Charles University in Prague

Faculty of Sport and Physical Education

Summary of the disertation thesis

OPTICAL SIGNALS OF THE BRAIN

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SUMMARY

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SUMMARY

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The aim of this project is to introduce the topic of intrinsic optical signals and show different possibilities for the detection of changing optical properties of nervous tissue both in vitro and in vivo.

The detection system of both light source and attachment of the optical fiber to the skull of the animal for in vivo experiments have been designed. The proper experimental part of the study have been done in hippocampal tissue slices and shows the correlation of the tissue activity and the optical signal when affecting ion channels, glutamatergic synaptic activity, glial component of the signal and mitochondrial signal.

We are also dealing with the source of the noise that we inevitably record and should avoid while detecting these rather subtle changes of optical properties. We are introducing the software Vision Brain for synchronous detection of both electrical and optical changes.

Mapování optických signálů mozku

Mgr.Renata Konopková

Cílem této disertační práce bylo uvedení optických signálů jako zobrazovací metody mapující aktivitu nervové tkáně pomocí změn optických vlastností tkáně.

Součástí disertace je popis experimentálního zařízení pro snímání optických signálů, respektive návrh na jeho upevnění v lebce potkana spolu s návrhem zdroje světla.

Vlastní experimentální studie definuje vznik optických signálů v hipokampálním řezu potkana a ukazuje korelaci optických signálů a fyziologických dějů tkáně na úrovni iontových kanálů, glutamatergního přenosu, gliového a mitochondriálního signálu.

Dále se v disertační práci zabývám vznikem šumu, který detekujeme spolu s vlastním signálem, jeho odlišením a definicí. V neposlední řadě představuji software VisionBrain pro synchronní detekci elektrických a optických signálů tkáně.

1. INTRODUCTION

In neuroscience we learn about the elementary processes governing the function of CNS. Intrinsic optical signals are the signals reflecting changes in transmittance or reflectance of the light illumitating the tissue. Registration of such changes can be applied for the detection of physiological condition in the slices of biological tissues (in vitro) or in the whole animal (in vivo) and be used as one of the imaging technique (Pouratian, 2003).

There are many processes uderlying changing optical properties, the main component of these changes are ionic changes outside and inside of the cell which are coupled to the cellular volume changes and are cosequence of neuronal activity (Buchheim, 2005; Macvicar, 1991).

In vivo the detection of IOS is more demanding because of the presence of the respiratory and cardiac movements, hand in hand with the spontaneous movements of the animal and the flow of the blood present in the tissue makes difficult to distinguish the subtle optical changes from the noise. In vitro imaging is used especially for the experimental purposes to define some of the events or mapping the spread of the activity especially in the cerebral cortex (Grinvald, 1991) or epileptic activity (Haglund, 2004).

In this work, I am presenting optical signals in context of different cellular compartments, biomechanical properties and design the device that would enable us to detect these changes in vivo as well as in vitro.

I defined the signal in vitro using pharmacological agents to influence the ionic channels, excitatory synapses, extracellular volume changes as well as glial and mitochondrial signal. The other part is a summary introducing design of the imaging setup for in vivo detection and the software for synchronous detection of optical signals, it is also a summary of the work that has been done so far at the Department of Developmental Epileptology as a part of the project dealing with the optical signals

IOS

Already in the forties, the first studies dealing with changing optical properties of the tissue appeared. These were the studies done on non myelinated fibers of nerve trunk, the observed increase in the scattering of white light were thought to represent axonal swelling during the activity of the nerve (Hill and Keynes, 1949). Some 30 years later, Lipton (Lipton, 1973) made the first more advances recordings of changing optical properties reflectance respectively on the tissue slices using photodiode. It has been suggested that lowering bath osmolarity, hypoxia, elevated K^+ and electric stimulation decreased the reflectance of the illuminated tissue, these changes were reported as a result of the volume of the tissue as a whole.

