by presenting his work that combines purinergic receptors and

neurotrophins, showing that there are interactions between purinergic receptors and the sorts of molecules that neurobiologists are more accustomed to thinking about in nervous system development.

ATP is important in communication among all kinds of glia in the brain as well as with neurons, including interactions with the vasculature, microglia, axons and synapses. We'll have a talk on neuro–immune interactions (by Francesco Di Virgilio) and I'll talk a bit about interactions between myelinating glia and axons (as will Beth Stevens). We'll also have a presentation on the role of glia in pain involving purinergic signalling (by Kazuhide Inoue).

Our goal at this meeting is to consider neuron–glia interactions and the involvement of purinergic signalling. We now realize that so many aspects of brain function involve interactions between neurons and glia that it is no longer possible to ignore the involvement of glia. Many of these processes involve purinergic receptors. We want to fuse two fields, bringing together neurobiologists and glial biologists. Stéphane Oliet doesn't work on purinergics, as far as I know, but he does beautiful work on neuron–glia interactions in remodelling of synapses in the CNS. We have the top glial biologists here: Martin Raff, Rhona Mirsky, Kris Jessen and Boris Zalc. Then we have people who work on purines in neurons, glia and other cells: Mike Schwarzschild, Mike Salter, Peter Illes and Stanko Stojilkovic. Our goal is to work to find a synthesis of these two fields (purinergic signalling and neuron–glia interactions) and explore the common ground between them. We want to produce a book that will be a tool for the field, which glial biologists can use to learn about purinergic receptors and those of us working in purinergic receptors can use to learn about glia.

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A brief look at glial cells

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Abstract. Glial cells are numerically the dominant cell type in the central and peripheral nervous system. They are intermixed with the nerve cells and are found in intimate contacts with neuronal cell bodies, dendrites, axons and synaptic contacts. Like neurons, glial cells are a heterogeneous population of cells that differ in developmental origin, molecular composition, structure and activity. When these cells were first discovered some 150 years ago they were viewed as a type of connective tissue support for nerve cells. They are now known to be essential for the development and function of the brain and other parts of the nervous system. They are also central players in a large number of pathological processes. We have therefore moved away from a view of the nervous system as a system of neurons, to the appreciation that it is a neural system where the contributions of both nerve and glial cells are intimately integrated, interdependent and obligatory.

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The nervous system is built from two broad classes of cells—neurons and glial cells. This is true equally for the brain, spinal cord, peripheral nerves and ganglia. In numbers and volume, the contribution of glial cells to the nervous system is on a par with, or exceeds that of the neurons, a fact that is often unappreciated and sometimes comes as a surprise even to neuroscientists. Thus, glial cells are thought to occupy about half the volume of the brain, and they outnumber neurones by some margin in most brain areas. In the peripheral nervous system (PNS) the number of glial cells per neuron is even higher than in the central nervous system (CNS) (Fields 2004, Fields & Stevens-Graham 2002, Jessen 2004, Jessen & Richardson 2001, Kettenmann & Ransom 2005, Miller 2005).

These histological facts alone are sufficient to suggest that we are unlikely to comprehend the development and function of the nervous system, and the brain in particular, without understanding glial biology and the interactions between glial cells and nerve cells. This expectation has been borne out in a striking way by many recent findings showing for instance that glial cells are intimately involved in the control of development and survival of neurons, have a role in synapse formation and synaptic function, are components of pain mechanisms, and act as regulators

of repair after CNS and PNS injury, to name but a few of the functions of glial cells that are of great current interest. This is in addition to the better established involvement of glia in meeting critical metabolic needs of neurons, controlling the homeostasis of the immediate neuronal environment and providing myelin sheaths for the acceleration of impulse conduction along axons.

Much like neurons, glial cells are a heterogenous group of cells that differ in developmental origin, molecular composition, structure and function. In the CNS, the major groups of glia are astrocytes and oligodendrocytes, in addition to microglia, that are of non-neural origin and related to monocytes/macrophages in the rest of the body, and ependymal cells that line the ventricles of the brain (Fig. 1). In the PNS, the best known glial cells are Schwann cells, while enteric glial cells are found in the enteric nervous system, satellite cells in other autonomic ganglia and in sensory ganglia and terminal glia at somatic nerve terminals. Distinct cells, olfactory ensheathing cells, associate with the olfactory nerve.

