

Symposium

~ Neurodevelopment and related disorders ~

Reykjavik Iceland on May 16th, 2013
European Month of the Brain

Report by **Athéna R. Ypsilanti**

This Symposium is part of Work Package 2
Development of a Strategic Research Agenda of the NEURON II project,
Work Package Leaders are Inserm and CNRS.

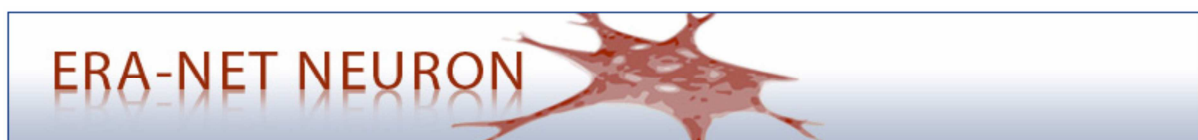


Table of Contents

Welcome	3
Dr. Katrin Valgeirsdottir and Dr. Marlies Dorlöchter	
Introduction	3
Dr. Etienne Hirsch	
1 st talk: General overview of nervous system development	3
Dr. Amparo Acker-Palmer	
2 nd talk: Myelin and glial cells in nervous system development.....	6
Dr. Klaus Armin Nave	
3 rd talk: Focus on Autism.....	8
Dr. Wendy Roberts	
4 th talk: Developmental disorders of sensory systems: the example of deafness	10
Dr. Karen Avraham	
5 th talk – Understanding brain development to treat brain disorders.....	12
Dr. Yehezkel Ben-Ari	
Welcome by the European Commission	15
Dr. Sigrid Weiland	
6 th talk - The bright side of the brain: the role of white matter in brain function and dysfunction.....	15
Dr. Ragnhildur Thora Karadottir	
Annex I.....	18
List of participants	

Welcome

Dr. Katrin Valgeirsdottir and Dr. Marlies Dorlöchter

The symposium on “Neurodevelopment and related disorders” was launched with a few welcoming words by Dr. Katrin Valgeirsdottir, the senior advisor in the International Division of the Icelandic Centre for Research (RANNIS).

Dr. Marlies Dorlöchter (PT-DLR, Germany) then welcomed participants on behalf of ERA-Net NEURON during the European “Month of the Brain” which is designed to raise awareness for neuroscience research and brain diseases. The various founding members, roles, partners and activities of ERA-Net NEURON were then described. An online version of Dr. Dorlöchter’s talk can be found online here:

http://www.neuron-eranet.eu/media/Bruessel_2013_Vortrag2.pdf.

Introduction

Dr. Etienne Hirsch

Dr. Hirsch presented the objectives of this symposium on research in the fields of basic and clinical developmental neurosciences. These were two-fold:

1. To discuss science and in particular developmental neuroscience
2. To inform the funding agencies of the important topics in neurodevelopment and related disorders and to identify the topics that still need to be addressed by scientists in Europe.

1st talk: General overview of nervous system development

Dr. Amparo Acker-Palmer

Goethe University, Frankfurt, Germany

Neurons are specialized cells of the human body that exhibit a great diversity in their structure and function. Nonetheless, most neurons typically share characteristic features such as the presence of dendritic trees through which they receive input signals. Neurons also possess long axons with sophisticated terminal branches, known as synapses, which enable a given neuron to communicate with other neurons or target tissues. One of the central challenges in the field of neuronal development is trying to understand how the incredible specificity and complexity of neuronal networks is generated. This can be achieved by, for instance, investigating the growth of axons, the formation of dendrites or the development of synapses. In this talk, Dr. Acker-Palmer focused on three subparts that are relevant for understanding brain formation and related diseases either at the cellular or molecular level. These topics are: 1) synapses which are the structures relevant for neuronal communication; 2) neuronal migration which is important for the formation of specific brain structures such as the cortex; 3) the neurovascular link which delves into the similarities in the development of the nervous and vascular systems.

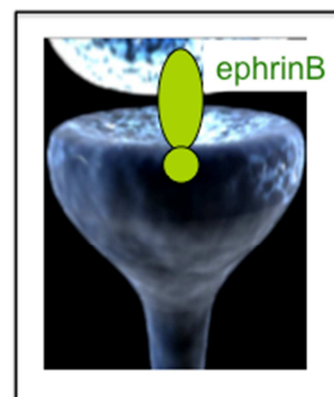
1) Synapses

Historically, synapses were first named by Dr. Ramon y Cajal as “espinas” or spines. Their morphology differs depending on the neuronal subtypes and organisms in which they are found. However, their role as input receivers for neuronal information is conserved across the board. What are the molecular players important for the generation of these points of communication between the pre- and the post-synaptic sides of two neurons? Investigating this question is crucial because, when these molecules are impaired,

the ensuing defect in spine maturation is the basis for a number of neurodevelopmental (e.g., Down's Syndrome) or neurodegenerative disorders (e.g., Alzheimer's disease).

Many molecular pathways are important for the development of a mature spine including EphB receptors and their ephrin A and B ligands. In this pathway, signalling is only initiated if EphB receptors and their ligands are clustered at the membrane. Moreover, this signalling is bi-directional since it also occurs downstream of the ligands. In fact, EphrinB is a membrane-bound protein with intracellular signalling domains such as tyrosine residues and a PDZ target site which can bind to other PDZ-containing proteins. Dr. Amparo's group discovered that ephrinB ligands are important in promoting spine maturation by activating downstream proteins such as rac. This signalling cascade then elicits the remodelling of the spine cytoskeleton thereby converting immature filopodia into putative synaptic contacts. Impairments in this pathway leads to an immature spine phenotype reminiscent of that observed in developmental diseases such as Down's syndrome.

Both in development and in adulthood, synaptic plasticity is crucial for numerous physiological functions such as memory storage. Synaptic plasticity is particularly important at a structural level to maintain a stable synaptic contact between mature spines. It involves recycling or "trafficking" of receptors which modulates communication between the pre- and post-synaptic neurons. At the post-synaptic membrane, there are a large number of molecular players such as ion channels like AMPA and NMDA receptors which transduce excitatory signals. There are also many PDZ-containing molecules which maintain receptors at the surface and also serve as a bridge between the membrane and the cytoskeleton. There are two forms of plasticity that are important for memory storage: long-term depression (or LTD) and long-term potentiation (or LTP). LTP is characterised by an accumulation of AMPA receptors at the synapse whereas, in LTD, AMPA receptors are rapidly removed from the membrane leading to the silencing of the synapse. It was shown that ephrins are important for the modulation of the synaptic strength by controlling AMPA receptor trafficking. Indeed, ephrinB binds to a PDZ-containing protein known as glutamate-receptor interacting protein (GRIP) that also binds to AMPA receptors. EphrinB signalling thus stabilises AMPA receptors at the surface. In the absence of ephrinB2, AMPA receptors are internalized causing LTP and LTD defects in knockout animals.