In the studies that followed this research studied properties of the nervous tissue and as the technologies advanced, the first experiments done on the animal (in vivo) advanced. Mac Vicars work on intrinsic optical signals are crucial in this field and set the IOS as a new imaging method, suitable for mapping the spread of activity in nervous tissue in time without the need to load the tissue with dyes (MacVicar, 1991). IOS further posses substantial spatial specificity allowing for example experimental evaluation of the neural mechanisms that mediate the response of different cortical layers to repetitive stimuli (Kohn et al., 2000) or mapping the cortical activity which can be further used in even in human epileptology (Pouratian, 2002)

AIMS

1. We would like to characterize and define the optical signal in our in vitro conditions with the aim to apply these data in the other experiments such as pharmacological studies and studies mapping the epileptic activity in vitro.

2. We would like to define the different sources of noise and to use this knowledge in the following experiments.

3. The last aim of this thesis is the concept of development of device for the clinical practice based on IOS, for the animal in vivo experiments enabling detection of both optical and electrical signals using our specialized software VisionBrain.

2. EXPERIMENT

2.1. Sources of noise

Primarily two different sources of the noise can be distinguished in our experiments, noise in the captured scene and the noise arising from the light detector (CCD camera Retiga 2000R, QImaging). The final quality of the measurement is characterized by **signal-to-noise ratio (SNR)**, the ratio of signal power to the noise power corrupting the signal. **SNR** higher than one indicates more signal than noise.

The following equation is commonly used to calculate CCD camera sensor signal-to-noise ratio (1)

$$SNR = \frac{IQ(e)t}{\sqrt{IQEt + Ndt + Nr^2}} \qquad \textbf{(1)}$$

I is the photon flux(photons/pixel/second),

$Q(e)$ represents the CCD quantum efficiency,

t is the integration time (seconds),

Nd is dark current (electrons/pixel/second)

Nr is read noise (electrons).

Accesing signal to noise ratio:

We have set the different conditions (5 scenes) and access the signal to noise ratios with the aim to reveal the biggest sources of the unwanted noise.

1. **scene** – empty recording chamber

2. **scene** – recording chamber filled with water

3. **scene** – recording chamber with floating water – flow and pulsation of water, gas bubble

4. **scene** – recording chamber with floating bubbled acsf – flow and pulsation of acsf, gas bubble

5. **scene** – tissue slice without stimulation – slice movement caused by acsf flow

Index	SRN [dB]
1	25.0394
2	24.1494
3	18.9537
4	23.5003
5	24.1979
average	**23.1682**

Table 1

Certain sources of noise can be eliminated. Several sources of the noise that can influence resulting signal can be easily avoided. Figure 1 illustrates impact of the surrounding light. The step corresponds to covering of faraday cage that prevents from detecting other light that the one from microscope light source. If we can't afford to have the door closed, we have to at least make the conditions constant.

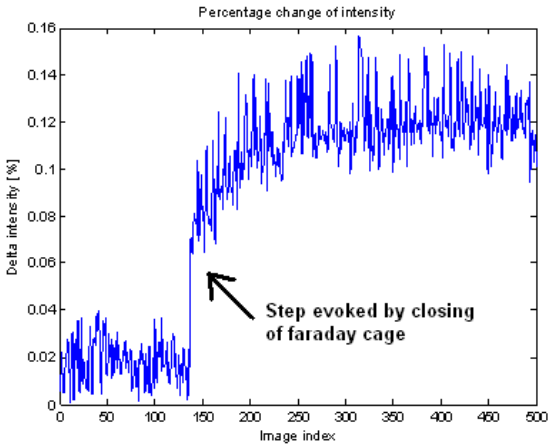

Figure 1

The other fact that has to be considered during recording is the transition curve that accompanies every change of intensity of the light source (Figure 2, Figure 3) illustrates those characteristic, measured during increasing resp. decreasing the intensity. The course has got decreasing signal in case the light has been decreased and the opposite course during increase of the signal. This problem can be easily eliminated when let sufficient period of

time period before we start the detection after the last change of the intensity of the light source. During experiments, we should avoid manual adjustment of the light source and correct the lightening by the time of the exposition. Or the other radical change is to use more stable light source like LEDdiode.

