Novel Method for *In Vivo* 3D Monitoring of the Hippocampal Neurodegeneration in Sleep Deprived Hairless Mice

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Abstract

With our novel *in vivo* method we are able to quantify the hippocampal neurodegeneration caused by sleep deprivation and to measure the neurodegeneration rate as a background of a possible memory loss.
Appeared on the market in 2011, Phontoimager Optima (PIO) is an optical imaging system for fluorescent and bioluminescent *in vivo* detection in small animal models. We performed the 3D fluorescent detection of neurodegeneration *in vivo* and non-invasively in the hippocampus of SKH1 hairless mice (Fig.1.). For fluorescent detection we used the lipophilic fluorescent dye DiR after stereotaxic IHC injection (ex/em: 750/800-1000).
A possible memory loss and also regeneration was measured using the Passive Avoidance system and finally, tissue viability was measured with MTT assay on *ex vivo* hippocampal tissue.
This method and model might be an appropriate source of a better understanding the quantitative neuronal viability background of sleep disturbances and memory loss and might also provide a model for AD investigations.

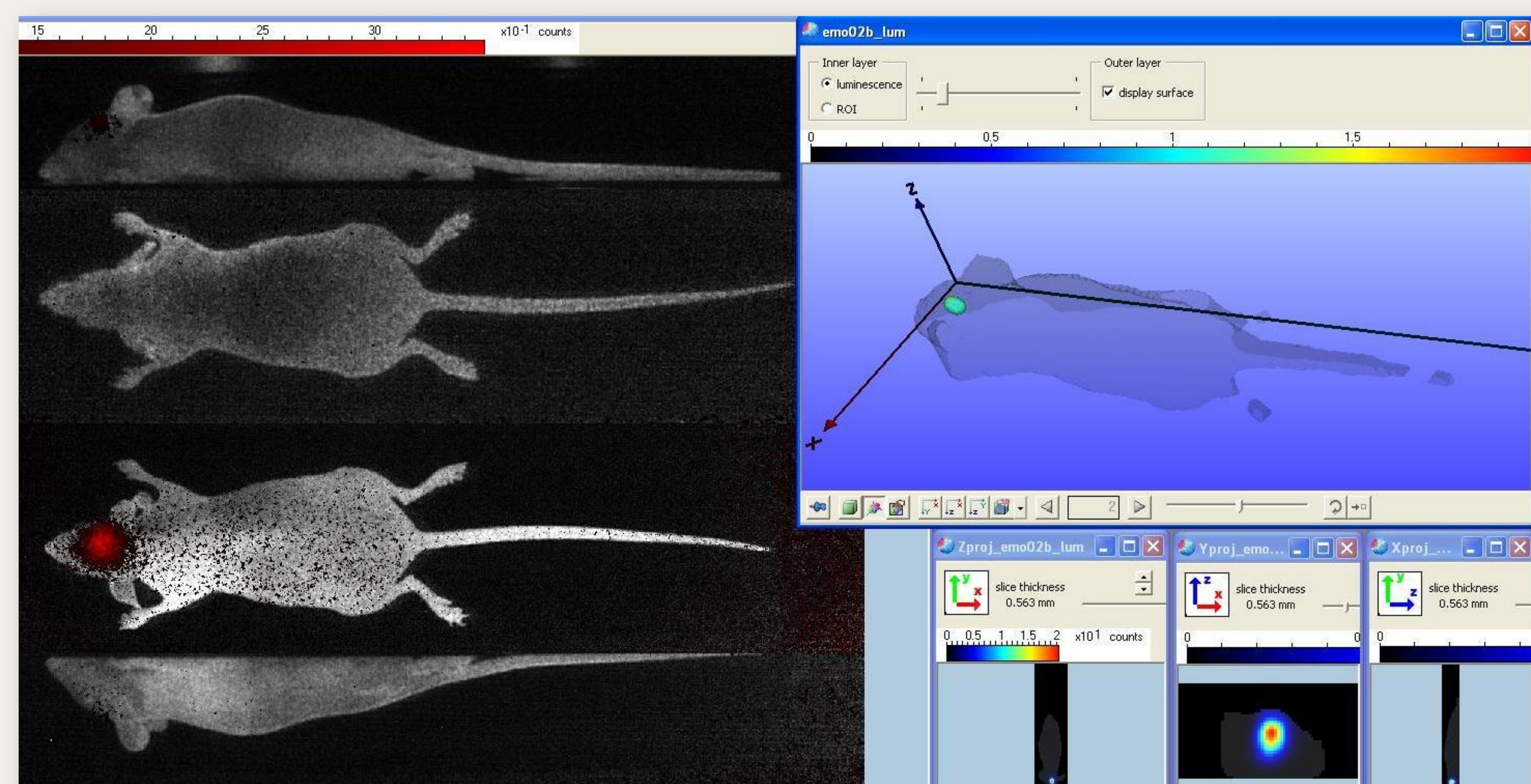

Figure 1. 3D Display of DiR Signal Detection in Mouse Hippocampus

Brief Methodology

1. Stereotaxic injection of the fluorescent dye, DiR in the hippocampus of SKH1 hairless mice

The solution (total 2.0 µl), 50 µM DiR diluted in ACSF with 10% DMSO was injected by Hamilton syringe (32 G) into the hippocampus unilaterally at rate of 0.3 µl/min, beginning 2 min after the needle was lowered. The following coordinates were used from Bregma point: AP: -0,13 ; ML: -20; DV: -0,18.

2. Sleep deprivation of SKH1 hairless mice

5 female mice were placed for 6 days into the sleep deprivation cage set up based on the classic platform method (animals are kept on small platforms surrounded by water of approximately 2 cm depth on the cage floor. Food and fresh drinking water were freely available during the sleep deprivation.)