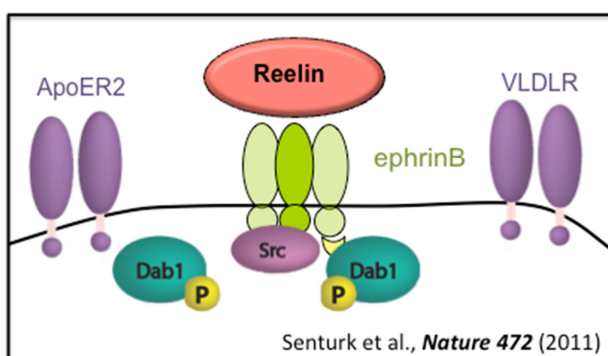


EphrinB regulates spine maturation and synaptic plasticity

2) Neuronal migration

There are many laminated structures in the brain such as the cortex, the cerebellum and the hippocampus. In the cortex, progenitor cells located in the proliferative zone migrate radially in order to colonise the different cortical layers. Therefore the cortex develops in an inside-out fashion whereby new neurons migrate through older ones to seed more superficial layers of the region.

In the cortex, neuronal migration is regulated by Reelin, an extracellular glycoprotein secreted by Cajal-



Reelin regulates cortical neuron migration and EphrinB is a regulator of Reelin signaling

Retzius cells located in the marginal (most superficial) zone of the cortex. Reelin signals to migrating neurons by binding to two receptors: VLDLR and APOER2. Reelin signalling leads to the activation of downstream components such as Dab1 and any interference in this signalling cascade causes drastic developmental defect in cortical layering. For example, the spontaneous mouse mutant for Reelin, *reeler*, exhibits an inverted cortical layering. In humans, a mutation in Reelin is associated with lissencephaly, a lethal condition in which there are no convolutions in the cortex. In addition, absence or reduction in Reelin levels has been associated with

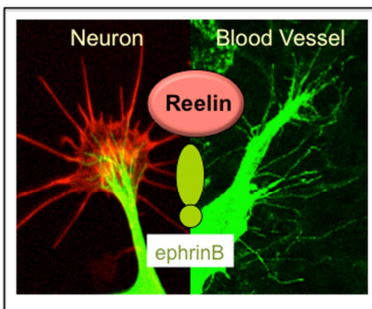
conditions such as ataxia, epilepsy and schizophrenia.

Although Reelin is such an important player in cortical layering, it must rely on a co-receptor other than VLDLR and APOER2 to exert its effects since one of the major downstream molecules in its pathway, Dab1, is phosphorylated by a kinase. However, neither VLDLR nor APOER2 is able to activate the Src kinases necessary for this phosphorylation. Dr. Amparo's group discovered that ephrinB ligands are actually major regulators of Reelin signalling. Reelin can directly bind to ephrinB which then recruits Src thus enabling it to phosphorylate Dab1. Moreover, removal of all ephrinB ligands in a mouse model leads to a cortical phenotype similar to that found in the *reeler* mouse.

3) Neurovascular link

In humans, the nervous and the vascular systems run in parallel and nerve bundles in the peripheral nervous system have been shown to secrete morphogens such as VEGF which attract emerging blood vessels. The structural components of both developing systems also bear uncanny similarities. For example, in neurons, axonal growth cones sense their environment using protruding filopodia which are also found on the endothelial tip cells of sprouting blood vessels. In nature, the same molecular pathways are often recycled to instruct the development of a large variety of systems. Since vessel and axonal guidance share many common features, an attractive hypothesis is that endothelial cells could potentially respond to the same molecular guidance cues as neurons.

Developmental angiogenesis is often studied in the mouse retina in which vascularisation occurs postnatally. At birth, astrocytes in the retina secrete VEGF which binds to VEGFR2 receptors located on endothelial cells. These receptors are then rapidly internalised which is required for signalling to occur and this signalling ultimately leads to filopodia extension and vessel sprouting. Ephrins are conserved in the endothelial system where they were shown to exert a major function by regulating the internalisation of VEGFR2 both during development and pathologic angiogenesis most notably in vascularisation of tumours. In addition, Reelin was also found to be a potent angiogenic factor. Upon stimulation with Reelin, endothelial cells, which express APOER2 and VLDLR, respond by forming tubes and migrating. *Ex vivo* experiments show that Reelin, like VEGF, induces tip cell filopodia extension in retinal blood vessels and that there is, in fact, a crosstalk between the Reelin and the VEGFR2 pathways.



Reelin and EphrinB: key regulators of both axon and vessel guidance?

In the developing cortex, is it possible for Reelin to be instructing both neuronal migration and vascularisation concomitantly? In adult *reeler* mutants, cortical vascularisation is disorganised and functionally impaired. There is also a loss in the integrity of the blood-brain-barrier (BBB) at the interface of the nervous and vascular system. Such disruptions have been implicated in a number of seizure and neuropsychiatric symptoms. Moreover, the BBB is not only important for maintaining the necessary extracellular environment of the central nervous system; it is also an important obstacle to the delivery of therapeutic agents into the brain. Dr. Amparo's group is therefore currently concentrating on studying ephrins and Reelin on the

development of the BBB and in particular on developing a strategy which would enable them to transiently open the BBB for therapeutic purposes.

A large amount of research efforts is still required to uncover the molecular and cellular mechanisms governing synapse formation, neuronal migration and the neurovascular link. This research would greatly benefit our understanding of cognitive, neurodevelopmental, neurodegenerative and neuropsychiatric disorders. A particularly exciting new field of research is the uncovering of potential instructive roles of the vascular system in the brain. This is an essential field of study as it is becoming increasingly clear that the vascular niche is important for neural stem cell development, connectivity and function and that it is also used by brain tumours during tumourigenesis.

2nd talk: Myelin and glial cells in nervous system development

Dr. Klaus Armin Nave

MPI Experimental Medicine, Göttingen, Germany

The brain is comprised of many more glial cells, or support cells, than neurons. These glia cells are uniquely different from the majority of neurons in that they are predominantly born during postnatal life with oligodendrocytes developing last. In the central nervous system (CNS), a single oligodendrocyte insulates several axons by enwrapping them in a myelin sheath. In the peripheral nervous system (PNS), the same function is performed by Schwann cells which myelinate single axonal segments. Diseases of myelin are amongst the most common and disabling disorders of neurology. Although, major defects in neuronal development are often incompatible with life, similar defects in glial development do not cause death but often lead to very serious diseases such as leukodystrophies. To gain insight into such diseases, it is primordial to first understand the normal development of glial cells. Our textbook view of oligodendrocytes and myelin is still incomplete and many basic questions remain unresolved. For instance, how is myelin wrapped around axons and what elicits myelination? In the PNS, axons release Neuregulin1 which activates a complex signalling cascade in neighbouring Schwann cells and triggers myelination. This molecular signal is not conserved in the CNS although the signalling cascade appears to be.

