The Royal Society of Edinburgh

Joint event with the French Embassy, London, Science and Technology Department

Franco–Scottish Seminar

Towards Repair in Multiple Sclerosis

Tuesday 28 October 2014

Report by Jennifer Trueland

The seminar was one of a programme of science events designed to explore areas where Scotland and France are particularly involved. They are intended to stimulate Franco–Scottish collaboration, present new ideas and increase awareness of activity in each country.

The event was limited to a relatively small number of participants to encourage participation and discussion. It was split into two sessions, morning and afternoon, including presentations from academic researchers and clinicians from France and Scotland, and opportunities for questions and discussion.

In the evening, there was a public lecture called *Multiple Sclerosis: has research got us to the end of the beginning or the beginning of the end*?, delivered by Professor Charles ffrench Constant, Director of the MRC Centre for Regenerative Medicine at the University of Edinburgh, and Professor Catherine Lubetzki, Professor of Neurology at the Université Pierre et Marie Curie, and Head of the Neurology Department at the Salpêtrière Hospital in Paris.

A separate report of this lecture is available on the RSE website at: http://www.royalsoced.org.uk/1154_October2014.html

Morning Session

The event was introduced by Professor Peter Brophy FRSE, Director of the Centre for Neuroregeneration at the University of Edinburgh, who chaired the morning session, and Dr Claire Mouchot, Senior Science Officer in the Science and Technology Department of the French Embassy.

Dr Mouchot spoke about the work of her department, which includes encouraging collaboration between UK and French scientists and bringing French scientists to give talks in the UK.

France and Scotland have “a special relationship”, she said, partly because of the “Auld Alliance”, but also because scientists from the two countries work together and have respect for each other.

She spoke about the reasons behind choosing multiple sclerosis as a seminar topic. Although prevalence is higher in the UK than in France (and is even higher in Scotland), the disease is a problem for all nations. “There’s a lot of work still to do,” she said.

Speakers

Dr Benedetta Bodini MD, PhD, ECTRIMS
Post-doctoral Research Fellow at ICM, Université Pierre et Marie Curie, Paris
Unveiling myelin damage and repair in multiple sclerosis with [11C]PIB-PET

Demyelination and remyelination are the central concerns of this seminar, but we have very little data on how this works in humans. Quantitative imaging is crucially needed in MS, said Dr Bodini, so that we can measure the effect of emerging treatments.

There are limitations with current methods using MRI scanning, including myelin water fracture (MWF), which has been used as an indirect measure of myelin content, and magentisation transfer ratios (MTR). But there are limitations with these indirect measures because there is limited reproducibility (with MWF) and suboptimal specificity with MTR, said Dr Bodini.

Moving forward, there is potential in molecular imaging, she said, in particular PET imaging using a high-resolution research tomograph (HRRT) camera. There are a number of myelin-specific PET compounds which have been successfully used in animal models. For example, PET imaging with the myelin marker BMB has identified white matter myelin in baboons, whilst others include BDB, DBT and MEDAS.

Studies are showing that it is possible to measure what is happening with myelin in animal models, she says, but, in humans, quantification is a big issue, as it's hard to get comparable information. Arterial sampling is too invasive, she said, so the aim is to find a non-invasive way of defining remyelination.

Dr Bodini described an unpublished study based on the hypothesis that [C-11] PIB (a thioflavin derivative) is a myelin marker (a hypothesis since confirmed by an independent study) and could be used successfully to measure myelin in humans.

Researchers set up the SHADOWTR study, which aimed to explore the extent, localisation and clinical impact of myelin loss and repair in MS through a combination of [C-11] PIB PET on HRRT and advanced MRI. The study included 20 patients with active relapsing–remitting MS and ten healthy volunteers, and involved using MRI and [C-11] PIB PET scanning on HRRT, over a period of three to four months. The study looked at four particular areas: data acquisition; segmentation and lesion detection; reference region extraction; and quantification and statistical analysis.

