KULEUVEN, DEPARTMENT OF PHYSICS AND ASTRONOMY

Bachelor Thesis

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October 31, 2015

1 EMAILS

1.1 NERF

Hi Davide
How are you? (I hope you still remember me, physics student with an internship at your lab two years ago. We met again I think last year? Yes, I′m sill very sure about becoming a researcher in neuroscience. I finished the second year of physics successfully; another step closer to what I want.
Third year of Bachelor means Bachelor thesis and it is in groups of two. I convinced my colleague (Hannelore Verhoeven) to pick the topic about neuroscience *Light distribution and thermal effects in the rat brain under optogenetic stimulation* .
Our host is Barbara Gysbrechts. Perhaps you know her? I saw on the website of Nerf that she participates/participated in the lab of Francesco Battagalia. Hannelore and I are now both reading publications and papers about optogenetics to get to know the field. Our work will be purely theoretical though; modelling the properties of light in different tissues (on the computer).
So here′s my question for you. Do you think it would be possible to show me and especially Hannelore around in the lab to see some of te practical aspects of neuroscience? It helps to get the whole picture of the experiment. But more importantly, concerning our bachelor thesis, do you think somebody from the lab, who′s working with optogenetics, would be willing to show us some of those experiments and explain his techniques?
And if it′s possible, could that happen next week or the week after? I know it′s soon, but we got our topic yesterday and our first evaluation is (already) 4 november.
Thanks a lot and see you soon (hopefully)!
Lies Deceuninck

Hello Lies
Of course I remember you. Nice to hear from you! I'm glad your interest for neuroscience hasn't subsided and that you are spreading your interest to your co-students. If you bring Hannelore to Nerf, I can show her the lab and explain a bit of what we do.
I saw Barbara around the lab, but I don't know her personally. Anyway you chose a very interesting topic: congrats!
At the moment we have only one person actively working with optogenetics in our lab. That's JJ, our post-doctoral researcher (in CC). Unfortunately, he's not going to perform optogenetics experiments until the 2nd half of November, but he can answer some of your questions regarding the practical use of this technique in freely behaving animals, together with the scientific aspects of its use (which questions about brain physiology he's addressing with it).
Hope to see you soon too, and let me know if I can be of any other help.
Cheers,
Davide

Davide
Hannelore and I are alway available on Wednesday and Friday
Perhaps you could introduce us to the lab and your techniques first and JJ could continue in the afternoon?
Let me know what suits you best.
Thank you for doing this!
Lies

Hello Lies
Shall we do it on 21st October then?
JJ will be available in the morning, as in the afternoon he's busy with experiments. You can come in the middle morning, we talk a bit, I'll show you the lab, you talk with him and then we have lunch together.
If there's chance (but I don't promise as it doesn't depend on me) in the afternoon you can attend some of the experiments going on in the lab.
Let me know.
Cheers,
Davide

2 Notes

2.1 On Light distribution and thermal effects in the rat brain under optogenetic stimulation

Optogenetics refers to the integration of optics and genetics to achieve gain- or loss-of-function of well-defined events within specific cells of living tissue[3]
There are different ways to record and studie brainsignals. Electrical with electrodes and with photons–>Optogenetics (*Optical brain stimulation.* That is what we will do.
Different organisms have beautifully evolved to obtaining mechanisms that can harvest light, using it to maintain their membrane potentials or identify suitable life environments. [1] An important class of these light-sensitive proteins (microbial opsins) are transmembrane *rhodopsins*. These proteins are useful

- activity control by light
- encoded by only one gene
- fast kinetics

Mostly they are ion channels used to regulate the membrane potential. Over the years, many research has been done to genetically modify mammalian neurons to have these light-sensitive proteins expressed in the cell. Consequently, we have the ability to control cell activity of neurons and record action potentials in the brains of mammals.
The Opsins are also modified to have a specific funcitonallity and to be sesitive for a specific wavelength.
why photogenetics?

- superior cell-type specificity
- no electrical artifact
- minimized tissue damage

In order to do experiment correctly and safely, we need an stimulation protocol; *What is the ideal intensity to optimize stimulation and minimize damage?*
To target a region in the brain, they use optical fibers (multi-mode (100 to 200 μm) or single mode + a fiber for stimulation and recording at once+ multiple brain regions). The goal is to minimize the illumination of braintissue–> High spatial resolution. The influence on te brain depends on the light intensity profile in the tissue. This profile depends on several things.
Variables for the lightsource

–>the propagation of light in the different areas of the brain; what kind of ifluence has light on the neurons.[**?**]
refractive index : n=$\frac{c}{v}$, Snell's law: $n_1 \sin\theta_1 = n_2 \sin\theta_2$
Scattering coefficient: how much attenuation occurs by scattering

- wavelenght
- sourcePower
- NA (numerical aperture)
- core diameter d_{core}

Variables for the braintissue (Wavelength dependent)

absorption < Scattering in tissue. a)Rayleigh scattering–> isotropic, b) Mie regime–> anisotropic–> in cells. g determine how anisotropic.