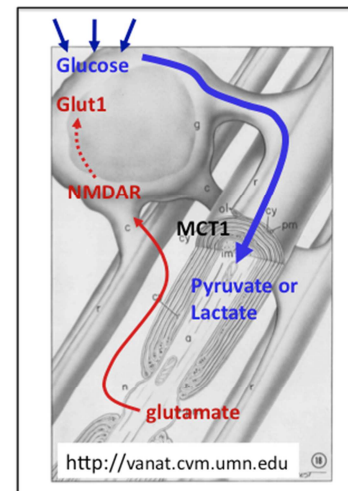
Evolutionarily, virtually all nervous systems, from insects to higher vertebrates, display a close interaction between axons and glia. Myelination is a late “invention” in vertebrate evolution and is found exclusively in higher vertebrates (from fish to mammals). As vertebrate evolution progresses, the proportion of myelin (or ‘white matter’) in the brain increases. Its main purpose is to increase the speed of electrical conduction in the nervous system thus acting to strengthen connectivity and reactivity. The human brain is composed of fifty percent of white matter; but what do oligodendrocytes and myelin actually contribute to our higher cognition? Although their contribution is likely to be important, it is still poorly understood. Neurons in both hemispheres of the brain communicate through white matter tracts which, when challenged, alter the millisecond precision required for synchronicity or spike time-dependent plasticity. This will likely lead to changes in behaviour and, in fact, white matter changes have been reported in several psychiatric diseases such as schizophrenia. However, it is still not understood whether these changes in myelination are the cause or the consequence of these diseases.

At the forefront of myelin/glial research is the study of autoimmune diseases in which demyelination ultimately leads to neurodegeneration. In multiple sclerosis (MS), it appears that autoimmune T cells enter the brain and cause myelin lesions. However, the field of MS research is still divided about whether the immune aspect of the disease is the primary cause of the pathology. What is clear, however, is that myelin repair is compromised in MS and that axonal degeneration eventually ensues. Another unclear point is whether this degeneration is due to inflammation, myelin lesions or the lack of remyelination. Alternatively, axonal degeneration could be due to the loss of metabolic support.

One of the major bottlenecks in the nervous system is axonal length. Although only 1 μm in width, certain axons are several meters long. How are these axons maintained when their business end, e.g., the cell body, is sometimes so far away from their terminal? And how do their mitochondria provide them with energy? Indeed, their metabolic requirements are exceedingly high since energy-demanding electrical and physical transport processes are constantly going back and forth along the axon. We now know that oligodendrocytes in close association with these axons play an important role in insuring their survival. This was supported by studies in mice with mutations targeted specifically to oligodendrocytes such as the *PLP1-null*, *Cnp1-null* and *Cnp::Cre; Pex5* mice. To the great surprise of researchers, some of these mutations did not affect myelination but led to axonal degeneration over time. Indeed, some mutant mice developed axonal abnormalities including axonal swelling due to the accumulation of debris following the arrest of

motor proteins. Moreover, the phenotype in these mice was quite distinct from mice with no myelin such as the *shiverer* mouse (which is an *MBP-null* mouse).

Another important question is how do axons get access to metabolites when they are wrapped in long myelin sheaths? In fact, although axons remain in close contact with the oligodendroglial cytoplasm, there is a thin “periaxonal space” between the axon and the glial cell. This periaxonal space forms a micro-environment surrounding the axon. In the PNS, connections known as Schmidt-Lanterman incisures are present between Schwann cells and the periaxonal space. These connections have gap junctions to provide greater access of Schwann cell metabolites to the axon. In the CNS, such connections do not exist but oligodendrocytes are still ideally positioned to provide metabolic support to axons. When glucose from the blood supply is taken up by oligodendrocytes, it immediately gets phosphorylated. Therefore, only the end products of glycolysis, such as pyruvate and lactate, are potentially able to exit the cell and cross into the periaxonal space. Are axons then able to use lactate to sustain their metabolic needs? In fact, in an *ex vivo* optic nerve model, it appears that the mitochondria in axons are able to metabolise lactate just as readily as glucose without any measurable loss of function. However, is there any evidence that oligodendrocytes deliver lactate to axons? In order to test this hypothesis, mitochondrial respiration in oligodendrocytes was selectively impaired by inactivating a crucial protein named *Cox10*. When *Cox10* was inactivated in hippocampal neurons by using transgenic mice (*CamKII::Cre; Cox10^{flox/flox}*), massive neurodegeneration ensued. However, when *Cox10* was selectively inactivated specifically in oligodendrocytes, using *Cnp::Cre; Cox10^{flox/flox}* mice, no differences in white matter density or axonal degeneration were observed even in older adult mice. To confirm that, in the transgenic mouse model, *Cox10* was actually functionally invalidated, lactate levels were measured and found to be elevated in the conditional transgenic animals compared to controls as expected if mitochondrial respiration had effectively been impaired. This, however, only occurred in animals that were being anaesthetised by isoflurane which is an unspecific inhibitor of mitochondrial respiration. In these conditions, lactate piled up in the brain and became detectable. When isoflurane was removed, lactate levels rapidly returned to undetectable levels suggesting that it was rapidly being metabolised. This metabolism could not take place in impaired oligodendrocytes and was therefore most likely to be happening in the axonal compartment. Finally, in order for lactate to exit oligodendrocytes and enter into the axonal compartment, lactate channels must be present. By electron microscopy, the lactate transporter MCT1 can be observed in glial cells in close apposition to the periaxonal space. Moreover, when this lactate channel is impaired in glial cells, axonal degeneration can be observed.



Model of oligodendroglial support of axonal metabolism (high energy demand)

One of the dangers in using lactate to support metabolic needs is that it is very acidic and its overproduction can lead to acidosis. With that in mind, how do oligodendrocytes know how much lactate is required to support axonal metabolism? One clue is that glial cells express glutamate receptors, known as NMDA receptors, although there are no synapses in oligodendrocytes. Are axons and oligodendrocytes therefore forming a unique type of synapse? A working model for this is based on two hypotheses. Firstly, high frequency impulses should trigger axonal glutamate release. Secondly, this glutamate should activate NMDA receptors on glial cells causing them to enhance their glucose uptake and release lactate to provide axons with their increased metabolic needs. In fact, *in vitro* experiments demonstrated that when oligodendrocytes were exposed to NMDA (a glutamate analogue), their glucose transporters were relocalised to the cell surface leading to an increase in glucose uptake. *Ex vivo*, axonal conductance is rapidly lost when the axon is deprived of oxygen and glucose. After 60 minutes of starvation, the system is permanently disabled even after glucose and oxygen reintroduction due to excitotoxicity following glutamate

release. Surprisingly, in axons of mutant mice lacking the oligodendrocyte NMDA receptor, the recovery of the system was even more perturbed. This indicated that the NMDA receptor is effectively important in regulating the energetics of oligodendrocytes. *In vivo* experiments on the spinal cord of these mutant mice revealed that, after high frequency stimulation, the mutant mice were unable to maintain energy requirements. Moreover, older mutant mice showed early signs of axonal degeneration as well as functional deficiencies on behavioural tests such as the rotarod test.