The researchers used the findings to develop a global index of myelin change. They wanted to find out whether demyelination or remyelination contributes more to disease progression. They found that once demyelination has taken place, and potentially permanent damage has occurred, then it is the remyelination potential of the individual that determines how well they recover from the damage. The amount of remyelination correlates strongly with clinical scores/outcomes, such as the MS severity scale and expanded disability status scale.

Dr Bodini concluded that PET with [C-11] PIB makes it possible to quantify myelin *in vivo*, reflecting the histo-pathological myelin gradient from lesional to normal-appearing brain tissue. The remyelination index derived from the PET images has the potential to predict disease severity and could be of interest as an outcome measure in clinical trials testing therapies to promote remylination.

In question time afterwards, Dr Bodini was asked if it is not counter-intuitive to look at remyelination rather than demyelination. She responded that the worst outcome is where damage has occurred and remyelination does not 'kick in'. She said the researchers were surprised themselves at the strong correlation.

Asked if this has been tried with patients with progressive MS, Dr Bodini said that this would be interesting but has not been done so far. This study was a pilot and they can now start thinking about where to go next.

Dr Don Mahad
Senior Clinical Research Fellow and Honorary Consultant Neurologist,
University of Edinburgh
Axonal mitochondria and remyelination

Dr Mahad is a researcher and clinician, who spends around 20% of his working life looking after patients with progressive MS, for whom little can be done apart from symptomatic relief. He talked about observational studies carried out in post-mortem tissue over the last few years in Edinburgh, leading to a hypothesis on axonal degeneration in progressive MS.

He is interested in long nerve fibres (axons) that carry electrical impulses away from a cell body. These axons are so long that if a cell body was in Edinburgh, and an axon was as wide as a Eurostar train, it would stretch “way beyond Paris” to transport this one cell. If you have an energy failure in the axon, it can no longer do its job.

The question is what drives the energy failure in MS. One of the problems can be the loss of myelin – and remyelination should help – but are there other things that drive the energy failure?

Dr Mahad spoke a little about mitochrondria – often called the powerhouse of a cell – which operates in networks, and is very dynamic. If you look at the ‘business end’ or mitochondria in terms of energy production, you can see that it’s not as protected as it could be, so it’s not surprising that it develops mutations.

He and his team looked at enzyme activity in the mitochrondria and found that the mitochondria changes following loss of myelin. The volume considerably increases within the axons and they become longer. Four different groups of researchers have found the same changes.

He spoke of a study (currently in press) where they prevented mitochondrial fission. Before axon degeneration, the mitochondria fragments into small parts. If you prevent mitochondria fragmentation, you protect against axon degeneration in *in vitro* studies. This gives some evidence that manipulating the mitochondrial parameters in the neuronal component could be a therapeutic aspect of preventing axonal degeneration.

He believes that it’s convincing that mitochondrial axon response is a good thing and, therapeutically, we should enhance and protect it. The question is what disrupts this response in MS. It could be that toxins from inflammation cause the problem in relapsing and remitting MS. In progressive MS, there seems to be an additional mechanism. The inflammatory mechanism would still be carrying on, but the additional mechanism is driven by the grey matter, the cell body.

Signs of inherited mitochrondrial mutations in the brains of people with MS are quite common, although not every patient has them. Mitochondrial injury appears to stop cells using oxygen properly. There is also speculation that axonal length plays a role. Essentially, over time, the inflammatory process adds to the damage of the mutations and leads to progressive disease. The next question is what happens when you remyelinate, and they found that you still require more mitochondria to maintain it.

Putting it all together in the axon and neuronal cell body, particularly in the long projection axons, there seem to be two divergent things going on. One is the demyelination of the axon, requiring more energy from the mitochondria. At the same

time, the cell body is no longer able to supply the axon with healthy mitochondria. It is this combination of lack of supply and increasing demand that makes progressive MS “pretty unique”, he said.

Questions

Asked why it is that if the mitochondria is inherited from the mother by males and females, males are far less likely to develop MS, Dr Mahad said he did not think that the mutations “drove” MS, but they might drive the response and possibly susceptibility.

