STSM Scientific Report

COST STSM Reference Number:	COST-STSM-BM1406-33744
Period:	2016-04-17 to 2016-04-24
COST Action:	BM1406 Ion Channels and Immune Response toward a global understanding of immune cell physiology and for new therapeutic approaches (IONCHAN-IMMUNRESPON)
STSM type:	Regular (from Poland to Germany)
STSM Applicant:	Ms Iga Wasilewska, International Institute of Molecular and Cell Biology in Warsaw, Warsaw (PL), iwasilewska@iimcb.gov.pl
STSM Topic:	Role of STIM2 splice variants in calcium homeostasis.
Host:	Barbara Niemeyer, Saarland University, Homburg (DE), barbara.niemeyer@uks.eu

1. STSM purposes:

STIM1 and STIM 2 localized in the endoplasmic reticulum (ER) mediate the process of store-operated calcium entry (SOCE) by interacting with the ion channels in the cell membrane – Orai. This process is the major Ca^{2+} influx pathway in non-excitable cells, however recent body of evidence indicates that SOCE plays also important role in brain. There are known three Orai isoforms and two STIM isoforms with Orai2 and STIM2 dominating in brain. Prof. Niemeyer has shown in her recent paper that in T cells there are two splice variants of STIM2 – STIM2.1 and STIM2.2, which play opposite roles in regulation of SOCE.

Our group is investigating the role of SOCE in neurodegenerative diseases. We are working on mice model with Orai1 neuronal-specific overexpression. Tissue and cDNA samples coming from these and wild-type mice in three different stages of development – about 2-month, 5-month and 2-year old animal were sent to Barbara Niemeyer laboratory. We isolated mice brain and separate particular brain structures to investigate expression of SOCE-related genes, including recently described STIM2 splicing variants .

The purposes of this STSM was:

- to assess the expression level of SOCE-related genes in particular mouse brain structures and the ratio between STIM2.1 and STIM2.2 in them,
- to investigate the age-related changes in these genes expression
- to check if disruption of calcium homeostasis by Orai1 neuronal-specific overexpression would affect STIMs and Orais expression.
- to open the possibility of collaboration between our and prof. Niemeyer laboratory