Astrocytes

In the early postnatal brain, astrocytes originate from precursors in the subventricular zones (SVZ). Earlier in development, astrocytes are generated from radial glia that span the wall of the developing brain, having a cell body in or near the ventricular zone and a process that reaches the brain surface. In addition to generating astrocytes, another long established function of radial glia is to support and

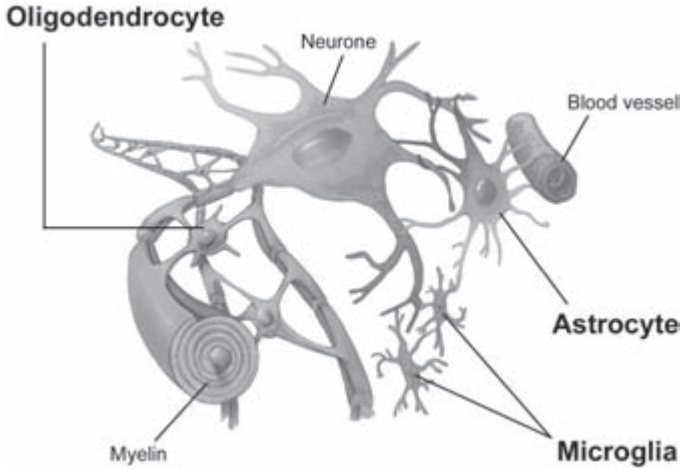

FIG. 1. Schematic illustration of the main glial cells in the CNS and their relationship with neurons and blood vessels.

guide the extensive neuronal migration that takes place during brain development (Goldman 2001).

A major surprise has come from recent studies of radial glia (Kriegstein & Götz 2003). These cells show certain features that have classically been associated with mature astrocytes. It now appears that these cells and another astrocyte-like cell, the subventricular zone astrocyte, can act as precursors to a number of cell types in the developing and adult brain, respectively, generating, in addition to astrocytes, neurons, and even oligodendrocytes and ependymal cells. Related observations have been made on the Schwann cell precursor in the PNS (Jessen & Mirsky 2005). These findings have given rise to the novel concept that cells with the phenotype of early glia can act as multipotent progenitors during nervous system development, and that astrocyte-like cells function as neural stem cells in the adult brain.

Astrocytes are the most numerous of CNS glia. They have been assigned more different functions than any other glial type and are, in fact, likely to consist of several distinct subtypes. An important function of these cells is to provide neurones with essential metabolic support (Pellerin & Magistretti 2004). Astrocytes carry numerous processes that branch among neuronal cell bodies and processes and often terminate on blood vessels. This might relate to astrocyte-mediated control of bloodflow in the brain to ensure adequate blood supply to active areas, and to the control of the blood–brain barrier. Other astrocyte processes lie in close association with synapses. Provocative new evidence suggests that these perisynaptic astrocyte processes carry out an important function by secreting substances that control the strength of synaptic transmission. Astrocytes also have high affinity uptake sites for neurotransmitters and help in this way to clear excess transmitter from the extracellular space. Together these observations indicate that astrocytes might be much more directly involved in information processing in the brain than most people had envisaged (Colomar & Robitaille 2004, Zhang & Haydon 2005). Another exciting link between astrocytes and synapses has been made in developmental studies that indicate that signals from astrocytes have a role in promoting synapse formation (Ullian et al 2004). These findings also raise the possibility that failure of appropriate glial signalling could be one of the factors that contribute to the synaptic loss underlying age related or pathological memory loss or cognitive dysfunction.