Asked about Uhthoff’s phenomenon (where a change in core temperature can worsen symptoms in people with MS), Dr Mahad said that reaction to exercise can be a predictor or a symptom of progression in MS. He cited a marathon runner who used to be able to run 16 miles before beginning to drag his feet, but as the distance he could manage grew shorter, his condition worsened. It’s possibly that we can pick up progressive MS patients early, in the same way as you can pick up angina by putting chest patients on a treadmill.

Dr Anne Baron-Van Evercooren
Director of Research, PHD, Inserm
Stem Cells and Myelin Repair

Dr Baron-Van Evercooren said she has been interested for some time in the biology of stem cells in endogenous and exogenous myelin repair. Stem cells have a great plasticity and can be harvested from many different sources. They can also be grown into useful cells, including oligodendrocytes, crucial in MS.

Dr Baron-Van Evercooren posed two questions: are mouse-induced pluripotent stem cells-neuro progenitor cells (miPS-NPC) functionally similar to forebrain E-NPCs, and what is the fate of hufNPC (human foetal NCP) in inflammatory conditions.

Although it is possible to get pluripotent stem cells from a patient’s own blastocyst, there is still a lot of work to do to improve stability, safety and functionality of these cells. Using mouse models, the researchers looked at how these cells would react if transplanted into demyelinated Shiverer mouse spinal cord. They found that both cells act similarly *in vitro*, with both equally proliferating.

In vivo, they found that the cells differentiate into three CNS (central nervous system) types. Both types of cell act similarly, except that there seems to be more astrocytes from the brain-derived cells. The main interest is in differentiation to oligodendrocytes – no real difference was noted.

The other question was whether the cells would migrate in the same way – crucial for therapeutic purposes. Again, there is no real difference. The migration is very efficient. So would they have the ability to myelinate the spinal cord? Yes they have. The transplanted cells make a great deal of myelin, as do the endogenous cells. They also found there is high overlap of endogenous and exogenous myelin, indicating that they are competing strongly against each other.

They also wanted to know whether the structure of the myelin is preserved and whether it is functional. They used electronic microscopy to confirm this. They found that very immature NPCs have the ability to out-compete endogenous cells in forming new myelin.

So what is the fate of human foetal NPC in inflammatory conditions? They found (working with mice) that these cells are an efficient tool for carrying remyelination in the adult central nervous system, and that they improve the clinical outcome for mice, including longevity and motor ability. Remyelination is, however, slow.

She concluded that miPS-NPC and mE-NPC show functional homogeneity; that hNPC act through immunomodulation and myelination; and that migration is an intrinsic property of the immature NPC, irrespective of the environment and their species. Myelination is also an intrinsic property of immature NPC, but is dependent on species, with the human ones being much slower.

Dr Anna Williams

Senior Research Fellow and Honorary Consultant Neurologist,
University of Edinburgh
Remyelination in MS – how to do it better?

Like Dr Mahad, Dr Williams spends 20% of her time in the clinic, but the rest of the time, she is looking at myelination. Her talk covered several points: why we want to improve remyelination; some of the roadblocks; and some of the molecules involved and how we can find more of them. Importantly, she wants to look at how we could move from moving these molecules from being targets for therapies and into drugs for patients and how they could be delivered.

Remyelination is necessary because people with MS start accruing disability as part of the progressive stage of the disease. There are drugs available for the early, inflammatory stage of MS, but they have no effect on progression.

What we don't have is anything to change neurodegeneration, so it is necessary to find neuroprotective therapies. There are different ways you can do this – including trying to promote axon survival. The alternative is remyelination. This is partly carried out by oligodendrocytes to put the myelin sheath back, in the hopes that it will restore support to the axon and allow it to survive. We know this happens in humans' brains, but that it is inefficient. She and her colleagues have been trying to improve the efficiency by trying to understand the process better.

