

SCIENTIFIC COMMENTARIES

A human cellular model to study peripheral myelination and demyelinating neuropathies

This scientific commentary refers to ‘Co-cultures with stem cell-derived human sensory neurons reveal regulators of peripheral myelination’, by Clark *et al.* (doi:10.1093/brain/awx012).

Peripheral myelination is an essential process in the development of the peripheral nervous system. Peripheral myelin permits saltatory action potential propagation, and also supports axons via an intricate system of interactions between Schwann cells and the axolemma. Both hereditary and acquired inflammatory processes can damage peripheral myelin and this is the pathological basis of demyelinating peripheral neuropathies such as Charcot-Marie-Tooth disease type 1, Guillain-Barre syndrome and chronic inflammatory demyelinating polyradiculoneuropathy (CIDP). The study of peripheral myelination and demyelination has relied heavily on animal models as systems that allow for dissecting the molecular mechanisms by which Schwann cells regulate myelin production, and for exploring the interaction between these cells and peripheral axons. Another classical approach to investigate peripheral myelination is based on *in vitro* co-culture systems of rodent dorsal root ganglia neurons and Schwann cells; however, the lack of an analogous human cellular system has limited the study of human peripheral myelination. In this issue of *Brain*, Clark and co-workers address this obstacle by using cellular reprogramming to

create a system to study peripheral myelination of human axons *in vitro* (Clark *et al.*, 2017).

Induced pluripotent stem cells (iPSCs) are generated by forcing expression of pluripotency factors in somatic cells such as fibroblasts, a technique termed cellular reprogramming. Human iPSCs can be differentiated into cells from all three germinal layers, including several neuronal types, and are an invaluable tool for modelling human neurological disorders at the cellular level. iPSCs have been used in the past to generate human motor neurons and to model inherited (Saporta *et al.*, 2015) and acquired (Harschnitz *et al.*, 2016) neuropathies. However, neurons behave quite differently in the absence of myelin. Juxtacrine signalling from Schwann cells helps organize the entire length of myelinated axons into a series of polarized domains centred around nodes of Ranvier, which are necessary for normal saltatory nerve conduction (Salzer, 2003). Axonal calibre, neurofilament phosphorylation and packing density, as well as axonal transport, are all disrupted when axons are ensheathed by abnormal myelin. This has been shown in elegant studies in rodent models (de Waegh *et al.*, 1992; Kirkpatrick and Brady, 1994), such as Trembler (Tr) mice, a naturally occurring dysmyelinating model caused by missense mutations in the peripheral myelin protein 22 (PMP22) gene (Suter *et al.*, 1992). Accordingly, many human disorders

cannot be accurately modelled using iPSC-derived neurons owing to the absence of myelin and axon-Schwann cell interactions. Human neuronal Schwann cell co-cultures have not been possible to date because of the inability to differentiate myelinating Schwann cells from iPSCs, as will be discussed below. However, in this issue of *Brain*, Clark *et al.* (2017) establish the first myelinating co-culture systems using human iPSC-derived sensory neurons and rat Schwann cells, and use them to demonstrate the important role of the neuregulin-ErbB signalling pathway in the myelination of human sensory axons. In addition, they use their system to demonstrate the effects of anti-disialosyl antibodies in myelinated axons, and thereby establish an *in vitro* system to model acquired demyelinating neuropathies.

By successfully establishing co-cultures of human iPSC-derived sensory neurons and rat Schwann cells, Clark *et al.* showed that two different species have enough molecular signalling homology to allow for efficient Schwann cell-axonal interactions and to initiate alignment, basal lamina formation and myelination. The authors also introduced adaptations to the classical protocol for co-culture assays, including the use of a dedicated myelination medium consisting of a neuronal medium (N2) supplemented with Matrigel® and ascorbic acid (Fig. 1). This simple modification yielded cultures with enhanced neuronal health and alignment of Schwann