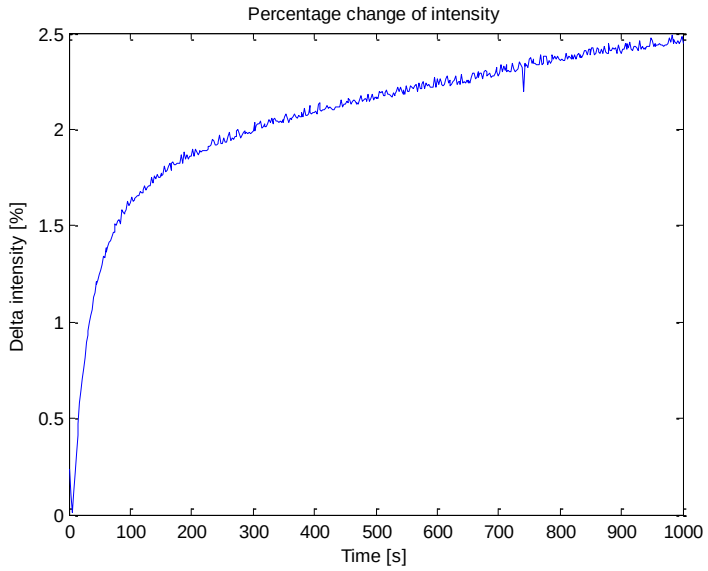

Figure 2

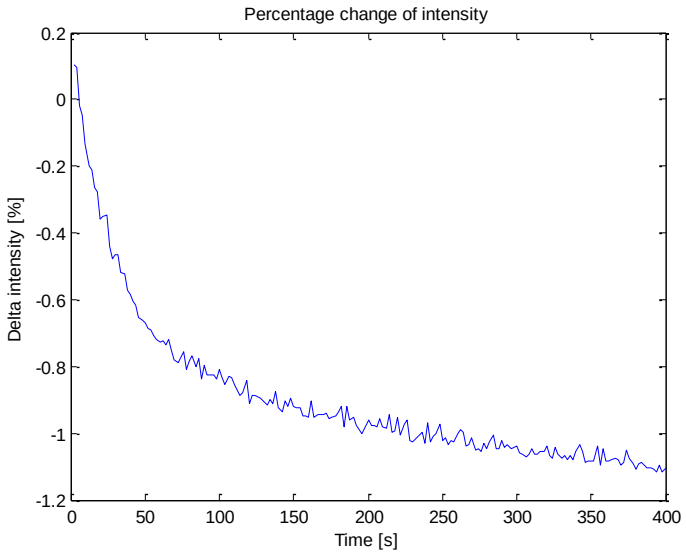

Figure 3

(Figure 2 Figure 3): Change of the intensity evoked by decreasing the intensity of the light source, Change of intensity evoked by manually increasing of intensity of the light source, x axes stand for time course, the capturing have been done with the pattern frames per 2seconds

Ilumination start[min]	Koeficient Linear Regression
0	0.0775
5	0.0026
10	0.0012
20	9.2275e-004
30	4.8190e-004
40	9.1709e-005
60	4.6662e-005

Table 2
For the further evaluation, coefficient of linear regression has been set to illustrate the slope of the curve. The coefficient is related to the angle θ that line makes with the positive axis of time via tangent function: $coef = \tan\theta$.

As we can see some noise components can be quite easily eliminated, some of them not. Such inevitable components are caused basically by time variance of the captured scene; water or ACSF flow, gas bubble movements, and natural movement of the slice. Several experiments (see results) were made to define the influence of the different components. Noise amplitude caused by those components is at least one digit place lower than amplitude of actual signal which is sufficient for the detection of the signal.

