Annual report 2013

FOM programme nr. 137
'Barriers in the brain: the molecular physics of learning and memory'

Foundation for Fundamental Research on Matter
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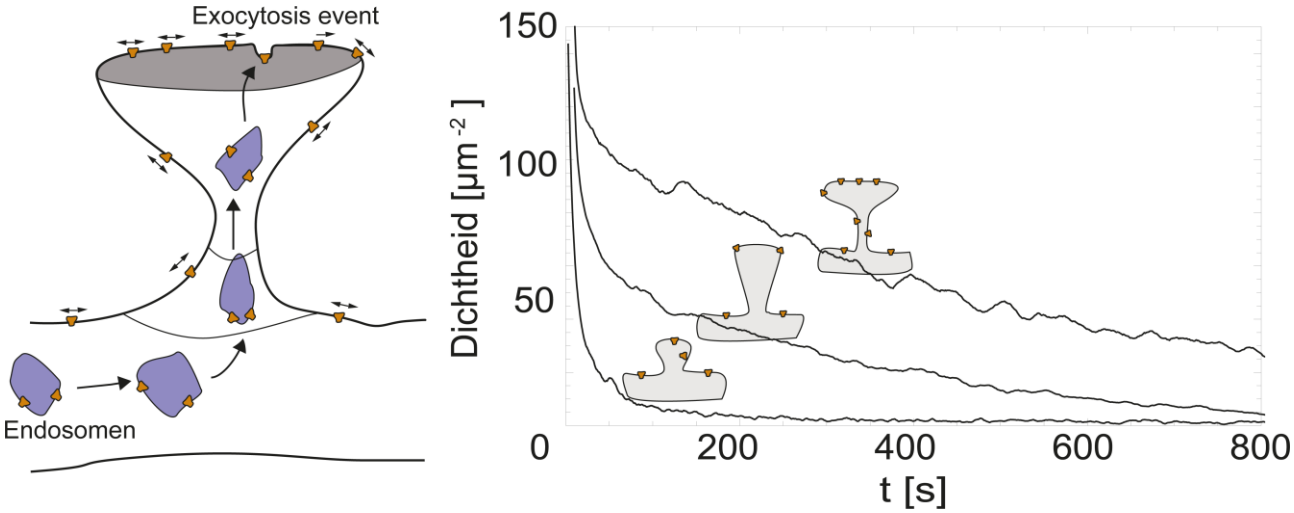

Evolution over time of the density of receptors on the upper side of the spine following the release of thousands of receptors at the top. Mushroom-shaped spines appear to retain receptors up to two orders of magnitude longer than elongated or stub-shaped spines. The shape of the spines therefore seems to regulate the diffusion of receptors across the membrane surface.

May 2014

Content

1. Scientific results 2013

The FOM programme 'Barriers in the Brain – the biophysics of memory and learning' is in its first full year. All personnel has been hired now and students and postdocs are now up to speed. In the case of the theoretical studies, nice, published results have already been obtained. In the case of the experimental studies, more time is required. Overall, the programme is still moving in the same direction as originally described. In more detail, progress in the individual groups has been as follows:

- **Storm/Kusters (TU/e).** Focus has been on the theoretical understanding of the effects of spine shape on the diffusive transport of AMPA receptors. We have shown that this shape could play a key role in delaying leak-out of the receptors by several orders of magnitude in time (published in Biophysical Journal, together with both Utrecht groups). A next step has been a more general consideration of how diffusion is affected by curved geometries. We have discovered that the non-equilibrium eflux on different sides of a curved constriction can be regulated by constriction shape (accepted in Phys. Rev. E.). We are currently focusing on another part of the project: how spine geometry affects the active transport of vesicles into (or out of) the spine. A manuscript containing Lattice Boltzmann simulations and analytical modelling is in preparation. Another focus of attention are Langevin-Molecular dynamics simulations of the effects of crowding in the membrane. It is planned that Kusters will spend 3 months in the lab of Prof. Mahadevan (Harvard) in the fall of 2014. There he will work on models describing the actin-cytoskeleton coupled growth and maturation of the mushroom spine
- **Schmidt/Pomp (Leiden)**. We have been able to produce vesicle-tube systems on a daily basis. Now we image, using fluorescence microscopy, individual, quantum-dot conjugated lipids in this topology, which allows long-time tracking. Tracking is achieved by quantum-dot conjugation to lipids. We have characterized lipid mobility on giant vesicles and compared those to mobility on tubes. In a next step we will monitor lipid mobility as a function of tube-geometry (radius) and results will be compared with the theoretical outcomes of the Eindhoven group.
- **Kapitein/Adrian/Katrukha (Utrecht).** We have started experiments monitoring receptor diffusion in spines, in order to relate diffusion behaviour to spine shape and compare with the theoretical predictions of the Eindhoven group. To allow high-speed and longer-term tracking, quantum-dot functionalization has been improved. Super-resolution microscopy is required to determine spine shape, localization algorithms have been improved and a theoretical analysis has been performed to optimize the conditions to obtain super-resolution images. In the coming year this will be applied to allow life super-resolution microscopy on spine morphology. Furthermore, we have developed an assay that allows light control of recycling endosome spine entry. This assay will be applied to study the dynamics of this process.
- **Peterman/Kushwaha (VUA).** We have generated a new kinesin construct optimized for *in vitro* assays to mimic vesicle transport in confined architectures. Furthermore, single-molecule assays have been optimized (including sample preparation and data/image analysis), as well as vesicle-preparation protocols (in collaboration with the Leiden group). The kinesin construct has been tested and works well. First attempts have been made to connect motors to vesicles. Further optimization will be required, but first moving vesicles have been observed. This assay will be further improved in order to track individual motors as well as vesicle shape.
- **Hoogenraad/Will (Utrecht).** To identify the molecules involved in spine-neck shaping, we will perform a systematic, candidate-based RNA-interference (RNAi) screen. In analogy with other compartmentalized cellular systems, we focus on conserved actin-binding/regulatory proteins, actin-dependent motor proteins, septin-family proteins, membrane-sculpting proteins and lipid-bound proteins. We have been able to clone (almost all) shRNAs (3 shRNA constructs/gene) and setup the neuronal system to determine the morphological spine characteristics. To address which of these specific genes are involved in shaping the spine neck we will, in a next step, transfect primary hippocampal neurons at 8 DIV and 18 DIV (at 8 and 18 days in