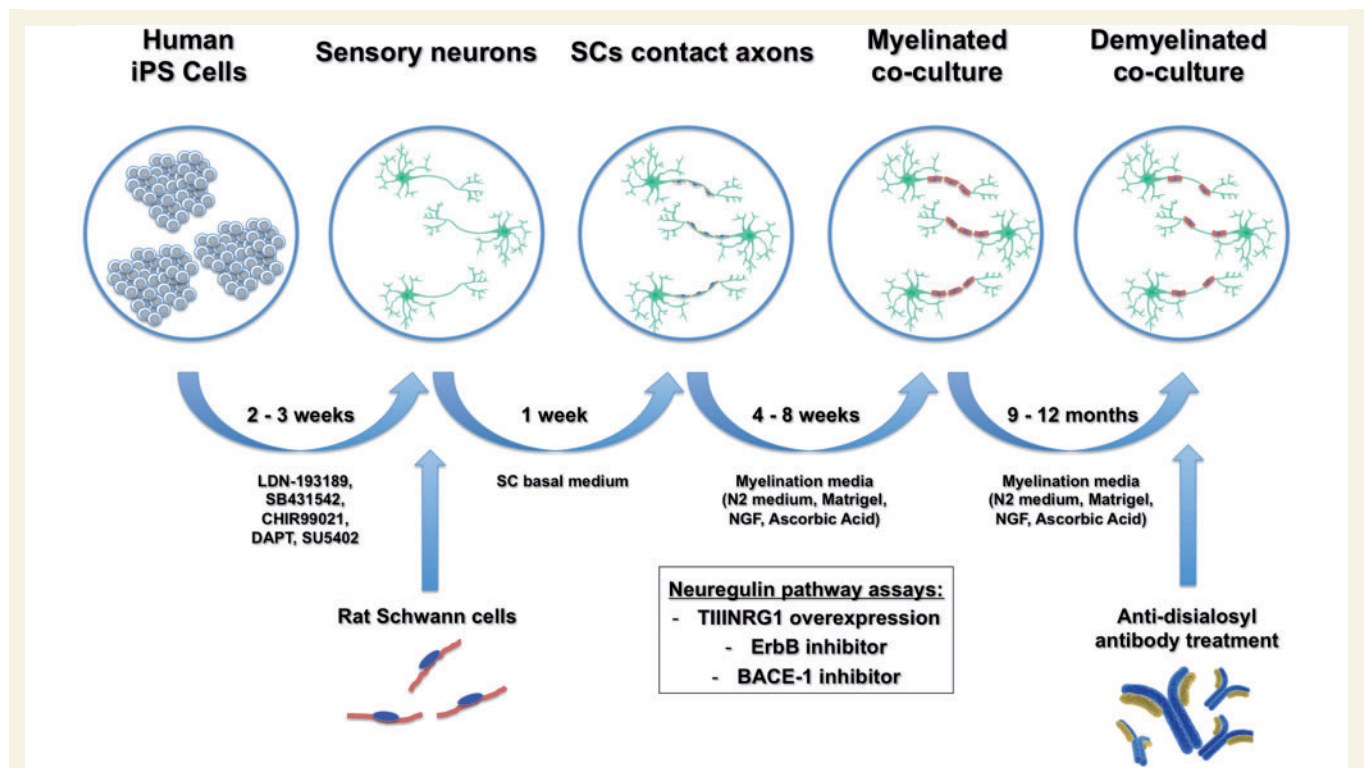


Figure 1 Schematic of experimental design. Human iPSC-derived sensory neurons were generated following a 14-day differentiation protocol based on small molecules. Rat Schwann cells were added and co-cultured with the human neurons in Schwann cell basal medium for 1 week to allow engagement with axons. Next, co-cultures were switched to a myelination medium consisting of N2 neuronal medium supplemented with Matrigel[®], NGF and ascorbic acid, which supported the co-culture for very long periods (at least 12 months). Assays to evaluate the neuregulin-ErbB signalling pathways were performed throughout the myelination period. When added to mature myelinated co-cultures, anti-disialosyl antibodies induced demyelination demonstrating the potential of this system to model acquired demyelinating neuropathies. SC = Schwann cell.

cells to axons as well as significantly increased levels of myelination. The fact that these co-cultures could be maintained for at least 12 months without any signs of axonal or myelin deterioration is also noteworthy. The authors further demonstrated that their co-culture system beautifully reproduced the molecular organization of myelinated axons in discrete compartments, including the node of Ranvier and the paranodal and juxtaparanodal regions, as previously demonstrated in rodent peripheral nerves (Salzer, 2003) as well as in human myelinated dermal nerve fibres (Li *et al.*, 2005).