Astrocytes are also involved in the CNS pathology that results from mechanical injury (Fig. 2). In this case, astrocytes respond by hypertrophy and structural reorganization that contributes to the generation of a fibrous glial scar around the injury site. While this may be important for reinforcing the traumatized region, the scar is also thought to form an effective barrier to axonal regeneration. Injury also prompts astrocytes, and oligodendrocytes (below), to express molecules that potentially block the regrowth of axons. These are the major reasons why the CNS

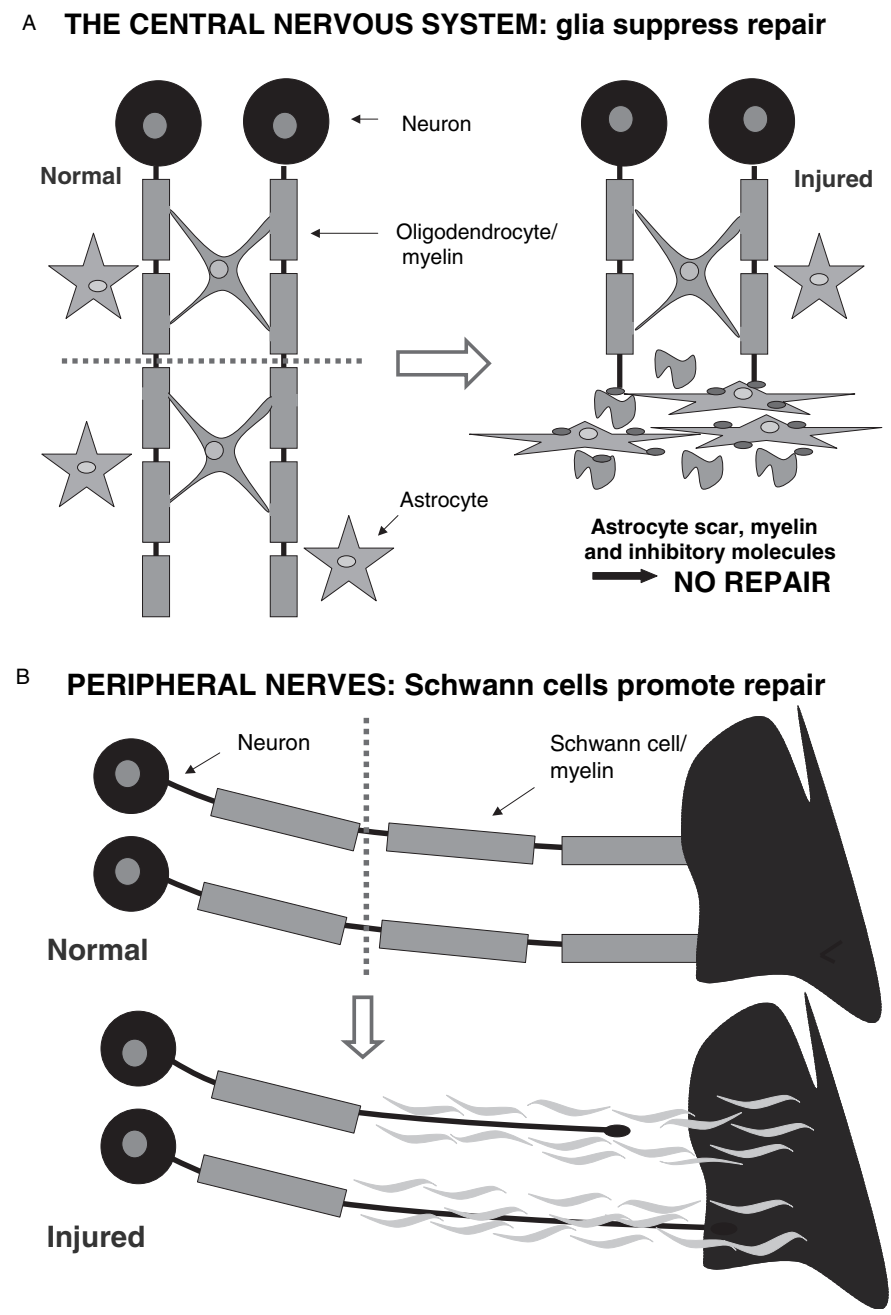

FIG. 2. (A) Injury (dotted line) to axons in the CNS leads to formation of an astrocyte scar and expression of potential growth-inhibiting factors. (B) Injury (dotted line) to a peripheral nerve triggers Schwann cell proliferation and de-differentiation that generates a growth-promoting environment.

is unable to regenerate severed fibre tracts following, for instance, spinal cord injury (Filbin 2003, Raisman 2004).