To allow remyelination, you need an oligodendrocyte precursor cell that needs to be recruited to the lesion. That means it has to know that there is a demyelinated lesion and that it has to be activated, and has to mature into a myelinating oligodendrocyte which has to recognise an axon and wrap a myelin sheath around it. There are potential roadblocks at all of these stages. At the moment, they are at the stage where there are some factors positive and negative for recruitment, and some for maturation.

The only target so far that has reached clinical trial is LINGO-1. There are anti-LINGO-1 antibodies currently in phase 2 clinical trials. She and her colleagues are trying to find more targets and are particularly interested in the recruitment site – what helps an OPC know that it has to act? They've been looking at what is released from injury sites in the brain to attract OPCs.

They have taken a bioinformatic approach, taking a new look at old data and have found around 150 secreted factors that might make targets. Some – chemokines – we know are involved in OPC migration, so that at least gives 'comfort' that they are on the right lines.

They have also used the data more cleverly to look for correlations and found that there are two waves: one at 24 hours post-lesion and one at a week. Different factors fall into the different patterns. They also looked at what sort of expression pattern they would want to bring OPCs into the lesion, then they used this to interrogate the data further to pick out more factors. They then used one chemokine which they know acts in this way (PDGF-alpha) to validate the approach and find others which work in a similar way.

The next step is testing the factors, but it's hard to get decent assays for OPCs. They have been using systems to find out how well the cells migrate through a membrane.

They have found the xCELLigence system a useful way of doing this because there's no imaging or counting involved, and it is a reasonably high throughput, to measure impedance.

Once you find the targets, you need to be able to deliver them to people. This means getting through the blood–brain barrier, because any drugs for progressive MS will have to get into the brain. They also want it to affect the right cells – OPCs in this case – and not other cells.

They have been looking at different therapeutic strategies, including very tiny nanoparticles. The clever thing about nanoparticles is that you can load them with things that, for example, increase myelination. They loaded theirs with LIF because they know it does this and that it encourages OPC maturation, but they wanted to deliver it a bit more cleverly.

It seems that the nanoparticles release their LIF effectively in the first two days. They looked at nanoparticles with different combinations of LIF and antibodies: empty; targeted; and non-targeted. Do they stick to OPCs? Yes, *in vitro*, the targeted ones do. Is the intercellular pathway activated? Yes, if full of LIF. And yes, the targeted LIF one increases OPC maturation. They decided to try it in mice and waited eight days to see if they could increase maturation after injecting it into the lesion. They found that the LIF-targeted particles led to thicker myelin.

This is a proof of principle study that shows that it's possible to promote myelination *in vitro* and *in vivo* by injecting it into the lesion, so it is clearly a promising delivery system. The next stage will be to try to deliver it in a better way, either intravenously or intra-nasally, which might be an interesting way of bypassing the blood–brain barrier. There are, however, still lots of unanswered questions, including whether it would work in humans, when to do it and for how long, and who needs which sorts of therapy.

Questions

Asked whether it is better to use TNTF than LIF, she said they had taken a pragmatic approach because they had the LIF particles and they knew they worked. In any case, they were more interested in the delivery system.

Asked how long it took to bind the particles to the cell, she said it is difficult to find them *in vivo*, and they were looking at using new forms of microscopy to do this.

Asked whether it would work *in vivo*, would you be able to target the lesion or would it spread more generally, Dr Williams said she is excited about trying the nasal route to avoid the blood–brain barrier. "We don't know if they will find the lesion but it's worth trying", she said.

Asked whether more OPCs are necessarily a good thing, she said we don't know, but that we need to get to the right number.

Afternoon Session

Chaired by Dr Bernard (Boris) Zalc
Director of Research Emeritus (Inserm)

Dr Dave Lyons
Wellcome Trust Senior Research Fellow, University of Edinburgh
Using zebrafish to study myelinated axons in vivo

As a developmental biologist, Dr Lyons is interested in how the brain is built in the first place. In particular, he wants to know more about oligodendrocytes and how they find the right cells to myelinate, and what are the signals and cellular behaviours that make this happen.

