EuroNeurotrophin Newsletter January 2021

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The EuroNeurotrophin Network

Neurodegenerative Diseases

What are they

Neurodegenerative diseases are a worldwide problem that keep increasing over the years due to the population's life expectancy and the fact that there are no effective treatments developed yet.

Many names and symptoms of these illnesses are widely known to the general public: Alzheimer's disease erodes memories, Parkinson's disease impairs mobility, Amyothrophic Lateral Sclerosis inhibits muscle control, Huntington's disease strips off various psychophysical abilities. Also very well known is the absence of a cure. Unfortunately, even if researchers shine more and more light over the mechanisms behind causes and symptoms, so far medicines can only mitigate symptoms and ease the patient's condition.

Neurodegeneration results from a progressive deterioration and loss of the nerve cells due to genetic faults, epigenetic factors, and, more generally, mere aging. Thanks to the advances of the Contemporary Era, our life expectancy has increased, and consequently these illnesses impact our lives more and more: by affecting us or someone dear to us.

Neurotrophins

Scientists all over the world are exploring various solutions to tackle these diseases. In the EuroNeurotrophin Network, we decided to focus our research on a family of large proteins, produced in our bodies, called Neurotrophins. These, by binding to their natural receptors (Tropomyosin Receptor Kinase), protect the neuronal cells and increase their survival. Unfortunately, so far Neurotrophins do not seem suitable to be used as drugs, because of their large size and protein nature. Hence, the need for developing small molecules capable of reproducing the effects of the Neurotrophins, without their short-comings, namely, a micro-Neurotrophin.

Dehydroepiandrosterone, a small steroidal compound we secrete, was found capable of improving neuronal survival, thus providing for a promising «lead» to be structurally modified in the quest for a potential new drug to treat neurodegeneration.

In our project, we work on producing such modifications, while in parallel exploring and modifying different non steroidal molecules and interrogating marine microorganisms extracts for solutions potentially already present in nature. We then test these compounds on biological models of neurodegenerative diseases and neuroinflammation to study their activity and, eventually, identify the most promising to be transformed into a medicine.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement no. 765704

The use of proteomics from *in vitro* to *in vivo* in neurotrophic research

Proteomics is a key technique in drug discovery.

The lack of therapeutic options to prevent or treat neurodegenerative diseases is of concern, as the prevalence and disability resulting from these disorders are predicted to increase significantly (Abrahams et al. 2019).

Since proteins are responsible for almost all biological processes in an organism, changes in the concentration and/or their structure are likely to reflect the effects of a disease (Davidsson and Sjögren, 2005). Specifically, the presence of protein aggregates in the cells of the central nervous system is a hallmark of neurodegenerative diseases. As a consequence, most drug targets are proteins, enabling proteomics for drug discovery, development and clinical practice (Tyers and Mann, 2003).

Neurotrophins are a family of four similar proteins: NGF, BDNF, NT-3 and NT-4 (Allen et al. 2011); that are important regulators of neuronal survival, development, function, and plasticity (Huang and Reichardt, 2001). Since, in EuroNeurotrophin, we are interested in the study of the therapeutic potential of neurotrophins mimetics, the solution we propose is to create novel small molecules that mimic neurotrophins.

At the Department of Molecular and Cellular Neurobiology, of Vrije Universiteit of Amsterdam, we attempt to use proteomics in order to study the effectiveness of the neurotrophins mimetic molecules that other ESRs are developing in the project. To do it, two different approaches have been established.

The first approach is focused on an *in vitro* strategy, where primary hippocampal neurons from embryonic wild type mouse have been used in order to create a cell-based model. These cultures have been challenged with APP/Tau/β-Amyloid to obtain an Alzheimer's disease (AD) model in which we can test the novel molecules that have been created. To analyse the effects of the compounds in our cell model, a high throughput imaging screening is used. This technique gives us information about morphological development, as for example, neurite outgrowth and length; cell proliferation, among many other parameters.

the compound treated cells versus the non-treated and provide information concerning if the compound is a good candidate for further studies.

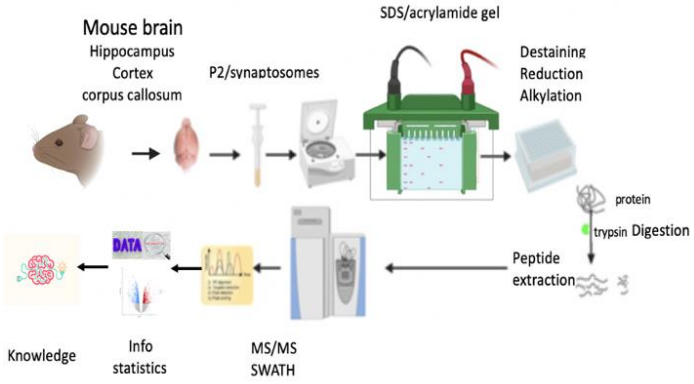

Figure 1: Proteomic procedure (ESR13)

The second approach is an *in vivo* one which focuses on the examination of the temporal effects of neurotrophin mimetics on the brain tissue and synaptic proteomes in mouse models of neurodegeneration (APPswe/PS1dE9 and 5xFAD transgenic mice for AD; cuprizone mouse model of MS). Large scale proteomics, gives the opportunity to gain deep knowledge with broad information. Data-independent acquisition (DIA) methods such as sequential windowed acquisition of all theoretical fragment ion spectra, SWATH analysis, is ideally suited to high-throughput tissue research.