3. In vivo fluorescent detection of the hippocampus of sleep deprived mice

7 days after the stereotaxic injection of fluorescent dye DiR and again after 6 days of sleep deprivation and 1 day resting, mice were analised with Photonimager Optima in 3D mode (Fig.1) and a quantification report was made on the signal of DiR fluorescent dye which labels only the intact neuron membranes and the signal remains stable only in non-deviding cells . Measurements were performed with the following settings: 3D (4 view) acquisition, fluorescent mode, Ex/Em: 750/800-1000.

4. Passive Avoidance Test

The passive avoidance test - performed one week after sleep deprivation - is a well-established experimental procedure used to assess short-term reference memory, which depend on cortical and hippocampal circuitries . An illuminated and a dark compartment is separated by a guillotine door. For the acquisition trial mice were placed in the illuminated compartment and the door between the two compartments was opened 30 s later. When the mice entered the dark compartment, the door closed automatically and an electrical foot shock (0.6 mA) of 1 s duration was delivered. 24 hours after the acquisition trial, the mice were again placed in the illuminated compartment for retention trials. The time taken for a mouse to enter the dark compartment after the door opened was measured as the latency time in both acquisition and retention trials, with a maximum of 298 s.

5. MTT viability test of ex vivo hippocampal tissue of sleep deprived mice

Bilateral hippocampi was removed from mice brain 24 hours after sleep deprivation and MTT viability test was perormed. 0.5 mg/ml MTT stock solution was added to the hippocampus tissue in ACSF. After 20 minutes the solution was removed and pure DMSO (0,5 ml/hippocampus hemisphere) was added to dilute the formazane resulted from MTT reduction. The optical density (OD) of the formazane –DMSO solution was measured in 96 well-plaets (70 uL/well) with Fluorescent Plate Reader.

