Grant agreement no. 642881

ECMED

The Extracellular Matrix in Epileptogenesis

MSCA-ITN-2014-ETN: Marie SkłodowskaCurie Innovative Training Networks (ITN-ETN)

D1.3 Advanced training course 1
A cell biologist's view on active synapses and the perisynaptic Extracellular Matrix

Work Package: 1

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Lead beneficiary for this deliverable: LIN
Contributors: UCL, DZNE

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Dissemination Level		
PU	Public	√
CO	Confidential, only for members of the consortium (including the Commission Services)	
CI	Classified, as referred to in Commission Decision 2001/844/EC	

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1. History table

Version	Date	Released by	Comments
	Nov. 5, 2015	Seidenbecher (LIN)	Planning of the course with all ESRs; narrative description of the course objectives and program
	Nov. 6, 2015	Walker (UCL)	Agreement on rescheduling the course to M15
	Jan. 22, 2016	Marsili (UCL)	Invitation and final program sent to all ECMED members
1.0	April 27, 2016	Seidenbecher (LIN)	1st version of the report sent to UCL
1.1	May 9, 2016	Marsili (UCL)	1st round of revisions
1.2	May 9, 2016	Dityatev (DZNE)	2nd round of revisions
	May 10, 2016	Walker (UCL)	Approval

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2. Definitions and acronyms

Acronyms	Definitions
4-AP	4-Aminopyridine
CAZ	Cytomatrix at the Active Zone
ECM	Extracellular matrix
ESR	Early stage researcher
HEK293T	Human Embryonic Kidney cells
SPECT	Single-photon emission computed tomography
TTX	Tetrodotoxin

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3. Introduction

To keep ESRs up to date with recent advances in synapse activity and plasticity in the field of epilepsy and translational neuroscience (CTD1), LIN organised the 1st Advanced Training Course of the ECMED ITN. Originally the course was scheduled for M12 (December 2015). However, due to a delay in recruitment of ESRs and the fact that the "Practical workshop 1: Analysis of neurodynamics using microelectrode arrays *in vitro* and *in vivo"* took place in November 2015 also in Magdeburg, the consortium agreed to reschedule the advanced training course 1 to take place in M15 from March 1st to 4th 2016 in Magdeburg. ESRs were actively involved in the organization of the course. In November 2015 they streamlined the topic and tuned the practical lab part according to their needs. Furthermore, they prepared posters to report on progress in their Ph.D projects.

The focus of the advanced course "A cell biologist's view on active synapses and the perisynaptic Extracellular Matrix" was on biochemical and cell-biological methods to measure and analyze synaptic activity and proteolysis. The program was composed of a theoretical part with lectures and a practical part where the ESRs formed teams to work on 4 suggested topics. Each group completed two out of the four topics, according to previous lab experience and interest. Finally, the teams chose one of the topics and presented their results obtained during the week in a slide presentation to all ESRs and faculty members. They presented their scientific projects to faculty and ESRs in a poster presentation. The best posters were awarded a poster prize.

Advanced Training course topics:

No.	Topic	Trainer	Groups
1	Biochemical ECM fractionation	Karl-Heinz Smalla	A, C
2	High end micro- and nanoscopy, image processing	Oliver Kobler, Werner Zuschratter, Rodrigo Herrera-Molina	B, D
3	Cell biological measurements of synaptic activity	Anika Dirks	C, A
4	Protease assays, Matrix-cell binding assays	Renato Frischknecht	D, B

Topics in details:

1. Biochemical ECM fractionation

Biochemical fractionation techniques are extremely useful tools to characterize cellular organelles and localization changes of biomacromolecules as for instance ECM proteoglycans, e.g. after status epilepticus. In the hands-on course students were familiarized with two different approaches: (i) preparation of synaptosomes using a protocol for subcellular fractionation and (ii) a sequential extraction protocol using increasingly harsh conditions for solubilization of ECM molecules. Each student produced

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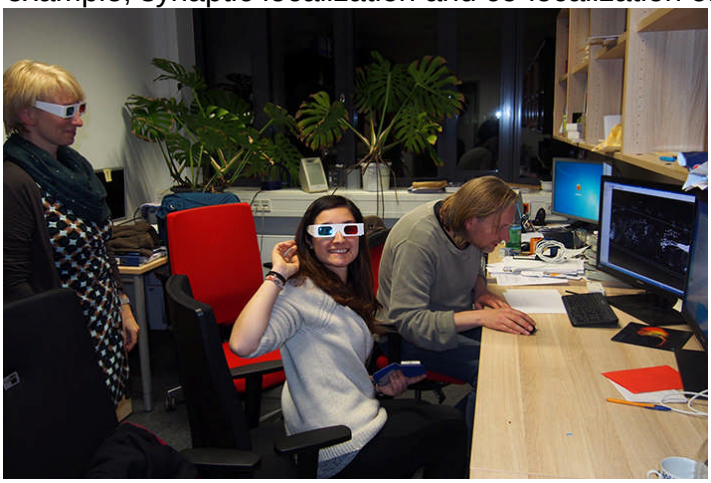
his/her own samples, which were separated on gels and transferred to blots and provided to the ESRs for further processing in their home labs.