Astrocytes probably influence many other CNS diseases through their ability to remove potentially cytotoxic amino acids and secrete numerous bioactive molecules, including immune modulators, nitric oxide and metalloproteases. In this way astrocytes are likely to be significant determinants of the events following stroke and of inflammatory conditions such as Alzheimer's disease and multiple sclerosis (below).

Oligodendrocytes

Oligodendrocytes are highly specialized for generating myelin sheaths around axons (Richardson 2001, Butt 2005). A single oligodendrocyte can extend 30–40 processes, each of which ends in a myelin sheath (Fig. 2). The sheath forms by spiralling movements of a flattened cellular process around the axon, in a process that demands a large amount of membrane synthesis leading to several thousand fold increase in membrane area. The resulting multilayered and compact membranous sheath provides electrical insulation around the axon. The meeting points between adjacent sheaths along a single axon are known as the nodes of Ranvier. In the nodal region, the axonal membrane is enriched in sodium and potassium channels. This means that electrically insulated and excitable segments alternate along the axon, an arrangement that leads to saltatory conduction of action potentials, which is about 10 times faster than impulse conduction along an unmyelinated axon of a similar diameter.

Perhaps the most widely recognized disease of glial cells is multiple sclerosis (MS), a condition that most obviously affects oligodendrocytes, although other cells in the CNS are also affected (Prat & Antel 2005). MS is an inflammatory disorder that characteristically involves the formation of multiple lesions in the CNS in which myelin is destroyed, oligodendrocytes die and axons eventually degenerate. The reasons for this catastrophic series of events are poorly understood. As mentioned above, oligodendrocytes are also likely to be responsible, in part, for the absence of axonal regeneration following CNS injury, since trauma activates the expression of factors with the potential to block axon growth (Fig. 3).

Microglia

Microglia originate from the monocytic lineage (Perry 2001). Developmentally, microglia are therefore related to tissue macrophages but unrelated to other glial cells. They migrate into the CNS during embryonic development, and are in the adult found throughout the brain and spinal cord.

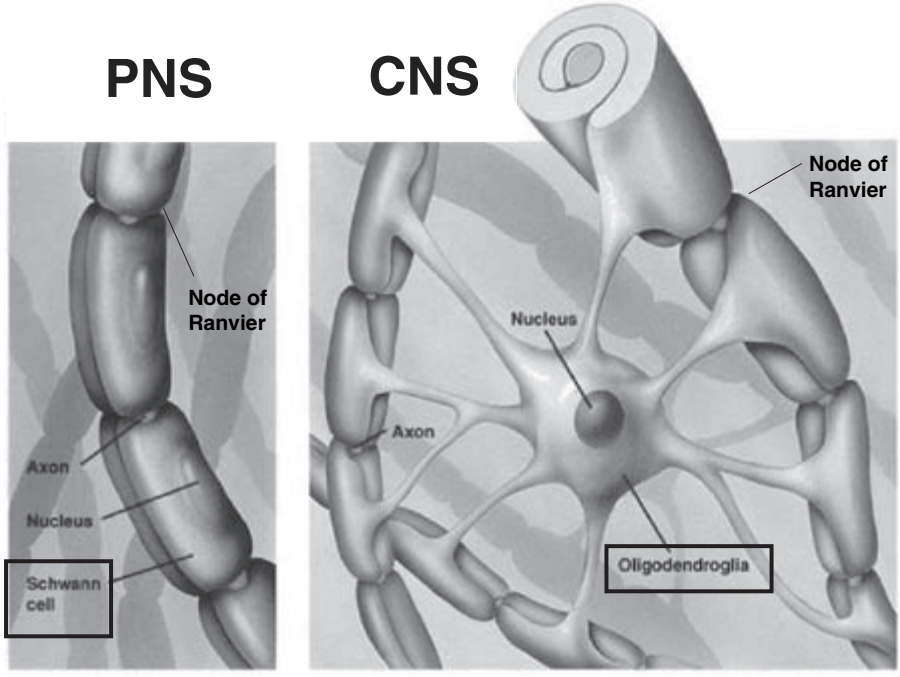

FIG. 3. Myelinating glial cells in the PNS and CNS. In the PNS, myelination is carried out by Schwann cells, each of which myelinates one axon only. In the CNS, myelination is carried out by oligodendrocytes that have numerous processes, each of which carries a myelin sheath.