Although myelin is important for making the nervous system fast, the role of oligodendrocytes goes far beyond myelination. In fact, we are only beginning to gain insight into the level of metabolic support provided by oligodendrocytes to axons. However there are a slew of unresolved questions in the field. For instance, what are the functions of oligodendrocyte precursor cells and why do they receive synaptic input from unmyelinated axons? Moreover, what triggers myelination in the CNS, what is myelin's role in cognition and how is the steady-state of myelin maintained over the years? Finally, what is the role of myelin pathology in normal brain aging?

3rd talk: Focus on Autism

Dr. Wendy Roberts

University of Toronto, Toronto, Canada

Autism is a neurodevelopmental disorder, which is defined as a disorder that alters the development of the brain thus interfering with growth and function over time. Apart from autism, there is a large variety of neurodevelopmental disorders such as foetal alcohol syndrome, traumatic or acquired brain injury (e.g., cerebral palsy) and a number of genetic disorders (e.g., Fragile X syndrome, Down's syndrome, Rett syndrome, Moebius syndrome etc.). These developmental disorders are not necessarily linked with intellectual disability (IQ < 70). In autism, certain patients have a moderate to high IQ, however, in many cases, the adaptive function of autistic people, e.g., capacity to perform on a day-to-day basis within their family or community can be greatly discrepant from their measurable cognitive level.

The core issues involved in autism are abnormal social function, impaired communication and language skills, as well as the presence of repetitive behaviours and restricted interests. Severely autistic people are commonly recognised by the display of certain stereotypic behaviours such as repetitive flapping behaviour, unusual vocalisations and sounds, and a preference for interacting around the activity instead of interacting with people. However, autism does not only include individuals that display such extreme

behaviours. In fact, certain autistic patients are very high functioning but retain an unawareness of social innuendoes. Dr. Roberts used several specific casestudies of patients to demonstrate the vast behavioural and intellectual ranges that have been associated with autism such as Carly (<http://carlyvoice.com/home/>), Temple Grandin etc. In fact, one of the central tenets of autism is that the disease can be defined as a



The autism spectrum disorder: dimensions of impairment

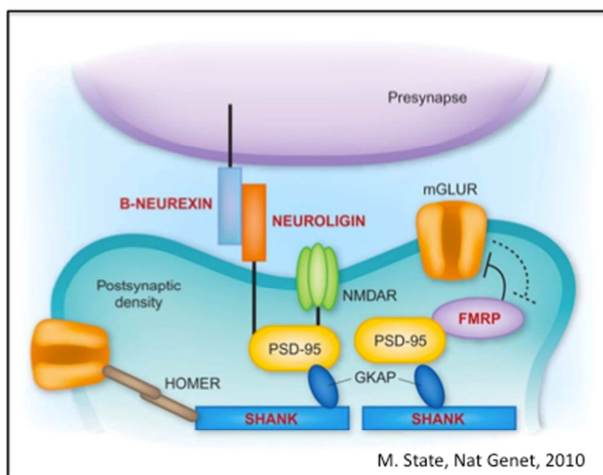
spectrum disorder (autism spectrum disorder or ASD) centred around social communication deficits, language deficits and restrictive/repetitive behaviours. According to this concept, autism is an integration of many different dimensions of impairment including intelligence, social anxiety, use and form of language, insistence on sameness, sticky and rigid behaviours and over- or under-reaction to sensory

stimuli. This leads to a very heterogeneous disorder that can be understood by dividing it into two main impairment areas: social communication and narrow interests such as sensory and repetitive motor behaviours and mannerisms.

How can one make sense of the aetiology of this heterogeneous disease? It is believed that autism is a genetic disease though some researchers argue that there are also contributing neuro-inflammatory or epigenetic factors. A complicating factor, is that in certain patients, several neurodevelopmental disorders can co-occur in patients (e.g., 30% of Fragile X syndrome patients also have autism) which strongly affects the study of these diseases. For instance, in the first genetic studies, researchers used to use Down syndrome as controls for genotyping autistic children although approximately 13% of Down syndrome patients also have autism. Moreover, this co-expression of several disorders can either have positive or negative implications for the developmental trajectory of patients and these confounding factors are still not very well understood. One example is that autistic patients who also present with epileptic syndromes have a more negative developmental course in terms of autistic behaviours.

The genetic aetiology of autism is supported by the fact that 50-90 percent of monozygotic twins have co-concordance of autism compared with only 10% of dizygotic twins. So far, various genes have been shown to play a role in autism. Approximately 10% of autistic patients have an identifiable Mendelian or genetic condition such as Fragile X syndrome or Rett syndrome. Moreover, another 5% of patients display unusual karyotypes with visible chromosomal rearrangements. Using microarrays, another further 5% of patients have been identified to have rare copy number variations (CNVs) in which small areas of a chromosome are rearranged, missing or added on. Finally, in another 5% of patients, mutations of rare penetrant genes were found in patients and families with ASD. Nonetheless, it is clear that genetics alone cannot entirely explain ASD and in fact environmental effects certainly play a role in disease expression. This is strongly supported by the fact that, in many cases, one monozygotic twin with autism expresses autistic symptoms more strongly than the other diseased twin. Moreover, there is an increased susceptibility of having a child with autism with increased paternal and maternal ages. It is also interesting that autism and other neurodevelopmental disorders are more prevalent in males than in females. In fact, the same deletion in Shank1 in a family segregates with a diagnosis of autism in males only while females remain asymptomatic.

A new avenue of research in autism is bent on characterising synaptic transmission in autistic patients. Indeed, neuron synaptic spine volume is reduced in ASD and many of the genes that have thus far been



**Many identified 'ASD genes' are involved in
The regulation and maintenance of synapses**

linked with ASD are involved in regulating and maintaining neuronal synapse development and plasticity. These include genes such as Neurexin1, Patched D1 and Shank1-Shank3. Researchers are currently using *in vitro* neurons generated from induced pluripotent stem cells obtained from patients and controls in order to study the structure and function of autistic synapses. This will hopefully yield interesting new concepts as to the causes of autism and it will also enable researchers to test new therapeutic agents in a human system. There is indeed a desperate lack of drugs specific for autistic symptomatology. In general, when a behavioural crisis occurs, clinicians prescribe drugs typically indicated for anxiety or neurolepsy. Although helpful in treating the

immediate symptoms, these drugs have a tremendous negative impact on long-term health as witnessed by the rise in the numbers of young patients who are developing serious side effects such as metabolic syndromes with obesity.