vitro) with shRNA constructs and GFP (to highlight spine morphology) and process the neurons 3-4 days later for imaging.

2. Added value of the programme

The collaborations within the programme have already been very valuable and plans for further extension are ongoing. More specifically:

- A joint publication by the Eindhoven/Utrecht groups on the connection between spine shape and receptor diffusion, so far focusing on theoretical aspects. This will be extended to a comparison between theory (Eindhoven) and experiments in neurons (Utrecht) and in *in vitro* membrane tubes (Leiden).
- We are taking a similarly unique multi-pronged approach combining *in vivo, in vitro* and *in silico* measurements in our study of active vesicle transport through the spine neck, which might act as a barrier.
- Once key 'spine-neck' shaping molecules have been identified and methods have been developed to inhibit them (Hoogenraad) the effect of these molecules will be studied on spine structure (with super-resolution microscopy) and their ability to act as barrier for actively transported or diffusing receptors (with single-particle tracking) (Kapitein).
- A general, vivid exchange of technology and knowledge: e.g. image analysis software, super-resolution technology and procedures, expertise on *in vitro* membrane / vesicle formation, expertise on modelling diffusion of membrane proteins.

3. Personnel

Personnel has been hired as planned in the original application, with one exception, in consultation with the programme leader Kapitein has hired a postdoc instead of a PhD student. Main reason was the availability of an excellent candidate. This will have no further impact on the actual science to be done.

4. Publications

- *Remy Kusters, Lukas Kapitein, Casper Hoogenraad, and Cornelis Storm,* Shape-Induced Asymmetric Diffusion in Dendritic Spines Allows Efficient Synaptic AMPA Receptor Trapping, Biophysical Journal, 105, 2743-2750, 2013.
- *Remy Kusters, Cornelis Storm,* Impact of morphology on diffusive dynamics on curved surfaces, Phys. Rev. E, accepted 2014.
- *Harinath Doodhi, Eugene A. Katrukha, Lukas C. Kapitein, Anna Akhmanova,* Mechanical and Geometrical Constraints Control Kinesin-Based Microtubule Guidance, Current Biology, 24, 322-328, 2014.

5. Valorisation and outreach

One of us (Peterman) is involved in the foundation of LUMICKS B.V., a company that will design, construct and market optical instruments for the scientific market. Initial products will focus on correlative optical tweezers – fluorescence microscopy instruments. In case our programme will result in technical innovations, LUMICKS B.V. might be a natural partner.

Research in the group of Peterman has received quite a bit of attention in the media, mainly because of his oration July 4th 2014 (http://youtu.be/e72r9vVlEVY). He has attended a radio show (Hoe?Zo!; http://www.npo.nl/hoezo-wetenschapscafe-05-07-2013/05-07-2013/WO_NTR_376946), a movie on his research has been made (http://fastfacts.nl/en/content/erwin-peterman-movement-cell) and he has been interviewed in FOM express (*Blik op de (zomer)week*).

The Hoogenraad group has produced a fascinating online movie, presenting their research to the wider audience (http://youtu.be/tMKlPDBRJ1E). In addition to a radio interview (Hoe?Zo! 'Verkeersregels' in zenuwcellen opgehelderd), an interview in *AD/Utrechts Nieuwsblad* and widespread media attention in response to publication in Nature on structure of botox with its receptor ('Utrechtse antistof tegen dodelijkste gif ter wereld').