Specifically, these kind of approaches result in comprehensive peptide data capture. Currently, the chance of analyses by various proteomic software pipelines with high confidence is available with false discovery rates (FDR) usage to control error propagation. The large-scale proteomics data can be hypothesis generating that guide the subsequent functional studies to explain the mechanistic aspects of the disorders and their rescues by the neurotrophin mimetics.

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Blood-Brain Barrier *in vitro* model to study neurodegeneration

The Blood-Brain Barrier (BBB) is a specialized and dynamic membrane that protects the brain from the external blood-flow. The blood contains and transports some molecules that can be toxic to the brain. The BBB is also essential in selecting which molecules can enter the brain and the spinal cord and these include therapeutic drugs, thus affecting patient treatment.

BBB dysfunction is involved in several neurodegenerative pathologies and by creating a model in the laboratory we may be able to explore it and identify alterations involved in the disease. A BBB model will be like an open window to explore the connection between the brain and the rest of the body.

Because of its complex function, different layers of cells, all with their characteristics and specialised roles, comprise the BBB. As part of the EuroNeurotrophin Consortium, we use pluripotent stem cells donated by patients affected by motor neuron disease/amyotrophic lateral sclerosis (MND/ALS). To reproduce the most accurate BBB model we differentiate patients stem cells into brain endothelial stem cells (BMECs). BMECs form the basic structure of the BBB, allowing us to analyse the differences between a healthy and diseased barrier so that we can better understand its impairment in MND. We will also explore the interaction between BMECs and astrocytes, reproducing the BBB environment. In a pathological context, we would able to explore how these alterations affect disease and analyse the toxic compounds that affect the function of the BBB.

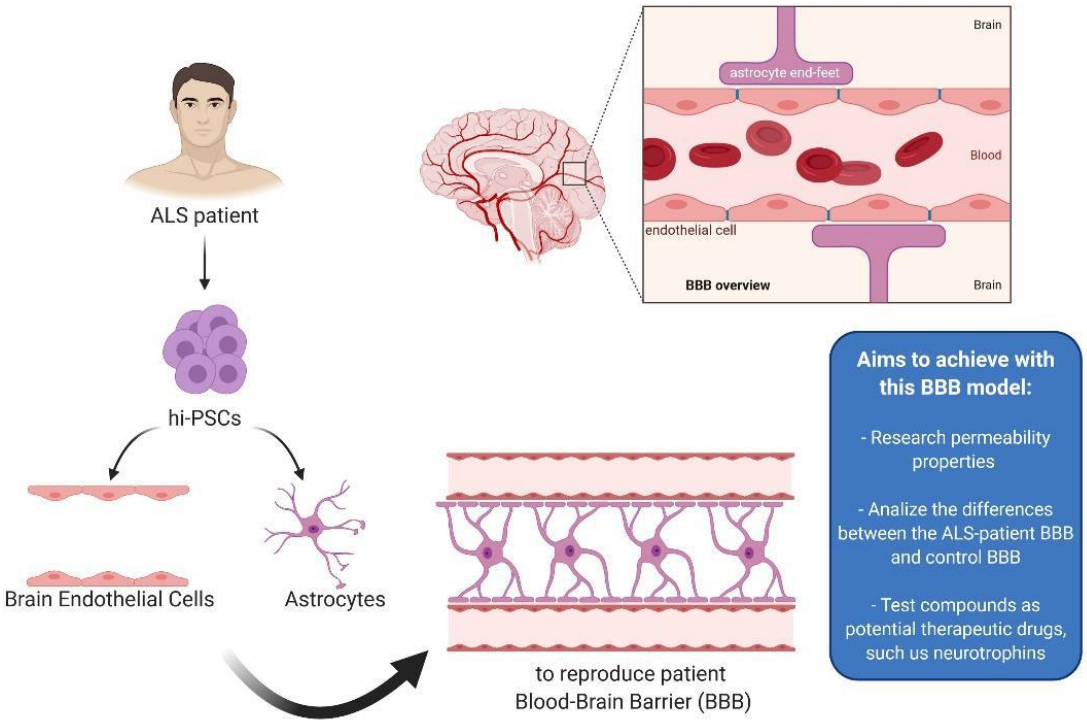

Figure 2: BBB-patient derived model overview. Figure created with BioRender

In terms of the EuroNeurotrophin project, we will use our laboratory model to identify the most potent neurotrophin mimetics that are also efficient in crossing the BBB. With our patient-derived model, we would able to test drugs as the neurotrophin molecules developed by other ESRs within the consortium, thus reaching the brain and spinal cord.