2.2. In vitro experiments

2.2.1. Experimental definition of IOS, origin of the signal

Strength of IOS signal

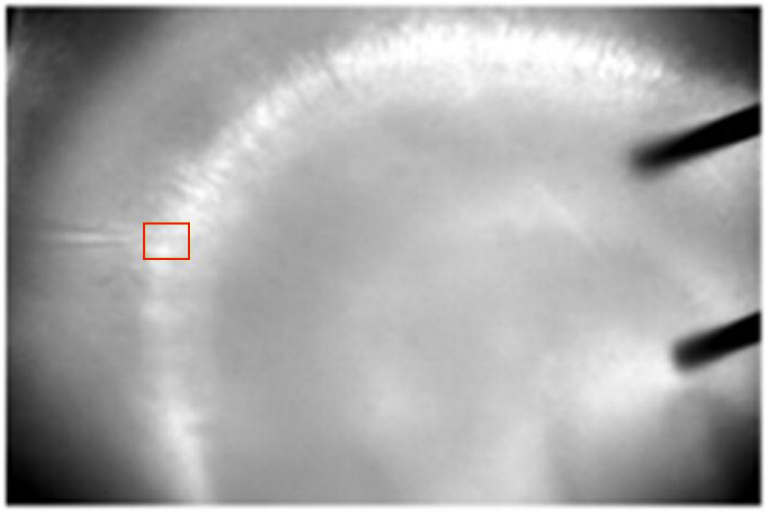

Fig. 4: On the right side, note the stimulating electrode placed in DG (dentate gyrus) of hippocampus, on the left, we can see a glass registrating electrode in the pyramidal layer of CA3 region of hippocampus. ROI was places in the region where the registration electrode was placed which was in CA3, see the yellow square. Fluorescence microscope image (20/0.5) (Olympus BX51WI)

The total number of 11 slices has been in the evaluation. The maximal increase in intensity of transmitted light achieved was determined and included into the evaluations. The first maximal intensity obtained during 2T (double of the threshold value) stimulation has been set as 100% value and the other intensities have been evaluated in percents of this value. There was statistically significant difference between both groups compared to the 2T (P=>0.001). The change in LT in the 3T stimulation was 150.6% compared to 100% for the stimulation 2T with standard error 16.3%. In the stimulation 4T the change in LT was 173.9% with standard error 21.8%.

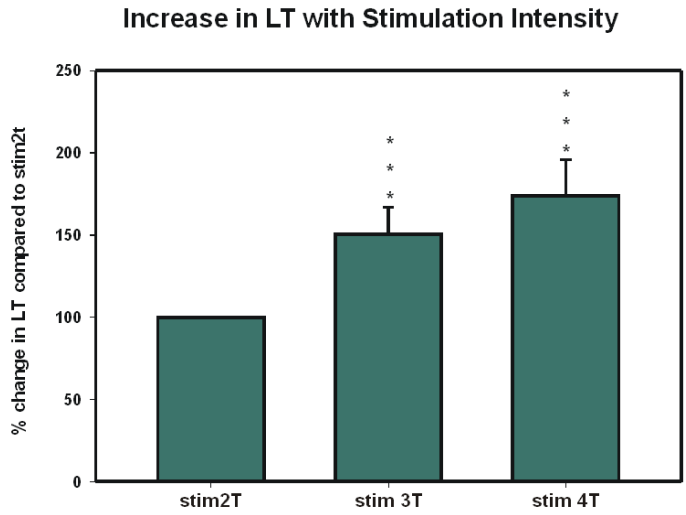

Figure 5 for the statistical comparison, Mann-Whitney test was used. There was statistically significant difference between the 2T and 3T group and 2T and 4T group (P=>0.001). With the following results: the change in LT in the 3T stimulation was 150.6% compared to 100% for the stimulation 2T with SE 16.3%. In the stimulation 4T the change in LT was 173.9% with SE 21.8%.