Membrane-bound type III neuregulin 1 (TIIINRG1) is a key regulator of peripheral myelination through its interactions with ErbB2-ErbB3 receptors at the Schwann cell membrane (Taveggia *et al.*, 2005). Clark and

co-authors investigated the role of TIIINRG1-ErbB signalling pathways in their co-cultures using a number of approaches. First, they established that overexpression of TIIINRG1 by iPSC-derived sensory neurons strongly promoted myelination. Second, they demonstrated that pharmacological inhibition of ErbB receptors caused a dose-dependent reduction in myelination. Lastly, inhibition of beta secretase 1 (BACE-1), an enzyme involved in the proteolytic activation of neuregulin 1 in the peripheral nervous system, also inhibited myelination. Taken together, these results indicate that the TIIINRG1-ErbB signalling pathway is also an essential regulator of human peripheral myelination. Of note, these findings raise concern that BACE-1 inhibition, a current treatment strategy for Alzheimer's disease, may be

complicated by peripheral demyelination, limiting its use as a human therapeutic.

Finally, the authors used their co-culture system to study the effects of anti-disialosyl antibodies on myelinated axons. Anti-disialosyl antibodies are associated with several acquired inflammatory demyelinating neuropathies, including acute sensory neuropathy associated with anti-GD1b IgG antibodies and CANOMAD (chronic ataxic neuropathy with ophthalmoplegia and M-protein, cold agglutinins and anti-disialosyl antibodies). Using both mouse and human anti-disialosyl monoclonal antibodies, the authors demonstrated impaired myelination of human sensory axons when antibodies were added to co-cultures at the onset of myelination. Even more strikingly, human anti-disialosyl IgM antibody caused demyelination in established

Glossary

Anti-disialosyl antibodies: Anti-disialosyl antibodies react with the disialosyl epitopes shared by gangliosides, sialic-acid containing glycosphingolipids that are abundant in the nervous system. Monoclonal IgM anti-disialosyl antibodies react with gangliosides GD1b, GQ1b, GT1c and GD3 and are typically associated with an ataxic sensory neuropathy, such as CANOMAD. IgG antibodies to disialosyl epitopes, including GD1b and GQ1b are associated with Miller-Fisher syndrome (acute ataxic neuropathy with ophthalmoplegia).

Induced pluripotent stem cells: Embryonic-like iPSCs capable of differentiating into a variety of cells in the body can be derived from somatic cells by the forced expression of defined factors, including KLF4, OCT3/4, SOX2 and C-MYC. Distinct combinations of factors, and strategies to induce their expression, have been used to generate iPSCs from a number of human tissues with varying degrees of efficiency. iPSCs can be coaxed into specific cell types by manipulating the culture environment. Growth factors, small molecules and extracellular matrix proteins can be applied in a sequential manner to emulate the normal development of the cell lineage of interest. Using this approach, investigators have been able to differentiate human pluripotent cells into lineages necessary for modelling neurological diseases, including cholinergic, glutamatergic and dopaminergic neurons, astrocytes, oligodendrocytes and Schwann cells.

Neuregulin signalling pathway: Neuregulins (NRGs) comprise a large family of EGF-like signalling molecules involved in cell-cell communication during development and disease. NRG1 plays important roles in the development of the nervous system, heart, and mammary glands and this diversity of *in vivo* functions is partially explained by the many different isoforms of NRG1. Neuregulins transmit their signals to target cells by interacting with transmembrane tyrosine kinase receptors of the ErbB family. *In vivo*, functional NRG1 receptors are heterodimers composed of ErbB2 with either an ErbB3 or ErbB4 molecule. The tissue-specific distribution of the different receptor types further contributes to the diversity and specificity of the biological functions of this signalling pathway. This pathway functions in early precursor proliferation, maturation, as well as in the myelination of Schwann cells, mainly by interactions between type III neuregulin I and ErbB2/B3 receptors.

co-cultures aged 9 to 12 months, quantified as a 10% increase in myelin loss compared to control cultures, as well as the presence of disintegrating myelin surrounding intact axons. These findings demonstrate the potential of this system to model demyelinating immune neuropathies such as Guillain-Barre syndrome and CIDP and to identify and validate new therapeutic agents for this group of acquired neuropathies.

Unfortunately, the ideal co-culture system composed of both Schwann cells and neurons derived from human iPSCs remains elusive. Such a system would be particularly useful in modelling inherited demyelinating neuropathies, where having Schwann cells and neurons with an identical genetic background to patients would be a valuable asset. Despite significant advancements in the development of Schwann cell differentiation protocols (Liu *et al.*, 2012), iPSC-derived Schwann cells have failed to demonstrate robust *in vitro* myelination when co-cultured with iPSC-derived neurons, a limitation also observed in primary human Schwann cells. This is the major roadblock that needs to be overcome to allow further development of this field.