- absorption coefficient μ_a
- Scattering coefficient μ_s
- refractive index n
- anisotropy factor g

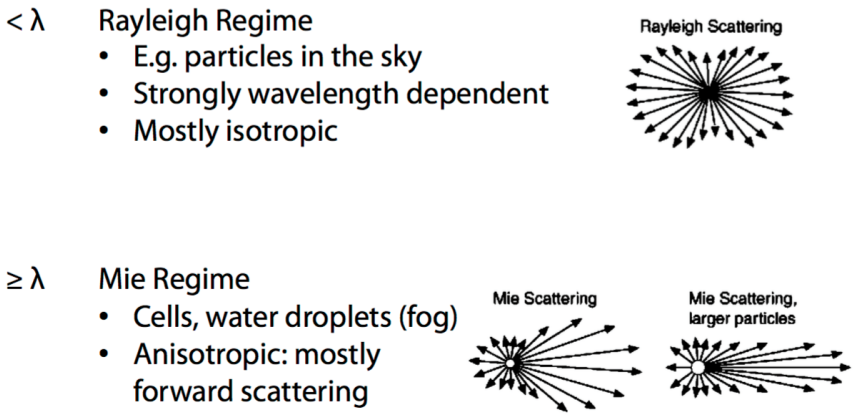

Figure 2.1: Different scatterings

In this studie, optical coefficients are measured in fuction of these properties for different rat brain tissues. *Contact spatially resolved spectroscopy* was used to study the brains. The different investigated brain regions are:

- cortex (CO)
- hippocampus (HC)
- striatuma (ST)
- thalamus (TH)

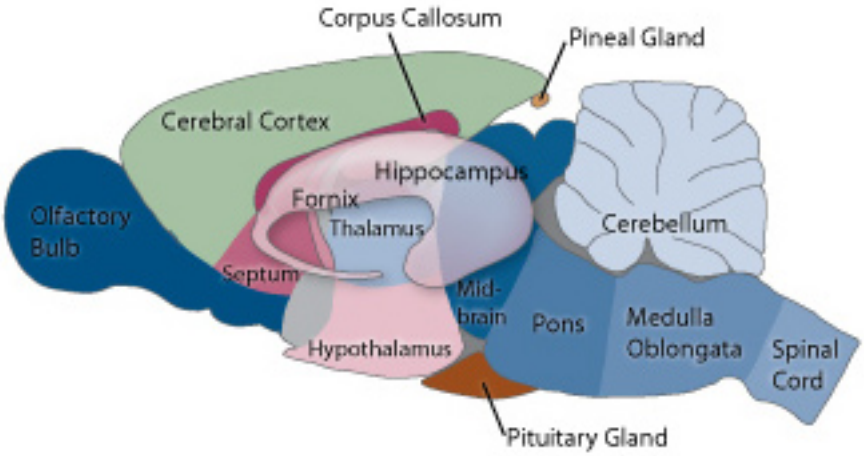

Figure 2.2: Schematic overvieuw of the Rat Brain

In all mesurements, the treshold for Opsin stimulation in $1 mW/mm^2$.

Measurements in each brain section

- preformed within 1 minute
- repeted 5 times (minimize noize)
- CO: 10 different brains
- HC, ST,TH: 7 different brains
- dark room
- within $4h$ after extraction

3 VOCABULAIRY

This section contains a list of domain specific vocabulairy [2]

Attenuation	The gradual loss in intensity of any kind of fluc through a medium	Attenuation can be caused by the quality of the glass in the optical fiber or by micro/macro bending
Optical fiber	flexible thin fiber used for data transmittance	An Optical fiber consists of a core and cladding both existing out of glass.It works on the principle of total internal reflection.
Single mode fiber	Optical fiber with only one lightsignal	Thin core that allows only one light beam
Multimode fiber	Optical fiber with more ligthsignals at once	Bigger core
Cutoff wavelength	the wavelength above which the wire will only support single mode signals	propertie of a single mode optical fibers
Mode field diameter	the diameter of the optical distribution in the fiber	propertie of a single mode fiber,some of the light (almost 30 %) propagates in the cladding layer. This is a measuere of how much propagation happens
Numerical aperture	the measure of the angular range in which the fiber accepts light	it depends on the refractive indices of the core and cladding glass
Core size	design propertie of the fiber	the larger the core, the more moving light can propagate throug the fiber (more modes)
Acquisition time	time necessary to get the data of a measurement ($140ms$)	the time between two measurements here was 1s

REFERENCES

[1] Ofer Yizhar Lief E. Fenno Satoshi Tsunoda Arash Kianianmomeni Matthias Prigge Andre Berndt John Cushman Jurgen Polle Jon Magnuson Peter Hegemann Feng Zhang, Johannes Vierock and Karl Deisseroth. The microbial opsin family of optogenetic tools. *Cell 147*, 2011.

[2] Corning Incorporated. Fiber 101. `https://youtu.be/N_kA8EpCUQo`, Juli 2013. Video,Visited on Friday 9^{th} October.

[3] THomas J.Davidson Murtaza Mogri Ofer Yizhar, Lief E.Fenno and Karl Deisseroth. Optogenetics in neural systems. *Neuron 71*, 2011.