LT increase is stimulation intensity dependent. The other experiments are essential to estimate the strength of the signal and its neural character.

Block of AP

TTX is potent neurotoxin, specifically blocking voltage-gated sodium channels on the surface of nerve membranes. Slice was submerged in ACSF, approximately 15 min of wash in with ACSF containing TTX (1μM). TTX has been washed out. The AP appeared 45min after wash out.

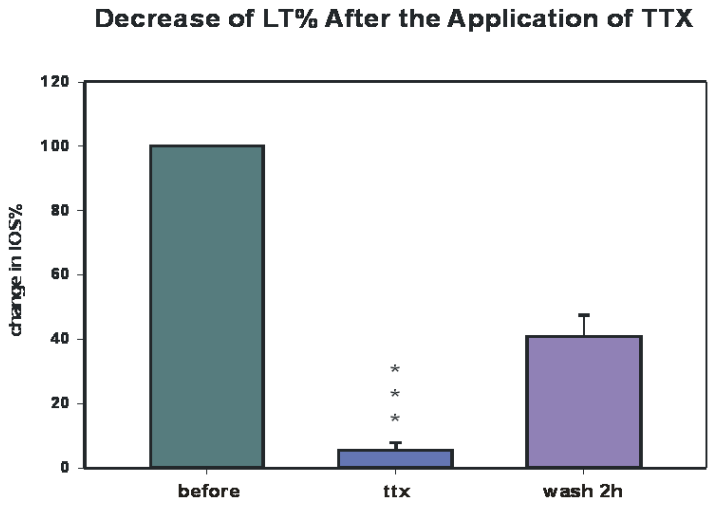

Figure 6 The changes in LT in CA3 pyramidal cell layer were almost completely abolished by TTX (1uM in ACSF), LT increase was led down to 5.4 ± 2.4% compared to the control measurement, indicating that the synaptic activation was responsible for the changes of LT. For the statistical comparison, Mann-Whitney test was used. There was statistically significant difference between in the group before and after the application of TTX (P=>0.001).

We determined the maximal change in LT in 4 slices with and without the application of TTX. LT has been decreased to 5%. If we block Na channels (generation of action potential), LT is significantly increased, which points to the fact LT is a consequence of the activity of the nervous tissue and action potential generation.

Pharmacology - IOS glutamatergic transmission block APV/CNQX

To distinguish between the synaptic transmission and non-synaptic activity of the nervous tissue, we completely blocked excitatory glutamate receptors using a selective NMDA receptor blocker APV 50uM (R-2-amino-5-phosphonopentanoate) and further AMPA/kainate receptor blocker 10uM CNQX (6-cyano 7 nitroquinoxaline-2,3-dione). Maximal LT was determined during supramaximal stimulation intensity.