This work by Clark and colleagues has created a wide range of

opportunities for the study of peripheral neuropathies. Their system is robust and durable and seems ideal for use in high-content platforms for drug discovery in demyelinating neuropathies and should also help expand our understanding of the basic mechanisms involved in peripheral myelination and Schwann cell-axonal interactions.

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Multi-modal imaging and photosensitive epilepsy: a link between resting brain rhythms and seizure genesis

This scientific commentary refers to ‘Photosensitive epilepsy is associated with reduced inhibition of alpha rhythm generating networks’, by Vaudano *et al.* (doi:10.1093/brain/awx009).

Photosensitive epilepsy refers to those epilepsies where some or all seizures are induced by flashing or flickering lights. It is neither a separate syndrome nor the result of a single aetiology. It is seen in a range of conditions that include focal occipital epilepsies, with or without visible focal occipital lesions on brain imaging, the idiopathic (genetic) generalized epilepsies (IGE) and the progressive myoclonic epilepsies, all of which share ‘hyperexcitability’ of the occipital cortex and visual system in common (Porciatti *et al.*, 2000; Parra *et al.*, 2003; Wilkins *et al.*, 2004). In addition to flashing or flickering lights, some patients will also experience seizures in response to high contrast striped or chequered patterns (Wilkins *et al.*, 2004). Photosensitive epilepsy is found in around 10% of patients presenting with seizures under the age of 20, and 3% across all ages. Seizure types in photosensitive epilepsy include focal onset occipital seizures, with initial visual symptoms, or generalized tonic seizures and myoclonic jerks seen in IGE. In this issue of *Brain*, Vaudano and colleagues combine EEG with functional MRI to obtain further insights into the neural basis of photosensitive epilepsy (Vaudano *et al.*, 2017).

A diagnosis of photosensitive epilepsy is supported by the presence of an abnormal photoparoxysmal response on EEG during intermittent photic stimulation. The photoparoxysmal response is classified according to the type and spatial extent of the abnormal EEG discharge. Broadly the photoparoxysmal response (spikes or spike wave) may be restricted to the occipital lobe and not associated with an increased risk of epilepsy, or show anterior spread to a generalized (bilateral) spike and spike-wave pattern, typically with a frontal predominance and in association with an increased risk of epilepsy. The latter is seen in around 40% of patients with juvenile myoclonic epilepsy. Whilst the overall prevalence of photosensitive epilepsy is relatively low, the condition offers unique opportunities for the study of seizure-generating networks using neurophysiological and functional imaging techniques.

Most studies of photosensitive epilepsy to date have focused on measuring cortical responses to visual stimuli. Patients with photosensitive epilepsy show an impaired cortical contrast gain control for pattern stimuli of high luminance contrast (Porciatti *et al.*, 2000), an increase in gamma band phase synchronization during intermittent photic stimulation (Parra *et al.*, 2003), and an increase in amplitude of the induced occipital gamma band response to high contrast visual gratings compared to patients with non-photosensitive epilepsy and controls (Perry

et al., 2014). However, studies of resting brain rhythms in the absence of external stimuli could also shed light on neural mechanisms of photosensitive epilepsy.

Vaudano and colleagues report findings from simultaneous EEG and functional MRI studies in photosensitive epilepsy (Vaudano *et al.*, 2017). They conducted a retrospective review of all patients who had undergone simultaneous EEG and functional MRI in their centre over a 7-year period. They selected three groups of epilepsy cases: IGE with photosensitive epilepsy, IGE with no history of photosensitivity, and focal epilepsy, alongside a matched control group. Inclusion criteria for further analysis included a 10-min resting EEG-functional MRI recording without evidence of sleep or frequent interictal discharges.

First they studied the haemodynamic correlates of the spontaneous EEG alpha rhythm. Fluctuations in posterior alpha power during the EEG-functional MRI scan session were extracted using an independent component analysis of the EEG signal. They selected the component with peak alpha amplitude and a medial-posterior topography of the mixing weights. The alpha frequency and its respective power fluctuation over time were calculated for that component, convolved with a haemodynamic response function and used as a regressor of the functional MRI signal analysis. A mass univariate