Research efforts are currently also trying to match up autistic genotypes with their phenotypes. These types of studies will enable researchers to match congenital anomalies with abnormalities in dysmorphology (including neuroimaging), medical co-morbidities, cognitive and behavioural profiles and other aspects of the autism phenotype including social affect, repetitive behaviours etc. As of yet, genetic mutations are only found in 20 percent of autism cases. Moreover, these data are generating major ethical and social debates. For example, it is still unclear whether siblings of autistic patients will pass the disorder on to their offspring leaving genetic counsellors unable to provide informed answers to affected families. Further ethical issues arise in cases in which the entire family has been screened and in which underage siblings that are carriers of the genetic anomaly have not been informed of their genetic predisposition. In order to study and recognise the early symptoms of autism, sibling studies are being carried out in which researchers are monitoring cohorts of newborn baby siblings of existing autistic children and assessing their developmental trajectory. This enables clinicians to make assessments of symptoms at younger ages and detect children at high risk of developing the disease at ages as young as one years old. What are some of the early signs of autism? Firstly, visual attention in children transitioning into autism changes from being focused on people to becoming fascinated with objects in their environment thus indicating an early problem with social referencing. Secondly, autistic babies display a decrease in babbling and social communication over time. Other signs are low reactivity, decreased orientation to their names being called and changes in play interests. Finally, the autistic babies typically begin to present atypical motor skills and behaviours such as unusual circular movements of the wrists. Simultaneously, new interventions are being designed to attempt to treat these high-risk siblings. Efforts in early prevention, using “pivotal response therapy”, are showing promising signs with over 80% of at-risk babies showing reduced symptomatology in time. A similar study is also looking at the reactivity and temperament of these babies by tracking their physiological responses with sensors.

The next steps in autism research need to address the gaps that currently exist in our understanding of autism. Firstly, researchers will need to identify the genes, the epigenetic factors and the environmental triggers that are implicated in autism in order to focus therapeutic responses. New therapies need to be developed and tested in both animal models and clinical trials. Moreover, new generalised policies should enforce early identification of autism and require preventative interventions in at-risk toddlers to reduce the developmental trajectory of the disease. Finally, the social and economic costs to families with affected members must be assessed and adequate support needs to be delivered. The future and major challenge of autism research will be in integrating hundreds of mini syndromes into a cohesive syndrome using integrative genomics.

4th talk: Developmental disorders of sensory systems: the example of deafness

Dr. Karen Avraham

Tel Aviv University, Tel Aviv, Israel

Hearing loss affects a large percentage of the population with approximately 1 in 1000 children being born with severe deafness. The incidence of hearing loss increases with age and it affects 4 percent of adults under 45 years old and around half the population by 80 years old. By 2015, the WHO reports that around 700 million people will be affected by hearing loss including 90 million Europeans.

Hearing is processed in the auditory system where sound waves are converted into mechanical impulses which are then translated into electrical impulses to be processed by auditory centres in the brain. There are a large number of cells involved in this process and in particular, in the inner ear, hair cells are the sensory cells responsible for mechanotransduction. In this complex system, there are many genes at play encoding proteins that are crucial for proper functioning at each level of the auditory pathway. In many cases, it is sufficient to knock out one of the genes for the whole cascade to fall and therefore, genetically,

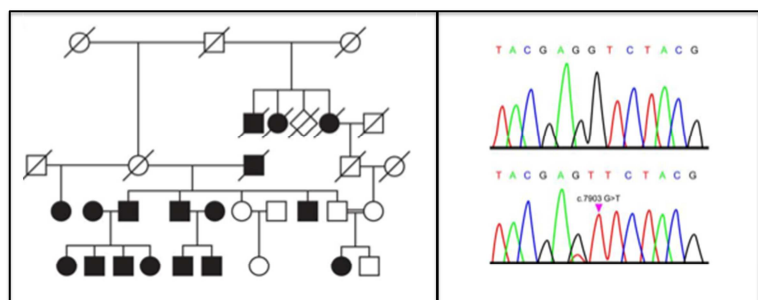
deafness is very heterogeneous. Certain forms of deafness are due to extremely rare mutations (61 genes so far) while others account for a large number of cases of congenital deafness (e.g., mutations in connexin-26 account for 40% of deafness in babies). Researchers are using many strategies to identify genes associated with deafness so as to uncover new mechanisms for deafness as well as to improve genetic counselling and develop new therapies for rehabilitation.

The field of hearing loss has benefited a great deal from mouse mutants such as the profoundly deaf *Slc26a4* mouse mutant. Studies in mice have not only enabled advances into the understanding of the mechanisms triggering hearing impairment but have also enabled researchers to identify new genes causing this sensory deficit. In fact, the inner ear of mice and humans retain similar functions and structures. Moreover, genes involved in hearing as well as their order on chromosome are often conserved between mice and humans. As of yet, 60 new genes involved in deafness have been discovered in spontaneous mouse mutants. For instance, the same mutations were found in humans and mice mutants such as the Beethoven or the Snell's Waltzer mutants. Several techniques are being used to uncover the mechanisms leading to hearing loss such as *in situ hybridisation*, immunohistochemistry, and scanning electron microscopy.

Why is it important to determine the genetic causes of deafness? Firstly, it is important in determining the prognosis. Based on the mutation present in the patient, it is sometimes possible to make predictions on the outcomes of the hearing loss. Moreover, it helps to identify associated risks and co-existing conditions. For instance, children with the mutation of Usher syndrome type 1F can expect to also lose their vision by the age of 10 and can therefore be counselled on being outfitted with cochlear implants to minimise sensory deprivation.

Current research has found mutations linked with hearing loss in genes belonging to a variety of protein families ranging from transcription factors to gap junction proteins and molecular motors. Moreover, these genes are expressed across all cell types of the inner ear although, thus far, researchers have not found many genes affecting auditory pathways or centres in the central nervous system. Historically, the genes have been discovered through linkage analysis but increasingly, researchers are turning to deep sequencing in order to discover new mutations. This method is highly powerful at identifying candidate genes for mutation but these mutations then need to be further analysed and proven to be causative of the disease.

A new approach to determining the genetic causes of deafness uses targeted genomics. By restricting deep sequencing to a subset of genes (e.g., 284 genes) known to be involved in human or mouse deafness, targeted genomics can detect variants called SNPs in all genes simultaneously in a cost-effective and timely manner. For each individual case, this deep sequencing approach will yield thousands of variants but a skilled bioinformatician can narrow these variants down to a dozen of relevant modifications. These variants can then be prioritised depending on how they affect protein function and whether they segregate with hearing loss in families. To validate whether the selected genetic variation is actually causing hearing loss, functional assays can be performed in cellular or animal models if they are available. Using this approach, a new mutation in cadherin-23 was discovered in a large family with severe to profound deafness. Cadherin is an important gene because in conjunction with proto-cadherin 15 (responsible for Usher syndrome and non-syndromic deafness), it forms the tip links located the tips of the stereocilia. Having confirmed this mutation with standard sequencing, the protein was then modelled in 3D and analyses were carried out to predict how the mutation would affect protein conformation. Ultimate validation of this mutation, however, needs to be carried out by generating a knockout mouse. In the meantime, the deep sequencing technique will continue to enable researchers to discover genes present in monogenic diseases and will greatly profit families with heritable hearing loss.



Deep sequencing identified a new mutation in cadherin-23 in a large family with individuals affected with severe hearing loss.