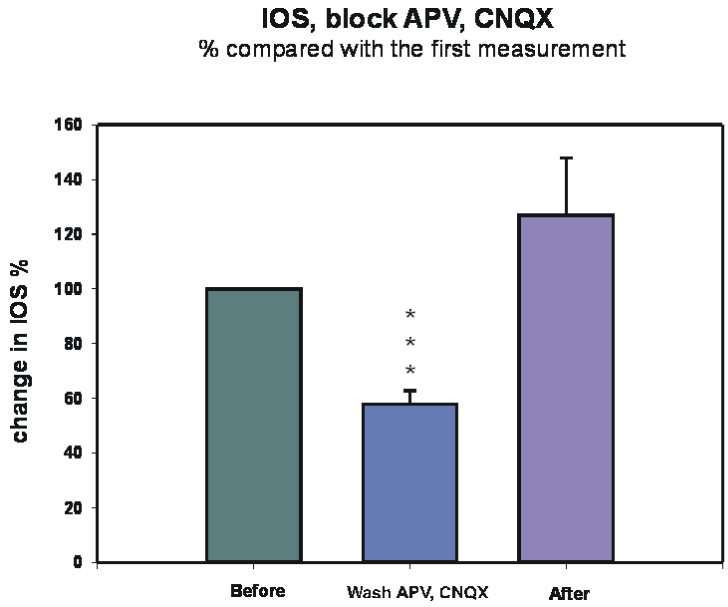

Figure 7 The changes in LT were led down to 57.9 ± 4.9% compared to the control measurement, indicating that the glutamatergic synaptic activation was responsible for the changes of LT. For the statistical comparison, Mann-Whitney test was used. There was statistically significant difference between in the group before and after the application of APV, CNQX (P=>0.001).

In this case LT was also decreased to 57.9% ± 4.9%.The first measurement, slice was submerged in ACSF, 40 min of wash in with ACSF with APV, CNQX in the concentration 50μM (APV) and 10μM (CNQX). After the second measurement, the wash out (40min), the third stimulation was being made. The LT signal was block to half which proves that the signal is glutamate dependent. The exact dependency of the signal is might be changed reflecting the fact we used glutamate receptor blocker that might compete over the binding site of glutamate and don't bind to all the binding sites.

IOS signal arising from glial cells

DL-TBOA in the concentration has been used in the perfusion (ACSF) to block glial glutamate reuptake. In the second set of experiments TBOA has been added into the perfusion in combination with APV, CNQX. Stimulation intensity: supramaximal, LT has been evaluated in the pyramidal cell layer of CA3.

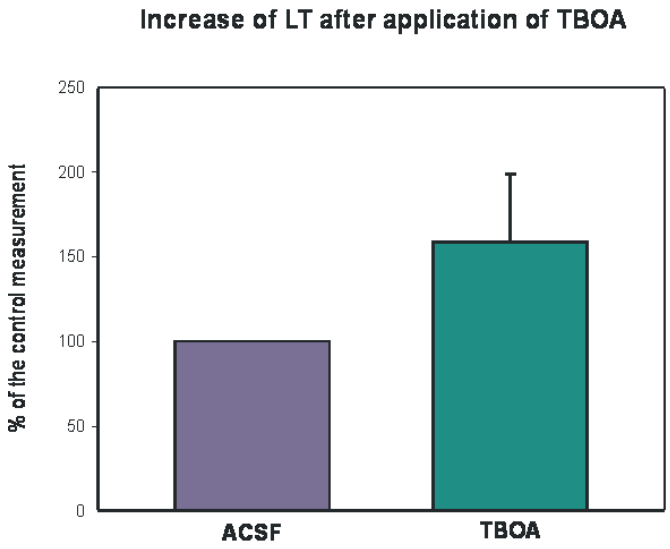

Figure 8, The graph represents percentage of the change of the maximal increase of the IOS during the stimulation. When adding TBOA into the perfusion media the optical signal during stimulation increased up to 159% ± 19.62%