Microglia carry out macrophage-like functions and are often regarded as the 'brain's immune system' (Streit 2005). In the developing brain, macrophages take part in removing dying cells (Mallat et al 2005). In the normal adult CNS, microglia are present in a resting state and their function is unclear. They are, however, remarkably sensitive to a broad spectrum of injury or perturbation of CNS tissue, and respond by switching to an activated state, characterized by altered morphology and molecular expression (Fig. 4). Activated microglia produce a variety of bioactive factors including potentially cytotoxic molecules, such as oxygen radicals and tumour necrosis factor (TNF) α, but also neurotrophic factors and cytokines. They also up-regulate the expression of major histocompatability complex I and II molecules. All of this generates an extensive potential for influencing pathological processes in the CNS and activated microglia are associated with a large number of diseases, including multiple sclerosis, Alzheimer's disease and AIDS. Activated microglia are also though to play a key role in neuropathic pain mechanisms (Tsuda et al 2005).

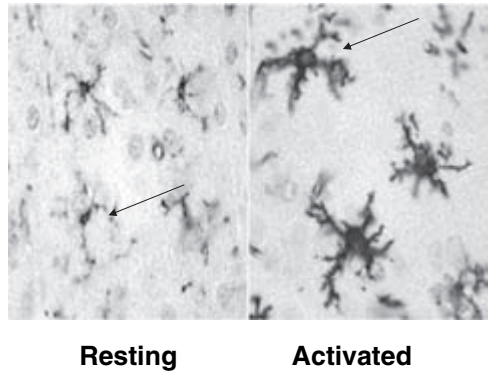

FIG. 4. Microglia are ubiquitously distributed throughout the CNS and are activated by a variety of traumatic stimuli.

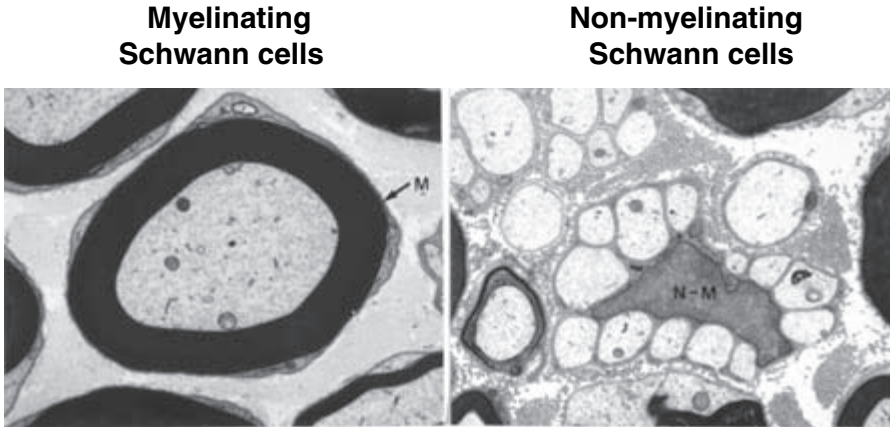

FIG. 5. Schwann cells as they appear in a transverse section through a peripheral nerve. A myelinating cell forms a compact, multilayered sheath (M) around a single axon. A non-myelinating cell (N-M) ensheathes several axons (here 13) each lying in a separate trough in the cell surface. Reprinted from Jessen & Mirsky (1999), with permission from Elsevier.

Schwann cells

Schwann cells envelop axons in peripheral nerves and are found exclusively in the PNS. They exist in two forms, myelinating and non-myelinating (Figs. 2 and 5). The myelinating cells form insulating myelin sheaths around all larger diameter axons, and this sheath has comparable structure and function to the myelin made by oligodendrocytes. The non-myelinating cells associate with the smaller diameter axons. They show similarities with the major non-myelin forming CNS

glia, the astrocytes, and are likely to provide neurones with metabolic and trophic support.

Schwann cells develop from the neural crest. They retain remarkable plasticity throughout life, a feature that is the major reason for the extensive regeneration potential of damaged peripheral nerves. Thus, after injury myelinating and non-myelinating Schwann cells are able to re-enter the cell cycle and de-differentiate to adopt an immature phenotype that forms a favourable substrate for axon re-growth due to elevated expression of trophic factors and adhesion molecules. This stands in marked contrast to the growth-adverse response of CNS glia to axon damage (above). It is notable, however, that in both cases it is the glial component of the nervous system that is the major determinant of repair after neuronal injury (Fig. 2).