In some cases, next generation deep-sequencing has already given new insights into new mechanisms for deafness as is the case for Nesprin 4, a member of the LINC (linker of nucleoskeleton and cytoskeleton) complex. These proteins are involved in connecting the nucleus to the cytoskeleton and Nesprin 4 has been shown to connect the outer nuclear membrane to the microtubules through kinesins. Nesprin 4 also interacts with the inner nuclear membrane through Sun1. In families with the Nesprin 4 mutation, which leads to a truncated protein, affected members suffer from high-frequency hearing loss either at birth or in adulthood. In knockout mouse models, *Nesprin 4-null* and *Sun1-null* mice are profoundly deaf by early adulthood. Further, electron scanning microscopy experiments revealed that at postnatal day 30, the mice exhibit a loss of outer hair cells although the inner hairs remain intact. Inner hair cells are the real sensory receptors of the inner ear and are the main producers of the neural signals that are then transmitted along the auditory nerves. Outer hair cells, on the other hand, are responsible for amplifying or increasing sensitivity to a given frequency. Sounds waves elicit an electromotile response of the outer hair cells which serves to convert quiet sounds into louder ones. In the *Nesprin 4-null* mutants, the nuclei of outer hair cells become progressively dislocated and a working theory is that this displacement affects their motility eventually leading to their death. Alternatively, since Sun1 interacts with chromatin, a mutation in *Nesprin 4* might simply lead to other types of physiological abnormalities and to their death.

Already, this new deep sequencing approach has enabled researchers to run increased numbers of sequencing experiments at a fraction of the cost and in a shorter time frame. This is strongly impacting genetic counselling since counsellors are now more readily able to offer informed guidance and/or diagnostic tools to affected families. Nonetheless, there remain a number of unresolved questions. Firstly, can the discovery of new genetic mutations be translated into therapeutic strategies? There are also new genetic frontiers to be explored such as the role of epigenetics in hearing loss. Environmental factors will need to be investigated to understand the cause of more complex diseases such as noise-induced or age-related hearing loss. New therapies for audition restoration are being actively pursued (mostly in the US) with recent studies using embryonic stem cells and antisense oligonucleotides that are already showing great therapeutic promises. Finally, some of the major issues that remain are ethical issues. Should carrier screening be offered? Should there be prenatal diagnosis? And what should researchers do with incidental findings?

5th talk – Understanding brain development to treat brain disorders

Dr. Yehezkel Ben-Ari

INSERM, Marseille, France

This talk was aimed at demonstrating that an understanding of brain development is the most effective way to ultimately treat brain disorders. The first part of the lecture put forward some conceptual theories and provided research data to support these concepts. The second part of the lecture demonstrated that a thorough understanding of the progress of brain development led to an unexpected therapeutic strategy in treating autism.

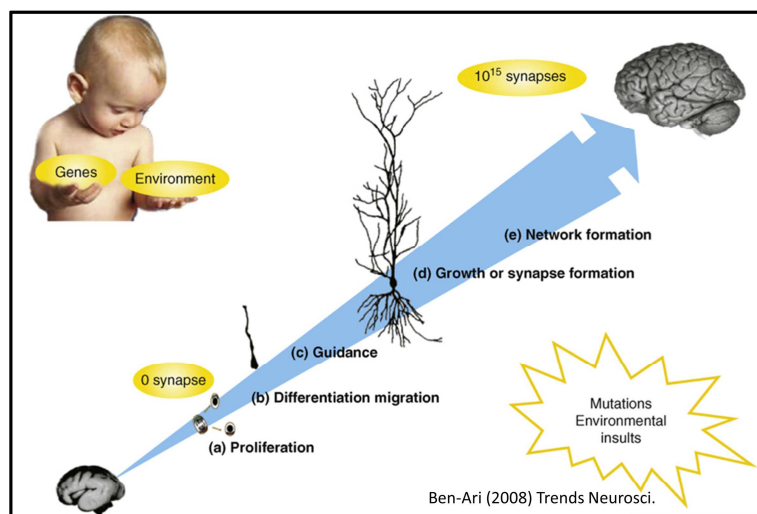
1) Conceptual theories of neuronal development: the check-point theory and the neuro-archeology concept

Every single step in brain development is associated with activity and, in fact, neurons start firing long before they are mature, even while they are still dividing or migrating. The immediate environment of developing neurons is teeming with electrical signals thus strongly implying that genetics are not sufficient to determine cellular development. Environmental factors and in particular neuronal activity must in all likelihood play an active role in the developmental process.

The “check point theory” postulates that electrical activity in neurons either positively or negatively controls the implementation of a cell’s inherent genetic program. This theory can be demonstrated by

observing the developmental sequence of ionic currents in the brain. Indeed, in young brains, ionic currents are slower and “sloppier” than in adult brains and serve another purpose. For instance, *in utero*, in the retina, there are long-lasting currents known as retinal waves which are incompatible with vision but are necessary for the subsequent development of the visual system. This retinal activity does not serve to sense but rather, it functions to enable retinal neurons to fire synchronously and generate appropriate synaptic connections. Similarly, recordings in the motor cortex of pre-term babies demonstrate that electrical currents follow motor movements rather than precede them; these currents also serve the function of wiring of the motor cortex. Finally, brain patterns also differ between immature young brain and adult brain slices. In fact, in embryonic brains, there are specific brain patterns, such as giant depolarising potentials, which are unique signatures of the developing brain that disappear at birth both in macaques and in humans.

The human cortex develops over a long period *in utero* and part of this construction process relies heavily on neuronal activity which provides a signature of the developmental stage of the brain. Further, this neuronal activity provides a checkpoint for the correct implementation of the genetic program. So, what happens if something in the process goes wrong? It falls to reason that, at any stage, the initial cause of the disorder (even if it's a single gene) is not the only consequence that will need repair down the line. Indeed, as the brain develops, it will react to the causal problem and the initial glitch (whether it be in migration, proliferation or differentiation) will lead to the deviation of a whole series of developmental programs. Typically, neurons that do not fulfil their program remain "frozen" in an immature state corresponding to the state where they were stopped in their development. It was shown in embryonic cortical slices that, if one thousand neurons are screened, there is normally an early-born neuron which fires systematically before other neurons of the system. This one neuron's activity was shown to be able to control and modulate the activity of the entire network of neurons and, as such, disrupting its activity could perturb the activity of the entire network.



At all stages of development, both genetic and environmental factors can dramatically impact proper brain development.