Results

1. In vivo fluorescent detection of the hippocampus of sleep deprived mice

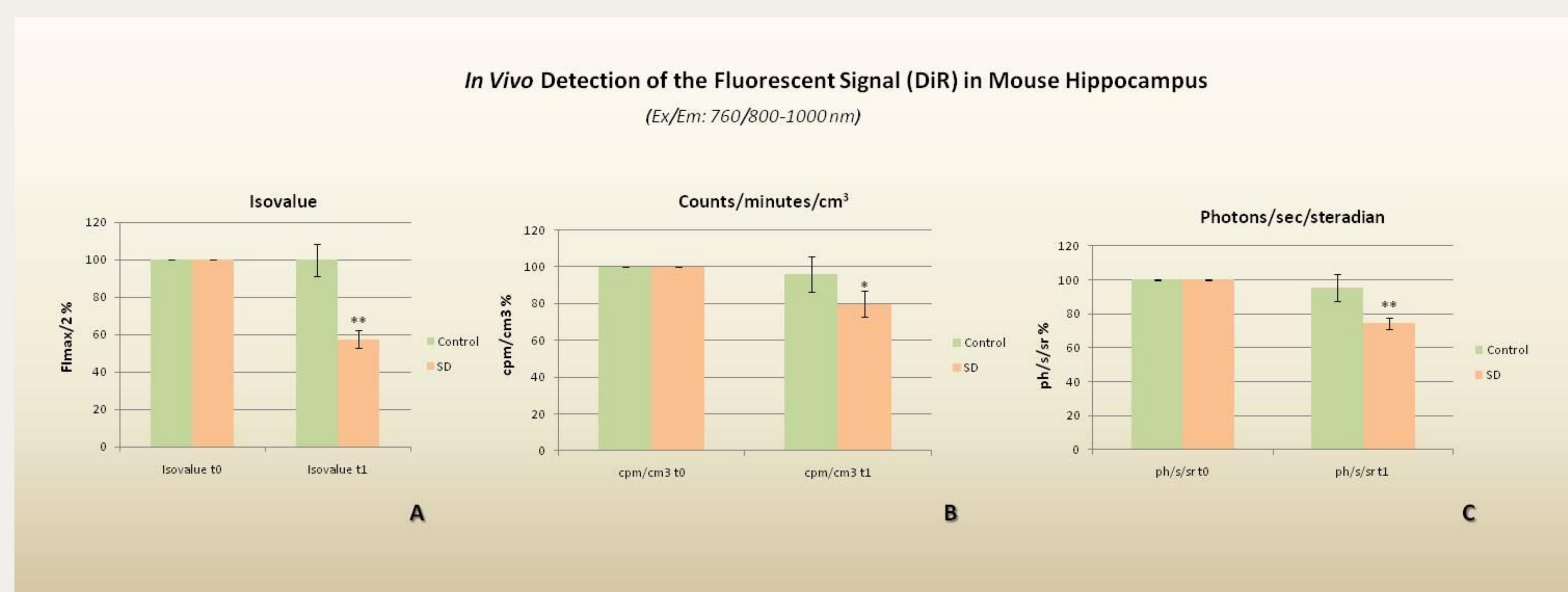

Figure 2. Quantification report of in vivo fluorescent detection of sleep deprived hairless mice

The quantification report of the *in vivo* frluorescent detection – performed with Photonimager, a small animal tomograf - of unilaterally DiR labeled hippocampus of SKH1 hairless mice was obtained by comaparing the results of the first measurement (t_0) before sleep deprivation (SD) and the second (t_1) measurement of the same animal (self-control trial). The avarage of the obtained data are illustrated in the percentage of t_0 results. ***DiR only labels intact neuron membranes*** and the difference between the fluorescence intensity of the measurment before and after SD can reveal neuron-loss in hippocampus. In all three (A, B, C) cases paired *t*-probe was performed, see p values above. N=5 control and 5 SD mice.

A: Isovalue is the half of the maximum fluorescence intensity (FImax/2), quantified by the software of the photonimager. p=0.0039 ; SEM of Control: 8,63, SEM of SD group: 4,66.

B: Counts/minutes/cm³ reveals the photon number detected per minute in 1 cm³ of the fluorescent signal. p=0,0436 ; SEM of Control: 9.62, SEM of SD group: 6,95.

C: Photons/s/steradiane reveals the scattering of photons in the tissue. p=0.0017; SEM of Control: 7.82, SEM of SD group: 3,32.