Interdependence of neurons and glia

CNS and PNS glial cells are closely related in function and often in structure and molecular composition. Our ideas about these cells are in a rapid flux and are changing in a similar direction. For both CNS and PNS glia, new and unexpected glial functions have been identified and glia are increasingly recognized as producers of factors that are necessary for survival, development and function of CNS and PNS neurons. The emerging idea that glial cells can act as multipotential progenitor cells also appears to be true for both systems.

It is increasingly clear that the nervous system develops and functions as a neural system where the contributions of nerve cells and glial cells are intimately integrated and interdependent. Developmentally, this has recently been revealed by the unexpected finding that glial-like cells are the precursors of large numbers of brain neurons and that astrocytes give rise to neurons in the adult brain (Götz & Barde 2005). Another example of an intimate relationship between neurons and glia during development is seen in the PNS (Jessen & Mirsky 2005). In this case it has been found that if Schwann cell precursors and early Schwann cells are removed from developing nerves by genetic manipulation, large numbers of early motor neurons and dorsal root sensory neurons die. This suggests that glial cells provide indispensable trophic support for developing neurons. A recent illustration of glial signals influencing neuronal development is also seen in synapse formation (Ullian et al 2004). A crucial developmental relationship where the key signalling is in the opposite direction—from neurons to glia—is evident in the well known, and absolute, dependence of Schwann cell myelination on axon-associated signals. Other developmental signals between axons and associated glial cells in the CNS and PNS depend on electrical activity of the axon (Fields & Stevens-Graham 2002, Coman et al 2005). In the mature system, the emerging role of glial cells in controlling synaptic function is likely to have considerable implications. Important

bi-directional signalling between neurons and Müller glial cells is also seen in the retina (Newman 2004), while the recently defined interactions between microglia and neurons in neuropathic pain mechanisms are likely to be of significant clinical importance.

So far, faster progress has been made in demonstrating unequivocally crucial *in vivo* relationships and interdependence between neurons and glia using developing systems than in the adult. To reveal more clearly the role of glial cells in the mature nervous system it will be important to design experiments that allow *in vivo* analysis of nervous system function following inactivation of defined glial signals or the ablation of distinct glial groups in the adult.

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The acquisition of myelin: a success story

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Abstract. The myelin sheath, and hence the myelin-forming cells (i.e. Schwann cells in the PNS and oligodendrocytes in the CNS), have been a crucial acquisition of vertebrates. The major function of myelin is to increase the velocity of propagation of nerve impulses. Invertebrate axons are ensheathed by glial cells, but do not have a compact myelin. As a consequence, action potentials along invertebrate axons propagate at about 1 m/s, or less. This is sufficient, however, for the survival of small animals (between 0.1 and 30 cm). Among invertebrates, only the cephalopods are larger. By increasing their axonal diameter to 1 mm or more, cephalopods have been able to increase the speed of propagation of action potentials and therefore adapt nerve conduction to their larger body size. However, due to the physical constraint imposed by the skull and vertebrae, vertebrates had to find an alternative solution. This was achieved by introducing the myelin sheath, which leads action potentials to propagate at speeds of 50–100 m/s without increasing the diameter of their axons. Not all vertebrate axons, however, are myelinated. In the protovertebrates (lancelets, hagfishes, lampreys), which belong to the agnathes (jawless fishes), axons are not ensheathed by myelin. Among living vertebrates, the most ancient myelinated species are the cartilaginous fishes (sharks, rays), suggesting that acquisition of myelin is concomitant with the acquisition of a hinged-jaw, i.e. the gnathostoma. The close association between the apparition of a hinged-jaw and the myelin sheath has led to speculation that among the devonian fishes that have disappeared today, the jawless conodonts and ostracoderms were not myelinated, and that myelin was first acquired by the oldest gnathostomes: the placoderms. I also question where myelin first appeared: the PNS, the CNS or both? I provide evidence that, in fact, it is not the type of myelin-forming cell that is crucial, but the appearance of axonal signals, rendering axons receptive to inducing an ensheathing glial cell to wrap around the axon. Under certain circumstances or in some species, invertebrate ensheathing glial cells wrap around axon to form a pseudo-myelin sheath. Therefore, to form myelin it was not compulsory to 'invent' a new cell type. Hence my conclusion that myelination has most probably started simultaneously in the PNS and the CNS, using pre-existing ensheathing glial cells.