They use zebrafish as breeding machines for the embryos they study, for imaging, genetics and chemical biology. The zebrafish is useful for imaging because it goes from single cell to little animal in three days, when still only 3.5 millimetres long. They are remarkably transparent, which makes them “fantastic” for imaging. So in just a few days you can image entire biological events, essentially in real time.

Zebrafish are used for reverse genetics to help target genes of interest. They’re good for chemical biology because they produce lots of eggs. Using an imaging tool, it’s possible to see in great detail the spinal cord in live animals, including the myelin sheath.

He spoke first about how imaging is helping us to understand how individual oligodendrocytes generate their myelin sheaths in the animal. We myelinate throughout life, he said. We (humans) start myelinating around birth. We lose myelin as we age. But how does any one oligodendrocyte contribute to that? Why do some myelinate and some don’t?

They looked at images of the fish to see oligodendrocytes’ myelinating behaviour. He showed images of axons myelinating, which he said happens very quickly. A single oligodendrocyte, once it has started to initiate any form of myelination at all, has a matter of a few hours in which to make its final number of myelin sheaths. Then the exploratory mechanisms are retracted – essentially this is what happens throughout life. We have never seen a mature oligodendrocyte make a new myelin sheath – not to say it’s impossible, but we don’t tend to see it, he said.

So why are some axons myelinated and others not? One candidate signal is neuronal activity. There’s evidence that synapses exist between axons and the processes of oligodendrocytes. In an experiment, they found that disruption in the synaptic activity results in fewer myelinated axons. It doesn’t change the number of oligodendrocytes, but does reduce number of myelin sheaths. Therefore, it appears that neuronal activity has an impact on myelin sheath production.

Then he turned to genetics. The field of zebrafish use has exploded for forward genetics – from phenotype to genotype. In the lab, the researchers look for defects in myelination, then look for genetic defects that might be implicated. In the past, the group has found genes involved in myelination.

In spite of the work done so far, however, we still know of no single ligand receptor in the central nervous system that’s required for myelination. There is, however, presumably a gene that stops myelination – that says “don’t myelinate me,” he said. The group is still screening to find out which factors are implicated in whether axons myelinate at any one time. Can we identify positive regulators of myelination, or is it all to do with inhibition?

He ended by talking about the use of fish as a chemical screening tool. They have been using a model that lets them count individual oligodendrocytes.

He cited work by Professor ffrench-Constant and others, published in *Nature Neuroscience*, that showed that retinoid X receptor gamma signalling accelerates remyelination of the central nervous system. That's the good news, but the bad news is that from a drug point of view, this would have too many side-effects, because it binds to too many receptors. The question is, can we find specific agonists that bond only to the relevant receptor and circumvent the side-effects?

They accessed a list of around 450 compounds (from a lab in Cambridge) that ought to bind to this receptor, and performed an assay using zebrafish (by counting the oligodendrocytes manually). They found around 20 potential compounds, nine of which robustly increase myelinating oligodendrocytes. At the moment, the group is trying to confirm that they work through the targets they are supposed to.

Asked about myelination of the optic nerve (which doesn't have synapses), Dr Lyons said the optic nerve might be an 'outlier' and doesn't work in the same as the rest of the CNS.

Dr Anne Desmazieres, PhD, CR2 Inserm
Institut du Cerveau et de la Moele Epinière, Paris
Formation of nascent nodes of Ranvier in the central nervous system

Nodes of Ranvier are gaps in the myelin sheath, and multiple sclerosis is a demyelinating disease associated with alteration of the nodes of Ranvier. Many labs have been working on nodes of Ranvier in CNS pathology, but there is still a long way to go to get a full understanding. Dr Desmazieres described some recent work to show the involvement of the nodes in MS, and the impact on functional problems.

Although repair (to myelin) is possible, it is only partial, and with neurodegeneration come permanent deficits in patients. The CNS 'story' is not clear yet, she said. But she described how colleagues were looking at the molecular organisation of the nodal and prenodal domains in order to get a better understanding of what's going on, including what the mechanisms of development and repair are.