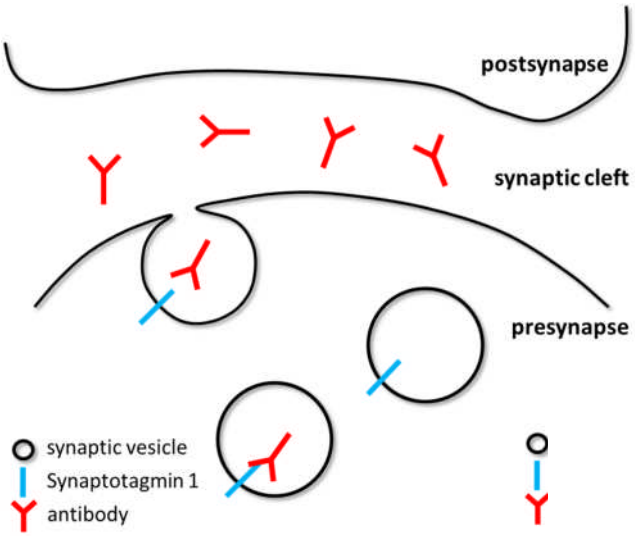
2. High end micro- and nanoscopy, image processing

Observation of molecules is an extremely useful tool to unravel mechanisms involved in the function or dysfunction of neuronal synapses. But for decades, classical optic laws have limited this experimental approach and reduced our capacity to describe the micro-world of neurons. Recent advances in the microscopy field i.e. super resolution techniques as 'Stimulated emission depletion' (STED*)* microscopy have circumvented these laws changing the way how we see molecular processes at the nanoscale level. In this theoretical and practical course, attendees learned the bases and performed 2-channel STED microscopy, advanced image processing, and deconvolution procedures to uncover nano-distribution and co-localization of a number of proteins important for synapse structure and function. For example, synaptic localization and co-localization of the cell adhesion molecule Neuroplastin with synaptic markers was assessed in cultured mature neurons after indirect co-immunostaining with a series of STED compatible fluorophore-conjugated antibodies. After this, students performed a full routine of image processing after producing publication-quality images.



3. Cell biological measurements of synaptic activity

Synaptic activity enables the communication between neurons, which is essential for learning and memory processes. Changes in the synaptic activity alter the Cytomatrix at the Active Zone (CAZ) composition and the neurotransmitter release. One established method to measure synaptic activity is the Synaptotagmin 1 antibody live-uptake, labeling endocytosed vesicles of active synapses. This method is useful to investigate presynaptic changes upon epileptogenesis. Thus, ESRs treated mature dissociated cortical cultures with either 4AP and bicuculline or TTX for 30 minutes before and during



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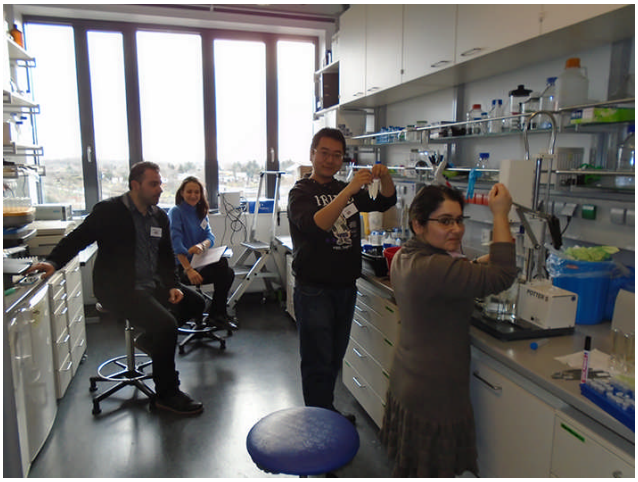
the live uptake of the antibody towards the lumenal domain of the synaptic vesicle protein Synaptotagmin 1. This antibody is fluorescently labeled with Oyster 550, which allowed the detection of recycled vesicles by fluorescence microscopy. Less recycled vesicles were detected after the silencing of neurons with TTX and the opposite was noticed after disinhibition of cultures with 4AP and bicuculline.