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The myelin sheath, and hence the myelin-forming cells (Schwann cells in the PNS and oligodendrocytes in the CNS), have been a crucial acquisition of verte-

brates. The major function of myelin is to increase the velocity of propagation of nerve impulse. The speed of propagation of action potentials along axons can be increased in two ways: either by increasing axons diameter, or by ensheathing them with a membrane: the myelin sheath. Invertebrate axons are ensheathed by glial cells, but do not have a compact myelin. As a consequence, action potentials along invertebrate axons propagate at about 1 m/s, or less. This is sufficient, however, for the survival of small size animals (between 0.1 and 30 cm). Among invertebrates only the cephalopods have a larger size. By increasing their axons diameter from 1 μm or less, to 1 mm or more, cephalopods have been able to increase the speed of propagation of action potential and therefore adapt nerve conduction to their larger body size. However, due to the physical constraint imposed by the skull and vertebrae, vertebrates had to find an alternative solution. This was achieved by introducing the myelin sheath, which leads action potentials to propagate at speeds of 50–100 m/s, without increasing the diameter of their axons. All vertebrates, however, are not myelinated. In the protovertebrates (lancelets, hagfishes, lampreys), which belong to the agnathes (jawless fishes), axons are not ensheathed by myelin (Bullock et al 1984). Among living vertebrates, the most ancient myelinated species are the cartilaginous fishes (sharks, rays) (Kitagawa et al 1993), suggesting that acquisition of myelin is concomitant with the acquisition of a hinged-jaw, i.e. the gnathostoma (Richardson et al 1997).

When, during evolution has myelin appeared?

The close association between the apparition of a hinged-jaw and the myelin sheath has led people to speculate that among the devonian fishes that have disappeared today, the jawless conodonts and ostracoderms were not myelinated, and that myelin was first acquired by the oldest gnathostomes: the placoderms (Zalc & Colman 2000).

Interestingly enough, in cartilaginous fishes, both the CNS and PNS axons are myelinated. In sharks myelin in the PNS is formed by a Schwann-like cell, i.e. a cell myelinating a single internode on a single axon, while in the CNS the myelin forming cells look like oligodendrocytes, i.e. multiprocess cells enwrapping several axons. Assuming that myelination has appeared in the placoderms, one can ask whether those axons were also myelinated both in the CNS and the PNS. If this has been the case it can be hypothesized that for myelination to occur has required the evolutionary appearance of new cell types, the myelin-forming cells. However, since Schwann cells derive from the neural crest and oligodendrocytes from the neural tube, the simultaneous generation of myelin forming cells in two different tissues is unlikely. Alternatively, it can be speculated that placoderms were hemi-myelinated either in the PNS or in the CNS, only. PNS nerves, from the dorsal root ganglion for instance, navigate also partially in the CNS where

they form the first synapse. If myelination had started in the PNS, these axons would have been myelinated in their peripheral portion only. Similarly to what is happening in demyelinating diseases, when a portion of an axon is demyelinated, this would create a conduction block along those axons when they enter the CNS. This is therefore an unlikely possibility. The same reasoning holds for the CNS axons, like the motor neuron axons, which travel both in the CNS and the PNS. However, in the CNS some tracts are strictly central. Hence it is conceivable that myelination has started in the CNS. However, one has to keep in mind that the evolutionary advantage of myelination is mostly related to motor functions. Rapid nerve conduction is critical to increase survival by rapid escape manoeuvres and for efficacious predation in a large animal. This requires motor tracks to be myelinated both in the CNS and in the PNS. It is therefore unlikely that placoderms, which have been the kings of the oceans for over 200 million years, would have been hemi-myelinated. Assuming that myelin has appeared both in the PNS and the CNS, how can we reconcile this hypothesis with the unlikely possibility that two myelin-forming cells emerged simultaneously in the neural crest and neural tube?