2. Passive Avoidance Test

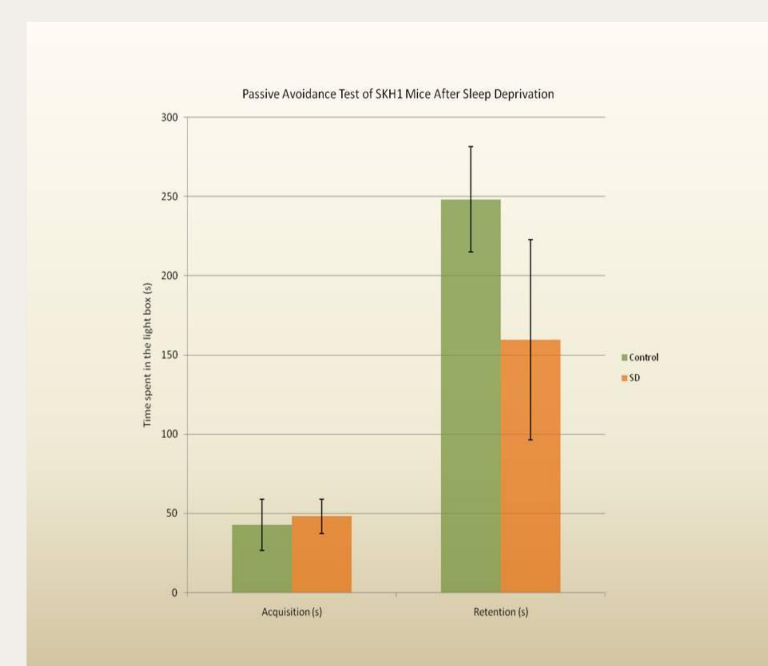

Figure 3. Results of the Passive Avoidance Test of Sleep Deprived Mice

The passive avoidance test - performed one week after sleep deprivation - is a well-established experimental procedure used to assess short-term reference memory, which depend on cortical and hippocampal circuitries. Obtained data reveal a mild difference in the latency time of sleep deprived (SD) and control group, however the difference is not significant. Further experiments with higher case number should be performed. SEM of Control: 33,23; SEM of SD group: 63,07. (paired *t*-probe)

3. MTT viability test on *ex vivo* hippocampus tissue

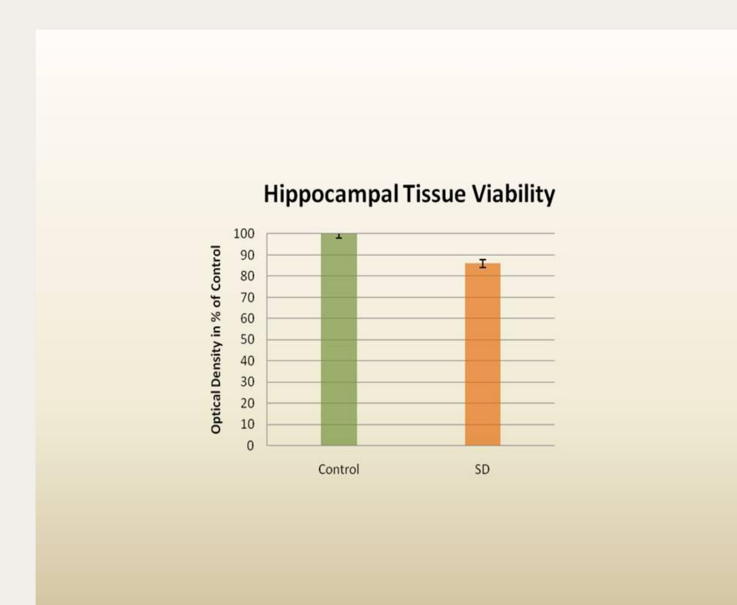

Figure 4. Ex vivo hippocampus tissue viability of sleep deprived mice . REPRESENTATIVE DATA

Classic viability test – MTT – was performed in order to compare the hippocampal tissue of sleep deprived (SD) and control mice. The test was performed 24 hours after 6 day S of SD. Data shown is from a pre-study, further investigations are required.

Conclusion

After further investigations this model might be an appropiate source of a better understanding of neurodegenarative background of sleep disturbances and can provide a neurodegenration model for Alzheimer's disease investigations. Based on literature there is a connection between the lack of sleep and the development of AD, however the mechanism yet is unclear. Subsequently we aim to study also the connection between β-amyloid toxicity and sleep deprivation *in vivo* and *ex vivo*.

HUNGARY'S RENEWAL

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