The “neuro-archeology concept” states that presymptomatic electrical or architectural signatures of future brain disorders exist and can be used to predict developmental disorders. This concept can be tested in several disorders. For instance, double cortex is a disorder in which a genetic mutation in doublecortin (Dcx) leads to a loss of cell migration and the development of severe phenotypes including the presence of two cortex and mental retardation. Inactivating Dcx in rats during their development leads to a defect in migration. Moreover, hippocampal neuron activity recordings demonstrate that those neurons display completely immature currents. Other neuronal disorders were shown to present with the same phenotype. For instance, in other diseases such as Parkinson’s disease and Huntington’s chorea, pre-symptomatic signatures exist which precede the development of clinical symptomatology. In addition, in tuberous

sclerosis, a disorder caused by mutations in either Tsc1 or Tsc2 genes, cells do not migrate but form heterochromatic cell masses in both rats and humans. The affected cells that have not migrated have very long-lasting NMDA currents that should normally have disappeared during development. Therapeutically, applying a selective antagonist of a subunit of the NMDA receptor (NR2C) in adult rats completely removes these immature currents. However, reintroducing the mutated gene in the adult would not have any effect since the gene is not functionally able to modify these immature currents. Similarly, although reintroducing Dcx in mice at P0 or P5 can partly correct the migration problem in a knockout model, reintroducing Dcx into the system at a later point does not correct the problem because the brain has rigidified. Therefore, although in some cases neuronal disorders are caused by a single genetic mutation, gene therapy does not appear to be a good solution for fixing neurodevelopmental problems. In fact, gene therapy, when employed after developmental programs have terminated, cannot possibly redress all the additional malfunctions that developed as a consequence of that initial developmental error.

2) GABA, intracellular chloride saga

In adults, GABA is known as an inhibiting neurotransmitter because it promotes the entry of chloride ions into neurons thus “inhibiting” them. During development, immature neurons have a much higher intracellular chloride concentration than in adulthood and, therefore, GABA acts to excite these immature neurons. When does this shift take place? It turns out that, during delivery, there is an abrupt shift in intracellular chloride concentrations. At the same time, blood concentrations of the hormone oxytocin are massively increased since the hormone serves to trigger labour. When oxytocin signalling is blocked during labour, the shift in chloride currents does not occur. Oxytocin exerts a neuroprotective role during delivery and in particular it has a protective role against anoxia. Moreover, oxytocin reduces calcium current in pain pathways thus exerting an analgesic effect during delivery.

It is known that GABAergic signals and oscillations are altered in autism. However, a drug that acts on GABAergic signalling, diazepam, often has paradoxical actions in treating autistic children. In a pilot study, five autistic children were treated with another drug which is a diuretic known as bumetanide. Bumetanide is a drug that has been used to treat hypertension for decades and has been shown to reduce chloride currents extremely efficiently much like oxytocin. Its side effects include diuresis and a treatable reduction of potassium concentrations in some cases. The pilot study showed promising results with kids improving significantly as measured by several diagnostic tools. A larger pilot study, with one hundred children treated during three years, has shown that bumetanide works very well as a treatment of autistic behaviours in a subset of children. This effect was also verified in a small group of Asperger’s adolescents and the results showed that, after treatment with bumetanide, there were improvements in the eye tracking of emotive figures and in the recognition of emotive faces. Moreover, MRI scans showed that previously inactive brain regions (such as regions implicated in fear response) were being activated after treatment. This promising treatment has just been approved for clinical trials in phase II in 4 countries.

Could this neurodevelopmental disorder thus be caused by complications occurring during delivery? It is known that complicated deliveries are associated with an increase in the incidence of autism. In fact, knocking out oxytocin in mice leads to the development of “autistic” mice. Moreover, oxytocin intranasal injections ameliorate visual communication in autistic adolescents. Although the bumetanide showed effects in autistic children, it is still unclear whether chloride is elevated in autistic children. However, in autistic and fragile X rats, chloride remains elevated and GABA is excitatory throughout adulthood. Although autism may be triggered *in utero*, delivery may play an important role. The next crucial step would be to test whether maternal-infant communication could be ameliorated by diuretics like bumetanide in very young patients.

In conclusion, genetic approaches have several limitations both in terms of understanding a disease and in treating it. For instance, there are expected to be one thousand mutations found in autism. However, in order to treat brain disorders, it is essential to determine when and where a problem occurs in the neurodevelopmental sequence. To truly understand disease, researchers need to first determine what

happens early on in development to trigger the defect in neurodevelopment. Then, researchers must expose the effect that this initial defect will impart upon the whole system.

Welcome by the European Commission

Dr. Sigrid Weiland

Directorate of Research and Innovation, European Commission

The European Commission is interested in promoting knowledge and research into brain research. This is because brain disorders are amongst the biggest societal challenge that is being faced by contemporary Europeans for the following reasons:

- 1. Suffering of patients and their families:* a third of the European population is affected by brain disease in its lifetime. This affects patients and their caretakers and often leads to the exclusion of patients from society. This is especially dire since many brain disorders cannot be adequately treated as of yet.
- 2. Science itself:* the brain is a complex structure comprised of 100 billion neurons with each establishing 10,000 connections. The brain is also a dynamic system thus adding another layer of complexity to the system. In that regards, neuroscience can therefore be viewed as one of the last frontiers of science.
- 3. Industry:* Several pharmaceutical companies have recently closed down their neuroscience departments because the development of drugs for brain disorders is a long, risky and expensive process. Pharmaceuticals companies do not consider it worthwhile anymore to invest in basic neuroscience research and development.
- 4. Costs related to brain disorders:* in 2010, brain disorders cost 800 billion dollars across Europe. This cost is likely to keep on increasing as Europe is faced with an aging population.
- 5. Chronic and progressive brain disorders:* The long-term nature of brain disorders creates a burden for health care systems which European countries are unable to cope with.

The European “Month of the Brain” is an initiative by the European Commission, which took place in May 2013, aimed at raising the public awareness of brain disorders and alleviating the stigmatisation of mental disorders. There are around 50 events organised across 16 countries including the conference of European Brain Research in Brussels and the conference of “Healthy Brains” in Dublin. This European “Month of the Brain” was particularly important as the European Commission is preparing for the next framework program for European Research and Innovation (2014-2020) and for budget. This talk then summarised some of the past funding endeavours of the European Commission.

6th talk - The bright side of the brain: the role of white matter in brain function and dysfunction

Lay audience presentation in frame of the “European Month of the Brain”

Dr. Ragnhildur Thora Karadottir

University of Cambridge, Cambridge, UK

The birth of neurophysiology began with the work of Dr. Benjamin Franklin on electricity. Later, Dr. Galvani connected dead frogs to a rod with a wire and observed that the frogs twitched whenever lightning hit the wire. He postulated that electricity (“bio-electricity”) inside organisms could control them. In fact, in the

brain, electrical current passes through neurons. Neurons are formed of two main sections: the input part of the neuron is termed the dendritic tree whereas the output section of the neuron is the axon. Electrical current flows along the axon till it reaches its terminals where it gets converted into chemical signals by the release of neurotransmitters from vesicles. Ninety percent of the time, chemical release will be in the form of glutamate which can then bind to the glutamate receptor located on the dendrites of neighbouring neurons. These receptors will then open up and become channels through which ions can pass.