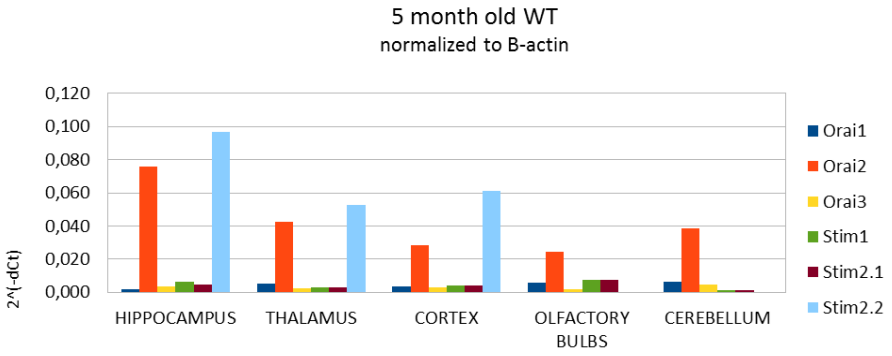
2. Description of the work

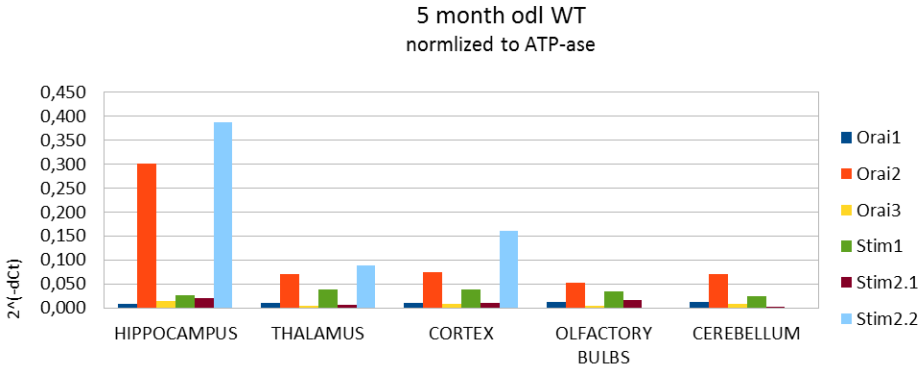
- Using quantitative real-time PCR the mRNA levels of Orai1, Orai2, Orai3, STIM1, STIM2.1 and STIM2.2 in cortex, hippocampus, thalamus, olfactory bulbs and cerebellum were assessed.
- Basing on data obtained from qRT-PCR the ratio of STIM2.2 to STIM2.1 was calculated for each of these structures.
- Using genetic material isolated from animals at different age the expression levels of all of the mentioned above genes in 5-month old and 2-years old animals were compared by qRT-PCR.
- Level of expression of SOCE-related genes in 5-month-old mice in hippocampus was compared between wild-type and ORAI1 overexpressing animals.
- STIM1, STIM2 and ORAI1 protein level in hippocampus, cortex and hypothalamus was compared between wild-type and ORAI1overexpressing mice in different ages.

3. Description of the obtained results

- Structure-specific expression pattern of SOCE-related genes.

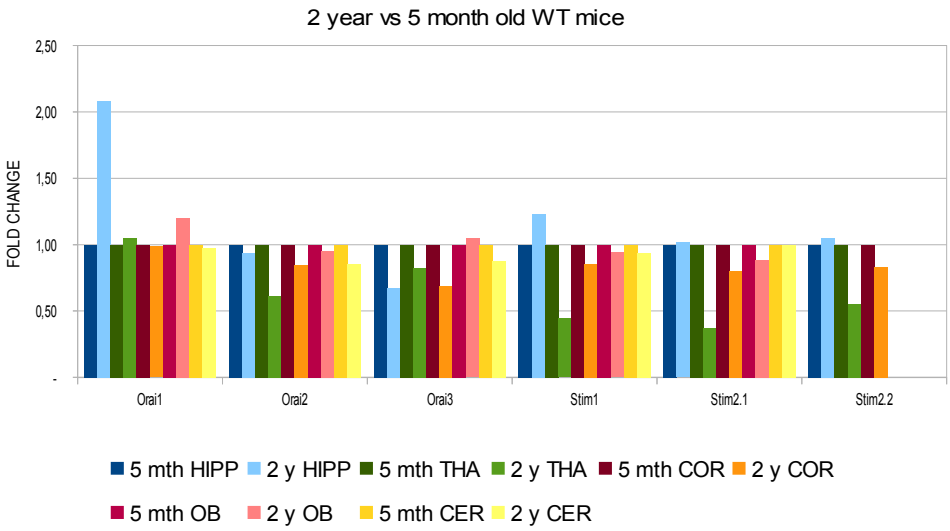
  By qRT-PCR mRNA level of Orai1, Orai2, Orai3, Stim1, Stim2.1 and Stim2.2 in hippocampus, thalamus, cortex, olfactory bulbs and cerebellum isolated from 5-month old mice. To quantify expression levels, data are presented as normalized threshold cycle (Ct) values of gene of interest to that of β-actin and ATP-ase using ΔCt method. The analysis has confirmed previously published data that Orai2 and STIM2.2 are the dominant isoforms in brain with especially high level in hippocampus. Due to technical problems any reliable data of expression of STIM2.2 in olfactory bulbs and cerebellum were obtained.