FOM - 12.0278/2
datum: 01-01-2014

APPROVED FOM PROGRAMME

Number	137.
Title (code)	Barriers in the brain: the molecular physics of learning and memory (BIB)
Executive organisational unit	BUW
Programme management	Prof.dr.ir. E.J.G. Peterman
Duration	2012-2016
Cost estimate	M€ 1.8

Concise programme description

a. Objectives

The programme focuses on the molecular physical processes underlying regulation of synaptic strength, with special emphasis on spines: the transmitting end of synapses. In particular we aim to answer the following questions:

1. What governs the morphodynamics of the spine neck?
2. How does the spine neck affect 2-dimensional AMPA-receptor diffusion?
3. Can the deformation of AMPA-receptor vesicles help to overcome the barrier posed by the spine neck?

b. Background, relevance and implementation

The human brain consists of more than one hundred billion neurons, intricately connected into functional neuronal circuits. These circuits enable us to feel, to express emotion, to sense the world around us, to move and to be creative. The basic structure of the neuronal circuitry is that of a network – individual neurons that interconnect at specialized cell-cell contact sites called synapses. At these sites, the action potential propagating along an axon (the long, transmitting protrusion of a neuron) triggers the release of small molecules – neurotransmitters – which in turn are sensed by the receiving cell using specialized receptor proteins embedded in the plasma membrane of dendrites (the receiving protrusions of neurons). Precise control of the development, connectivity and strength of synapses is critical for accurate neural network activity and normal brain function, including learning and memory formation [1]. In fact, most of what we learn or remember is encoded – permanently or transiently - by modulating the strength of the specific synapses. By using an interdisciplinary approach, ranging from single-molecule biophysics *in vitro* and *in vivo*, via soft-matter theory, to neuronal cell biology, we seek to quantify the basic, physical processes that are at the heart of synaptic strength-regulation. We will focus on three aspects. First, we will unravel the molecular interactions that regulate the shape of the dendritic spine, the transmitting end of the synapse. Second, we will investigate how diffusion of neurotransmitter-receptors in the membrane is modulated by spine shape. Third, we will study how vesicles containing neuro-

transmitters are transported by molecular motors and how barriers like the spine neck are overcome.

Funding

salarispeil cao per 01-07-2010

bedragen in k€	≤ 2013	2014	2015	2016	2017	2018	≥ 2019	Totaal
FOM-basisexploitatie	665	434	399	257	-	-	-	1.755
FOM-basisinvesteringen	-	-	-	-	-	-	-	-
Doelsubsidies NWO	-	-	-	-	-	-	-	-
Doelsubsidies derden	-	-	-	-	-	-	-	-
Totaal	665	434	399	257	-	-	-	1.755

Source documents and progress control

a) Original programme proposal: FOM-11.1197
b) Ex ante evaluation: FOM-11.1427
c) Decision Executive Board: FOM-12.0277

Remarks

The final evaluation of this programme will consist of a self-evaluation initiated by the programme leader and is foreseen for 2016.

EK par. HOZB

Subgebied: 100 % FL

Historical overview of input en output

Input	personnel (in fte)				finances* (in k€)
	WP/V	WP/T	PhD	NWP	
2012	-	0.3	0.6	-	40
2013	-	1.4	3.8	-	381

Output	PhD theses	refereed publications	other publications & presentations	patents
2013	-	4	6	-

* After closing the financial year.

PhD defences

2013
None.

Patents (new/changes)

2013
None.

Overview of projects and personnel

Workgroup FOM-E-10

Leader	Dr. C. Storm
Organisation	Eindhoven University of Technology
Programme	Barriers in the brain: the molecular physics oflearning and memory
Project (title + number)	Modeling of active ans passive receptor transportin spines 11BIB05

FOM employees on this project

Name	Position	Start date	End date
R.P.T. Kusters	PhD	01 September 2012	31 August 2016

Workgroup FOM-L-17

Leader	Prof.dr. Th. Schmidt
Organisation	Leiden University
Programme	Barriers in the brain: the molecular physics oflearning and memory
Project (title + number)	In vitro of receptor transport by diffusion 11BIB04

FOM employees on this project

Name	Position	Start date	End date
W. Pomp	PhD	01 February 2013	31 January 2017

Workgroup FOM-U-40

Leader	Prof.dr. C. Hoogenraad
Organisation	Utrecht University
Programme	Barriers in the brain: the molecular physics oflearning and memory
Project (title + number)	The molecular basis of dendritic spinemorphodynamics 11BIB02

FOM employees on this project

Name	Position	Start date	End date
L.M. Will	postdoc	01 August 2013	31 July 2014

Leader	Prof.dr. C. Hoogenraad
Organisation	Utrecht University
Project leader	Dr. L.C. Kapitein
Programme	Barriers in the brain: the molecular physics oflearning and memory
Project (title + number)	Imaging of spine morphodynamics and receptortransport in living neurons 11BIB03

FOM employees on this project

Name	Position	Start date	End date
M. Adrian	PhD	01 October 2012	30 September 2016
E. Katrukha	postdoc	01 September 2012	31 August 2015

Workgroup FOM-V-23

Leader	Prof.dr.ir. E.J.G. Peterman
Organisation	Vrije Universiteit Amsterdam
Programme	Barriers in the brain: the molecular physics oflearning and memory
Project (title + number)	In vitro studies of active receptor-vesicletransport 11BIB01

FOM employees on this project

Name	Position	Start date	End date
V.S. Kushwaha	PhD	01 March 2013	28 February 2017