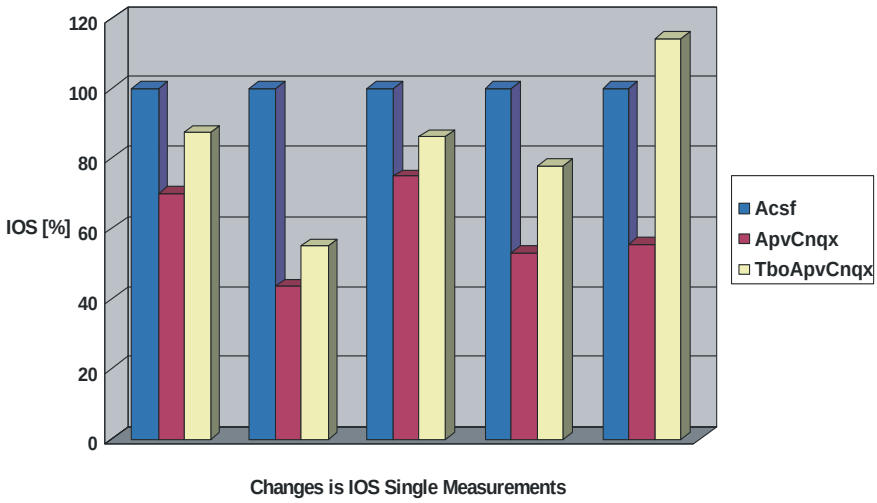

Figure 9 The graph represents percentage of the change of the maximal increase of the IOS during the stimulation. When adding APV, CNQX into the perfusion media the signals were reduced to 60.46% ± 7.2% and when adding TBOA the optical signal during stimulation increased up to 76.74% ± 7.48%

The changes of LT while inhibiting glial glutamate transport using TBOA showed the increase of LT 159% ± 19.62% in 4 slices. When using APV, CNQX into the perfusion media the signals were reduced to 60.46% ± 7.2% and when adding TBOA the optical signal during stimulation increased up to 76.74% ± 7.48% in 4 slices. So we can conclude that TBOA application results in the increase of the optical signal. While reducing the signal via APV, CNQX and subsequent application of TBOA into the perfusion media, the signal slight increase.

Furosemide- changes in ECS, volume changes

Furosemide was added into the perfusion in concentration of 0,2mM. IOS has been evaluated as the maximal increase of LT during supramaximal stimulation intensity in pyramidal cell layer

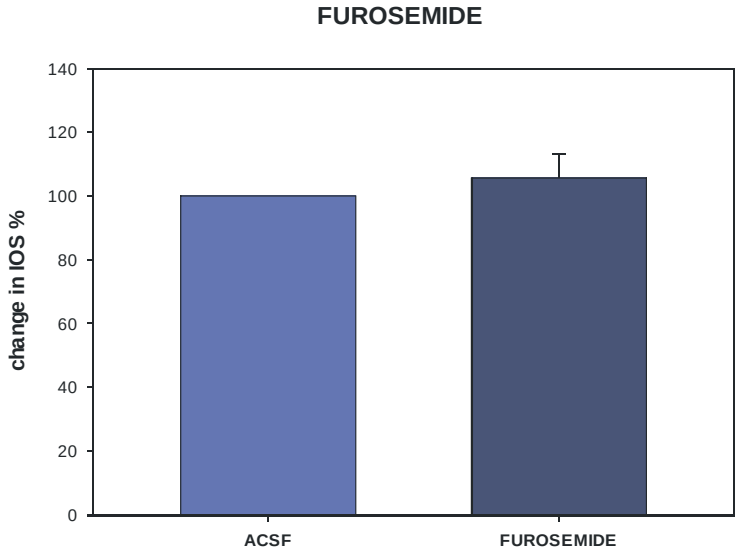

Figure 10 The graph represents percentage of the change of the maximal increase of the IOS during the stimulation. There was insignificant increase after application of FUR (111.88% ± 19.18%)

The change of the LT didn't show significant results after evaluation of 7slices. Although there was increase in the furosemide group 111.88% ± 19.18%. There was slight increase in LT after application of furosemide into the perfusion media.

Mitochondrial origin of the optical signal

The first measurement of LT in CA3 pyramidal cell layer was made during the stimulation 10s/20Hz with supramaximal stimulation intensity. Fluorescence (FAD) was excited at 490 ± 10 nm. Recordings were made with an epifluorescence illumination system (Olympus) that combines a fast driven excitation filter wheel and a triple band filter, allowing excitation FAD with a delay of 130 ms. FAD fluorescence images (emission 530 ± 10 nm) were recorded at 0.5 Hz using a CCD camera. Changes in FAD fluorescence are presented as changes in $\%\Delta F/F_0$, where F_0 is the averaged fluorescence of a 20 s period before stimulation of the tissue. The first maximal value from the beginning of the recording has been evaluated which corresponds to the initial fluorescence increase e.g. oxidation of FADH.