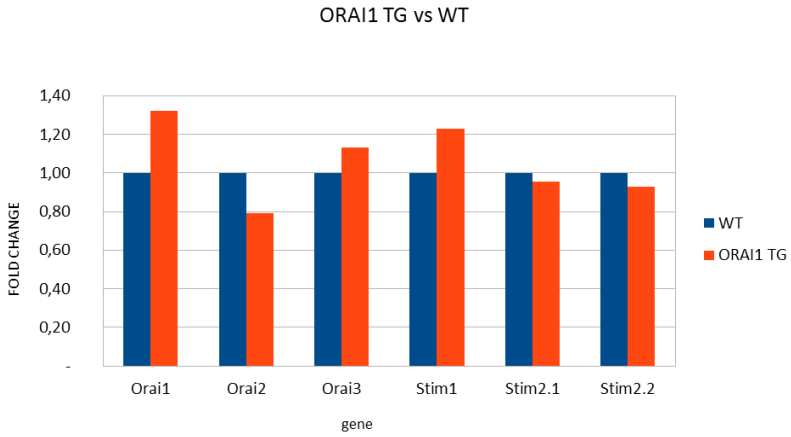
- Age-related changes in the expression of SOCE-related genes:

  The expression levels of all mentioned above genes in each structure were compared between 5-month-old and 2-year-old mice. The fold change in expression of genes of interest in old animals in relation to the young once was calculated. There is a small increase in Orai1 expression in hippocampus in old-mice, however it is worth to notice that Orai1 mRNA level in this structure is very low so this change does not seem to be significant. The decrease in all isoforms of STIM was observed in thalamus of 2-years-old mice.

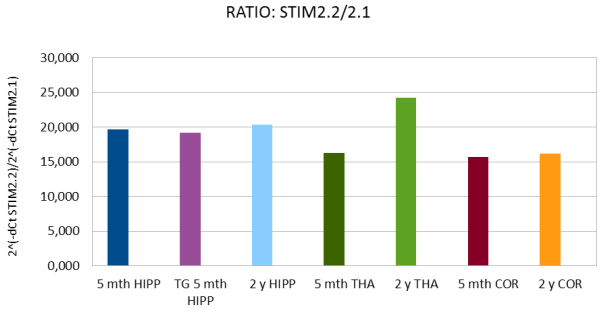


- SOCE-related genes expression in Orai1-overexpressing mice.

  The level of mRNA of mentioned above genes were compared between 5-month old wild-type and Orai-1 transgenic mice in samples coming from hippocampus. The data are presented as fold change of expression of gene of interest in transgenic in relation to wild-type animals. As no changes were detected no further comparisons between these genotypes were done.



- Ratios of STIM2.2 to STIM2.1 in all of analyzed structures were calculated. This analysis has shown that STIM2.2 is the dominant variant in the brain and level of the STIM2.1 isoform, detected by prof. Niemeyer in T-cells, is very low in all analyzed brain structures.

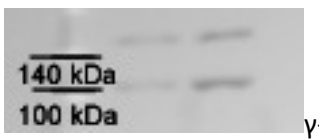


- Protein level of STIM1, STIM2 and Orai1 in Orai-1 overexpressing mice

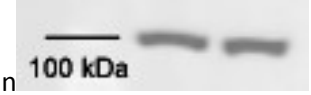
  The protein level of STIM1, STIM2 and Orai1 were compared between 5-month old wild-type and Orai-1 transgenic mice in samples coming from cortex. As expected, very high level of Orai1 protein was detected in transgenic mice, while in wild-types band for this protein is hardly visible. There are no significant changes in the level of STIM2 and STIM1 protein was not detected by used antibody. γ-tubulin was used as a reference gene.

ORAI1: 

TG WT

STIM2: 

TG WT

γ-tubulin 

TG WT

4. Future collaboration

The STSM has opened the possibility of collaboration between our and host laboratory and gave us the possibility to exchange our observations and knowledge. The work on the samples I have sent will be continued to refill lacking results.

5. Other comments

I would like to greatly thank prof. Barbara Niemeyer for hosting me in her laboratory and Dalia Alansary for her help and time dedicated to me.

I would like to record my appreciation to the MC of Cost Action BM1406 for granting the funding to allow me to carry out this STSM.