The relevance of this model as an approach of patients-BBB would offer us the possibility to test drugs as a potential MND treatment.

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Teaching at a EuroNeurotrophin School

Organizing a training week and teaching your peers: an opportunity to grow

Teaching activities of the EuroNeurotrophin Consortium

When someone thinks about doing a PhD, the first thing that comes to mind is doing extensive research on your selected topic, and collecting and presenting your findings in a dissertation. Performing good research is of course very important, however a PhD is also a time where you should learn soft skills, and build a network of your peers to prepare you for your career after the PhD. In order to equip all ESR's with broad scientific knowledge, and skills to prepare them best for their future careers, a large number of schools and workshops, as well as many secondments were proposed when building the consortium.

The three training weeks were composed of three schools, which were taught by our supervisors from different fields, each giving introductions to their fields of expertise. By our supervisors, we have been introduced to many different research approaches and techniques, computational, chemical and biological, which made communication and scientific discussion between fellow ESR's easier. Seeing how other re-searchers work is particularly important for interdisciplinary consortiums such as ours, and fosters the development of new research ideas and points us in new directions. In addition to the schools, workshops were given by members of our partner organizations from industry. We have also had the opportunity to learn how drug development works in a commercial setting from our partners at Novartis Hellas, and how patenting and commercialization of biomedical inventions can be achieved from Ventac Partners. These workshops provided valuable insight for those of us who would like to pursue a career in industry rather than academia after their PhD. We had two additional workshops planned, however were postponed due to COVID-19 pandemic, which we hope to complete next year.

Apart from the training weeks, all ESR's are participating in at least two secondments, many of them having one industrial secondment at an SME or a large company. These secondments enable students collaborating with each other to actually work with each other, learn new techniques from their host institutions and establish the techniques at their home institutions, and in case of industrial secondments, experience how research and production is conducted in an industrial setting.

Teaching at School 2: *In vivo* models for neurodegenerative disorders: strengths and limitations

Several of the ESR's helped the supervisors organizing and teaching at the EuroNeurotrophin schools. I, Canelif (ESR8) personally have had the opportunity to teach at "School 2: *In vivo* models for neurodegenerative disorders: strengths and limitations." My assigned lectures were on experimental techniques in neurodegenerative diseases, and Cuprizone model of MS, which I will also utilize in my research. It was particularly challenging to prepare the lectures, as all ESR's come from different backgrounds. I paid attention to keep them simple enough so that the students with less training in biology could easily follow, but also detailed enough for the biologists to be able to benefit from them, as well. In addition to the two lectures, I have prepared practical sessions, on the identification of different cell types in the nervous system, comparing differences between Cuprizone fed and healthy mouse brains, and quantification of demyelination and microgliosis on Cuprizone fed vs. healthy mice.

I've had a very good time preparing the practicals, picking good samples for inspection, and preparing and testing the walkthroughs was hard work, but was also fun. I felt very pleased guiding my fellow ESR's while they went through the practicals, and I hope they had a great time as well! I hadn't considered teaching prior to this experience, but seeing as I have enjoyed it so much, I will certainly apply to teaching positions as well once I have earned my PhD. The EuroNeurotrophin schools have proved to be an even more valuable experience than I expected for me, and I feel very lucky to be a part of this consortium.

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About the Authors

The EuroNeurotrophin December 2020 Newsletter was written by five of the Early Stage Researchers (ESRs) working on individual projects as part of the EuroNeurotrophin project.

ESR1: Daniele Narducci

Daniele is hosted at the National Hellenic Research Foundation, Institute of Chemical Biology. His research focuses on the synthesis of dehydroepiandrosterone (DHEA) derivatives substituted by five or six membered-17-spiro substituents.

ESR8: Canelif Yilmaz

Canelif's research focuses on the evaluation of small molecule neurotrophin mimetics in models of neurodegeneration and neuroinflammation. She is hosted by the Technische Universität Dresden, Faculty of Medicine, Department of Clinical Pathobiochemistry.

ESR9: Ana Aragón

Ana works at the University of Sheffield in the Sheffield Institute for Translational Neuroscience (SITraN). She is developing Human Cell-Based Models for the study of Blood Brain Barrier Molecular Permeability

ESR10: Débora Pita

Débora's research focuses on Cell-Based Models for Neurotrophic Therapeutic Testing. She is hosted at the Vrije Universiteit Amsterdam, Center for Neurogenomics and Cognitive Research.

ESR13: Evangelia Thanou

Evangelia works at the VU University of Amsterdam, Center for Neurogenomics and Cognitive Research in Vrije Universiteit Amsterdam, Netherlands. The main focus of the project is the examination of the temporal effect of the already existed neurosteroids and the new neurotrophin mimetics on synapse proteome and synapse density in mice models of AD (APPswe/PS1dE9 and 5xFAD transgenic mice).

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