In particular, they have been asking what the chronology is of the node of Ranvier formation with respect to myelin deposition in the CNS; which intrinsic or extrinsic cues are important for nodal clustering; and what the functional impact is of nodal clusters in the absence of myelination. We know that prenodal clustering occurs before myelination in neurons *in vitro*, and we know that it depends on some cues and factors. They found that the clustering doesn't happen on all neurons, so the question is what makes the difference.

So what are the cues? They found that in purified neurons there are hardly any axons with cultures, but if you add oligodendrocytes you can restore an important number of clusters. This is also observed in animals. *In vitro*, they found that prenodal clustering requires the presence of AnkG, but not Nfasc. They found that conduction velocity is increased in neurons with prenodes, and prenodal clustering is also observed in the hippocampus *in vivo*.

In summary, what they saw in this project using the *in vitro* culture was that some neurons reform their nodes at the same time as myelin is deposited, whilst some other neurons are able to produce prenodal clustering before myelination occurs. This depends in part on extrinsic cues, but we don't know yet exactly what they are, but we do know that AngK is required for prenodal clustering.

She went on to present some very early data on how nodal markers are transported and targeted to their nodes. The researchers sought to find out whether transport characteristics change with time/myelination, whether nodal/prenodal markers are co-transported, and whether a nodal organiser is required. They found that, with time, there can be slowing of movement around the neurons. They looked 'very

hard', she said, but could find no co-transportation, and found that transportation depends on the marker.

She finished by talking about mechanisms of nodal assembly during remyelination in the CNS. They found that prenodal structures are found during myelination and remyelination – and are not a 'rare event'.

In future, they want to see what is involved in the very early stages of myelination. Asked whether prenodes are required for myelination, she said it could be down to timing; sometimes myelination comes first, and sometimes after.

Professor Sue Barnett
Professor of Cellular Neuroscience (Immunology), University of Glasgow
A focus on astrocytes in multiple sclerosis

Professor Barnett spoke about the role of astrocytes (star-shaped glial cells in the brain and central nervous system). MS causes multifocal areas of demyelination, axonal damage, and astroglial scarring around plaques.

Astrocytosis, an abnormal increase in the number of astrocytes, is a pathological hallmark of CNS damage, but appears to have both a beneficial and harmful effect. In MS, astrocytes become reactive and hypertrophic, and upregulate GFAP (glial fibrillary acidic protein), nestin and vimentin and CSPG expression. They form a gliotic scar, which prevents oligodendrocyte precursor cells from migrating into the plaque and, importantly, stops immune cells getting into the normal CNS.

More positively, they may play a role in removing myelin debris, may modulate immune function, and have been shown both to inhibit and promote myelination. Astrocytes can, she stressed, have an important role in MS. It's important to look at all cells in context, and how they interact with other cells, and it's known that astrocytes can have an impact, for example, in mediating the blood brain barrier. Their role in MS, including interaction with other neural cells, was proposed (by Muller) as early as 1904.

There's a range of phenotypes of astrocytes, ranging from strongly reactive, through to reactive, to non-reactive. The strongly reactive astrocyte is at the centre of the lesion, forms the glial scar, and has adverse effects on cell survival. It's associated with inflammatory cytokine production, that inhibits neurite growth and kills oligodendrocytes. Less active astrocytes might be further from the lesion, and they promote neurite survival, secrete factors to aid remyelination, and elevate antioxidants. Non-reactive astrocytes aren't really quiescent, she said, because they maintain CNS homeostasis and the blood–brain barrier.

Professor Barnett split her talk into two main sections, talking about regulation of astrocytosis, and how astrocytes can modulate myelination.