When during development are myelin-forming cells generated?

Recent data have seriously questioned ancient established dogma. All textbooks say that during development oligodendrocytes are the last cells to be generated. This notion was supported by the observation that during nervous system development myelination is the last event to occur. This would fit well with Haeckel's hypothesis that development (ontogeny) recapitulates evolution (phylogeny). However, the late identification of cells of the oligodendrocyte lineage relied on identification of mature myelin-forming cells. Increased knowledge of the developmental origin of Schwann cells and oligodendrocytes, with the discovery of their precursors and progenitors, has pushed backwards the timing of their emergence. It is clear now that oligodendrocyte progenitor cells are generated at E2.5 in the chick, and E9.5 in the mouse, i.e. at about the same time or shortly after the first neurons have been generated (Perez-Villegas et al 1999). Similarly, Schwann cell precursors seem to have already been generated by the time neural crest cells are migrating (Jessen & Mirsky 1998).

What is needed for myelination to occur?

Myelination depends on axons. In the CNS, although oligodendrocyte precursor cells *in vitro* can survive, proliferate, differentiate and even extend large myelin-like membranous extension in the virtual absence of neurons in the culture dish, several lines of evidence have stressed the crucial role of the axon in the process of myelin formation. Both *in vivo* and *in vitro* only axons, and not the dendrites,

are myelinated suggesting the existence of a recognition signal at the surface of the axon (Lubetzki et al 1993), the nature of which still remains to be discovered. During early development, axons express the polysialylated form of NCAM, which has been shown to act as a negative signal that must be down-regulated for myelination to proceed (Charles et al 2000). It has also been shown that electrical activity along CNS axons is required as a positive inducing signal of myelination. This has been shown in oligodendrocyte–neuron co-culture using highly specific neurotoxins, which can either block (tetrodotoxin) or increase (α-scorpion toxin) the firing of neurons. Myelination can be inhibited by blocking the action potential of neighbouring axons, or enhanced by increasing their electrical activity, clearly linking neuronal electrical activity to myelin formation (Demerens et al 1996). Similarly *in vivo*, myelination of optic nerve axons starts at the time when retinal ganglion cells change their pattern of firing from embryonic to adult. Along the same lines, it has been shown that animals reared in the dark have a delayed onset of myelination while premature opening of the eye lid induces a precocious myelination. (Gyllensten & Malmfors 1963, Tauber et al 1980). Similarly, in the naturally blind cape mole rat, a high number of axons remain unmyelinated (Omlin 1997), suggesting that when retinal ganglion cells are not electrically active, myelination is not just delayed, it does not take place. Interestingly, in the medial forebrain bundle, dopaminergic axons from the nigro-striatal tract run along the descending myelinated motor fibres. Oligodendrocytes, which are, hence, in the vicinity of fibres firing at either 50–100 Hz (motor axons) or other, which are most of the time silent (dopaminergic axons) myelinate only the electrically active fibres. Similarly, in myelinating co-cultures of oligodendrocyte and neurons, we have never observed myelin deposited around neurites from dopaminergic neurons (tyrosine hydroxylase expressing cells) (Lubetzki & Zalc, unpublished observation). In the PNS, axonal signals are mandatory at all the stages of Schwann cell precursor development into myelin-forming cells; for example, it has been shown that proliferation, survival and differentiation of Schwann cell precursors does not occur in the absence of neurons (Jessen & Mirsky 1991, 1998). However, in contrast to CNS axons, electrical activity does not seem to be the inductive signal of myelination (Zalc & Fields 2000). Since the only difference between myelinated and non-myelinated axons is their different diameter, this has led to the suggestion that Schwann cell will be a sort of calliper rule, sensing the axon diameter. However, experiments by the group of A. Peterson, which has produced a transgenic mouse with no neurofilaments in the axons, demonstrate that myelination does not depend on the axon diameter (Eyer & Peterson 1994). Although the myelination signal that allows a Schwann cell to discriminate between axons to be myelinated from their neighbours that are not (type C fibres) remains to be discovered, in the PNS, like in the CNS, it is the axon which appears as the crucial element for myelination to occur.