4. Protease assay

Proteases are responsible for ECM remodeling and degradation in many tissues including the brain. There it has been shown that activity–dependent secretion and activation of proteases are important for synaptic plasticity. Unfortunately, proteases are of low abundance and therefore difficult to directly detect. Further they are mostly produced as so called zymogens, inactive precursors and activated by other proteases. Thus in order to measure abundance and activity of proteases simple localization studies are not sufficient. Therefore, the ESRs learned how to determine protease activity in HEK293T cells by measuring the appearance of the cleavage product of brevican, an important neural ECM protein. We used 3 different enzymes, potentially capable of cleaving brevican to determine their efficacy in cleaving brevican. The students were introduced into quantitative Western blotting and data analysis.



4. Activities and results

All course days started with morning lectures about topics spanning from synapse architecture, function and plasticity to protein transport, lateral diffusion and imaging of synaptic activity. The course also offered an evening lecture on “synapse to nucleus signaling”- how altered synaptic activity is transferred into gene expression. Together these lectures intended to mediate a deeper understanding of the cellular and synaptic events taking place during learning and adaption to altered synaptic and network activity. Malfunctions of these mechanisms are at the basis of epileptogenesis and thus important background knowledge for ECMED ESRs.
On the eve of the course we met with all arriving ESRs for a welcome dinner downtown at the restaurant “Franx” to get together in a relaxing atmosphere.

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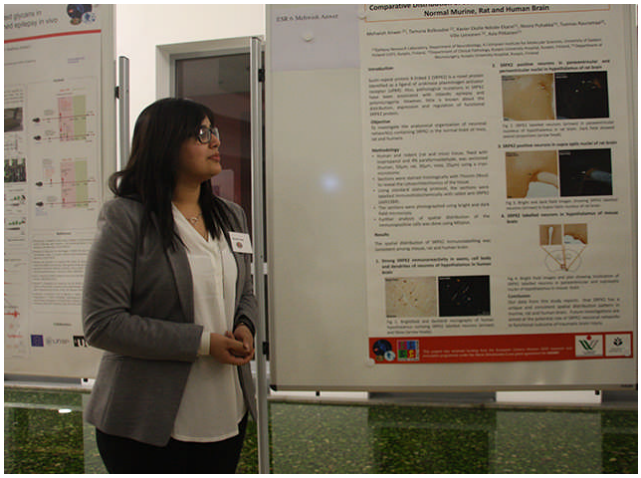
Day 1, Tuesday March 1

The students were welcomed by Prof. Eckart Gundelfinger at LIN. With his lecture “The Synapse” he introduced the basic molecular architecture of the synapse and mechanisms of synaptic plasticity. After the lecture the supervisors of the practical part gave short presentations about the four topics (table 1) offered to the students in order to form four teams. Each student chose two of the four topics according to personal interests and need and worked on each topic for two days.



In the evening, students presented posters of their scientific projects in short talks to faculty members and fellow ESRs. The best poster presentations were given by Mehwish Anwer from Kuopio (UEF) (see picture on the right), Eduardo Morais from Genova (IIT), and Anatoly Korotkov from Amsterdam (AMC).

Day 2, Wednesday March 2

The second day started with a lecture by Dr. Jürgen Goldschmidt entitled „Thallium measurement of neuronal activity and small animal SPECT“, giving an introduction into SPECT imaging and representation of neuronal activity. During the day the groups then continued to work on their first topic.

After dinner Dr. Michael Kreutz held the evening lecture „How synapses communicate with the nucleus“. He elaborated how synaptic signals lead to changes in protein expression and the molecular mechanisms involved.

Day 3, Thursday March 3

Day 3 started with a morning lecture by Dr. Martin Heine entitled „Tracking of single molecule dynamics“ giving an introduction into nanoscopy and single particle tracking, a method to measure single molecule dynamics in the neuronal membrane. The students then started to work on their second lab topic.

In the evening there was a Get-together at the historical place „Lukas-klause“ (see group picture at the right), with a guided tour and demonstration of Otto von Guericke’s original experimental setups. The dinner also took

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place at the historic site.

Day 4, Friday March 4

We started the day with a guided tour through the city of Magdeburg to pay a visit to the Otto von Guericke memorial (see group picture at the right) and to familiarize ECMED members with the course location - starting at the Cathedral and continuing at the remaining's of the city's historical town walls and some more modern architectonically important buildings in the city.
Then students continued their work and finished topic 2. In the evening each group of students presented one of the topics through power point presentations, followed by a short discussion about their results and technical background. Finally, the winners of the poster competition, elected by a committee consisting of the organizers, speakers and supervisors of the course were announced.

5. Conclusion

The goal of the advanced training course 1 was to mediate background of cell biology including synaptic plasticity, molecular composition of the synapse and transport and signaling mechanisms in neural cells. This goal was fully achieved, and beyond this, by interacting in small teams as well as in the whole group all ECMED ESRs got to know each other much better and formed a true corporate identity. The final version of the programme can be found here: http://www.ecmed-itn.eu/news-and-events/network-events. All material related to the course has been uploaded in the intranet section of the ECMED website.

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