The characteristics of astrocytosis/stress response can be studied *in vitro*, she said. She and colleagues have been looking for many years to see if OECs or Schwann cells (SCs) would be better for repair. She described a confrontation assay as a measure of astrocytosis, seeing whether OECs and SCs would mingle with astrocytes or form a boundary. This discovered that OECs (but not SCs) do not form a boundary with CNS astrocytes, and do not induce characteristics of astrocytic stress/scarring. SCs, but not OECs, secrete a factor (SCM) that induces boundary formation in OEC/As confrontation assays and increases astrocyte area and GFAP expression. SCM and OEC contact is necessary for the induction of these characteristics of astrocytosis and the factor also inhibits CNS myelination.

In summary, OECs show more promise in myelination as they promote it better and produce less of a scarring response.

They also found that inhibition of FGFR1 receptor activity increases the number of SCs interdigitating with astrocytes and reduces astrocyte hypertrophy. After testing with various growth factors, they decided to see if an antibody to FGF9 overcame boundary formation with SCs/As – this allowed the cells to mingle and break the boundary.

This suggests that this action is mediated by FGF9. Interestingly, she said, FGF can't bind to the receptor unless it is stabilised by the use of heparia sulphate proteoglycan (HSPG). Binding of FGF or heparan sulphate chains induces a conformational change that enables FGF to bind its receptor and HSPG appears to play a role in boundary formation. SCs secrete more highly sulphated moieties than OECs and the level of sulphation on HS affects boundary formation.

In summary, looking at the factors that induce characteristics of astrocytosis, FGF9 may be mediating the response, in part; highly sulphated heparan sulphates promote astrocytosis by the regulation of FGF9 (and preliminary data suggests that it can inhibit myelination); and low sulphated heparan sulphates can block astrocytosis. Targeting levels of sulphation around astroglyosis may be a novel approach for repair in MS, she said.

She then moved on to discuss regulation of astrocytosis/stress response, describing how the astrocyte can modulate myelination, and how myelination is reduced on quiescent astrocytes.

She said that astrocytes can affect myelination in a positive and negative manner; that CNTF, a known activator of astrocytes, promotes myelination; that 'quiescent' astrocytes inhibit myelination; and that a microarray gene expression profile identified CXCL10 as a factor secreted by astrocytes (plated on TnC) which can negatively affect myelination.

She concluded that the astrocyte can not only be a physical barrier to CNS repair, but is also an important regulator of myelin.

Dr Bernard Zalc
Director of Research Emeritus (Inserm)
Live imaging of demyelination and remyelination in a Xenopus *model of inducible targeted oligodendrocyte ablation*

Dr Zalc described a project to produce a genetically inducible model of demyelination in *Xenopus laevis* to create an *in vivo* medium-throughput screen of drugs favouring remyelnation. They wanted to measure the effect of a battery of molecules on remyelination in the model. The rationale is that tadpoles are transparent, the myelin composition is very similar to mammals, it's efficient (there are 3,000 eggs per laying) and the cost of the animal husbandry is low.

He showed the structure of a transgene which combines various elements, to create a pMBP-eGFP-NTR model. In this, the transgene is specifically and reliably expressed by myelinating oligodendrocytes.

He described how the loss and repair of myelin can be demonstrated using the antibiotic metronidazole, which shows spontaneous remyelination after treatment, and how it could be used to show the myelination potential of different molecules, which might be potential targets for MS.

They also looked at whether it's possible to develop a behavioural test in *Xenopus* tadpoles. If they are demyelinated, they should be blind, so should not see; so this should be a good way to see myelin status, he said. They used a cathodic (non-flat) screen, because tadpoles can't see things on flat screens, but this work is ongoing.

In conclusion, he said the *Xenopus* line MBP-GFP-NTR is a simple model for *in vivo* screening of drugs favouring myelin repair. They found that in this transgenic line, demyelination is inducible by treatment with metronidazole; that spontaneous remyelination occurs after demyelination; and that remyelination can be accelerated by pharmacologic treatment. The idea is to use the *in vitro* methods for high throughput, then test more promising molecules in tadpoles.

Concluding remarks and Vote of Thanks

Professor Neva Haites, Vice-Principal for Development at the University of Aberdeen and RSE Vice-President (Life Sciences), thanked the speakers and audience for their participation.