Some of the first experiments to understand neurophysiology were performed by Dr. Wiesel and Dr. Hubel who studied the visual system of cats in the 1960's. To determine how neurons responded to visual inputs, they recorded from single neurons in the visual cortex of a cat while the animal was looking at a screen. Dr. Wiesel and Dr. Hubel discovered that different types of neurons in the cortex responded differently to light. Certain neurons ("simple neurons") only responded to light located in a specific area. More "complex neurons" responded to the movement of light but only when it travelled in a unique direction within a specific area. In order to obtain a global picture of our environment it is necessary to integrate all this information and in fact, neuronal communication works by integrating signals from multiple neurons. In a lot of cases, in order for a message to get integrated, the signals from distinct neurons must reach their target destination (e.g., another neuron or a muscle) synchronously. A neuronal network therefore depends on all the neurons of the system working together. If some of the neurons die, as is the case in certain diseases, certain functionalities of the network will also disappear (e.g., memory in Alzheimer's disease). Since neurons do not regenerate, those functions might become permanently impaired.

In 2012, the Nobel Prize for Physiology or Medicine was attributed to two researchers, Dr. Gurdon and Dr. Yamanaka, for their discovery that adult cells could be reprogrammed to generate every cell type in an organism. Dr. Yamanaka's research work led to the discovery that adult skin cells can be coopted into generating immature cells, or induced pluripotent stem cells (iPS), which retain the ability to produce any other type of cells. In the lab, skin cells of patients with different diseases such as dementia can be harvested and reprogrammed into cortical neurons. Using this technology, researchers can then make observations to verify whether the diseased neurons are fundamentally different from regular neurons. These neurons can also be used to evaluate disease progression and to test new therapeutic molecules. For instance, in humans, leukodystrophies are a set of developmental diseases of the white matter that encompass 34 distinct types of dystrophies. Due to genetic abnormalities, the oligodendrocytes of affected children die causing severe motor and intellectual disorders. The study of leukodystrophy has been impeded by the lack of mouse models but new techniques such as iPS could enable research and therapy to move forward.

Although neuroscientists have largely focused on studying neurons in the grey matter, half of the human brain is actually composed of white matter. Whereas grey matter is computational, white matter is necessary for the maintenance and synchronisation of the 100 billion neurons of the human brain. The latter function is achieved by glial cells, which are composed of astrocytes, oligodendrocytes and microglia. White matter or "myelin" is made of oligodendrocytes that wrap around the axons of the neurons. This enables the communication between neurons to go remarkably faster and in fact myelination can increase the propagation time from 5 to 300 meters per second.

How is myelin made? During development, axons are unmyelinated, they are electrically active and they release glutamate. At the same time, oligodendrocyte precursor cells (or OPCs), which have stem cell-like properties, begin to proliferate and to differentiate into myelinating oligodendrocytes. OPCs remain in the brain in adulthood after development and they retain the ability to replace oligodendrocytes in diseases. Therefore myelination is a dynamic process that continues to occur well into adulthood. How is this process regulated both in development and in repair? In fact, it is known that OPCs can respond to glutamate through glutamate receptors. In the grey matter, during stroke, glutamate can kill neurons when it reaches high concentrations in the brain. However, blocking neuronal glutamate receptors can prevent this death.

In stroke, white matter is also damaged. This damage can slow down or stop axonal electrical conduction with potentially severe consequences. There are also several other diseases of myelination including a form of cerebral palsy known as periventricular leukomalacia (PVL), which occurs late during pregnancy or at birth. In this disease, oligodendrocytes are not generated because OPCs die prior to becoming oligodendrocytes. Normal aging is also associated with a loss of white matter. In fact as blood pressure

decreases with aging, oxygen and glucose are not as readily available to the white matter lying deep within the brain. Oxygen and energy deprivation causes little pockets of white matter to die off. When this process was mimicked in brain slices, it was found that glutamate release damages white matter and that OPCs die predominantly compared to other cells. Blocking glutamate receptors can prevent this cell death and, therefore, understanding glutamate signalling in disease is essential to understanding how to treat these disorders.

If glutamate is so toxic to oligodendrocyte, what is its role? To comprehend that, we first need to understand that myelination is an ongoing dynamic process throughout life and that new oligodendrocytes continue to be produced in the adult brain. Their role is to increase myelination and therefore synchronisation of functional neuronal networks. In fact, the size of white matter tracts is linearly proportional to the number of practice hours that professional pianists clocked in as children. In adults, white matter was also shown to be increased after medical students learnt to juggle. OPCs therefore seem to be able to sense changes in neuronal activity and can respond to them. In fact, neurons use glutamate to communicate with OPCs through structures that are similar to neuron-neuron synapses. However, even in the absence of neuronal activity or glutamate release, oligodendrocytes will myelinate axons. Oligodendrocytes also express another receptor, the Neuregulin receptor. Neuregulin is a molecule that is released by neurons in an activity-dependent manner. Upon binding to Neuregulin receptors on oligodendrocytes, the latter will respond by expressing an increased number of glutamate receptors thus increasing their sensitivity to glutamate and myelinating nearby axons. There appears, therefore, to be two modes of myelination: a developmental and an activity-dependent mode of myelination.

In disease, if the white matter, the myelin or the oligodendrocytes are damaged then the connection between neurons slows down and even stops causing mental and physical disabilities. Since OPCs are present in the brain throughout life, they can potentially be recruited to the sites of disease and differentiate into myelinating oligodendrocytes to remyelinate the sites of lesions. This partly explains the somewhat transient nature of symptoms observed in multiple sclerosis (MS). MS is the most common disabling neurological disease among young adults affecting around 2.5 million in the world. In this disease, the immune system attacks myelin and oligodendrocytes leading to a loss of function. In a model system, induced lesions of white matter are remyelinated so why is there only partial remyelination in MS? In fact, in the lesion, axons are releasing glutamate which activates and recruits OPCs. If neuronal activity is blocked by an agent, remyelination is prevented. In disease, the hypothesis is that the sensitivity of synaptic inputs or OPCs might be decreased. This avenue of research is currently being pursued actively by the group of Dr. Karadottir.

Annex I

List of Participants

Scientific workshop 'Neurodevelopment and related disorders', speakers

1. **Dr. Amparo Acker-Palmer** (Goethe University Frankfurt, Germany)
2. **Dr. Karen Avraham** (Tel Aviv University, Israel)
3. **Dr. Yehezkel Ben-Ari** (Inseri, Marseille, France)
4. **Dr. Ragnhildur Thora Karadottir** (University of Cambridge, UK)
5. **Dr. Klaus Armin Nave** (Max-Planck-Institute Experimental Medicine, Göttingen, Germany)
6. **Dr. S. Wendy Roberts** (University of Toronto, Canada)

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2. **Dr. Eero Castren** (University of Helsinki, Finland),
3. **Dr. Joab Chapman** (Sheba Medical Center, Tel Aviv University, Israel),
4. **Dr. Isabel Farinas** (University of Valencia, Spain)
5. **Dr. Fabrizio Tagliavini** (Istituto Nazionale Neurologico Carlo Besta, Milan, Italy)
6. **Dr. Ana-Maria Zagrean** (University of Medicine and Pharmacy, Bucharest, Romania)

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