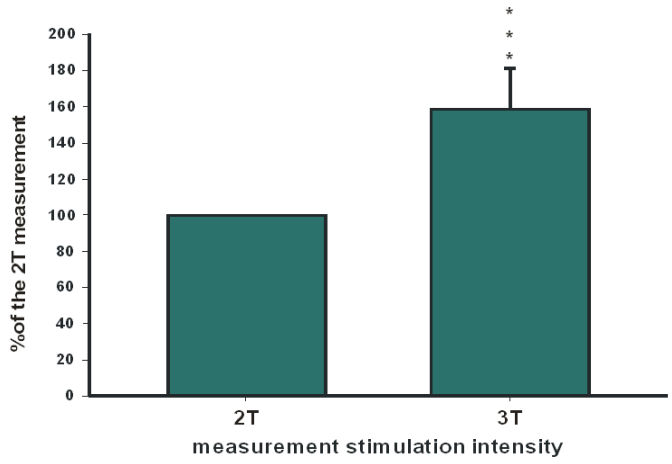

Figure 11 This graph represents the percentage of the maximal change in the intensity during stimulation of the 2T intensity.

There was an increase in the maximal value of FAD fluorescence corresponding with the increase of stimulation intensity (158.544 % ± 59.713%).

Increase of FAD fluorescence with stimulation intensity is probably the sign of increase of mitochondrial stimulation.

3. DESIGN OF IMAGING SETUP

3.1. General description of the system

We designed the device enabling detection of changes of IOS in vivo. This device enables the detection of FAD, NAD(P)H and hemoglobin in cortical areas of the rat, with the field of view 2-3mm in diameter, possibility to disconnect the device, minimal weight, biocompatibility and the size appropriate to the size. Device is attached to the animal during the experiment and enables easy reattachment. During the experiments, the device should enable the movement of the animal. For this purpose, we used optical device including optical fibre bound to the rotating connector with the solid attachement to the skull of the rat which includes the source and detection of the light with CCD camera. Optical connectors should are designed to be easily removed with the optical fiber and protect the fibers against destruction. Optic fiber transmitting the signal from the brain surface is connected through FC connector. The split of the parallel light beam going into the optic fiber is made by beam splitter. Different filters further enable to choose different wave lengths on both sides (light detector, light source). During surgery the device can be attached to the skull into the 4mm wide hole, this fixation probe should stay in the skull the rest of the device is removable with the help of rotatory connector (Horacek, 2008).

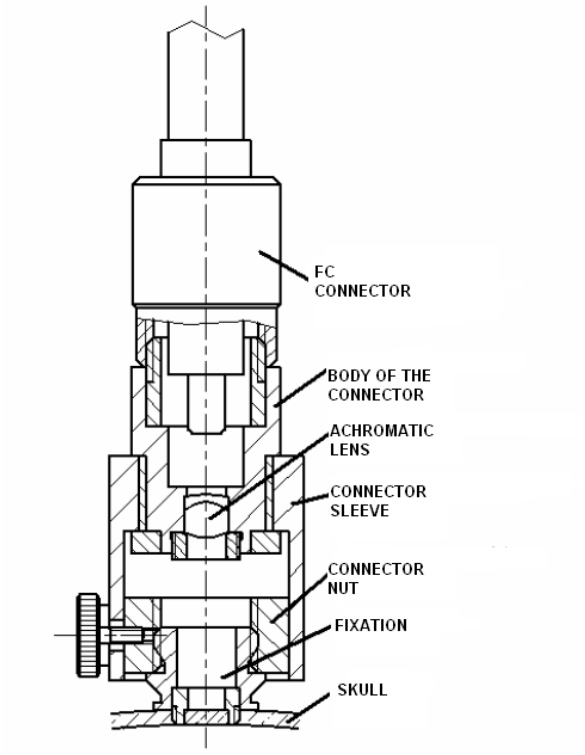

Figure 12

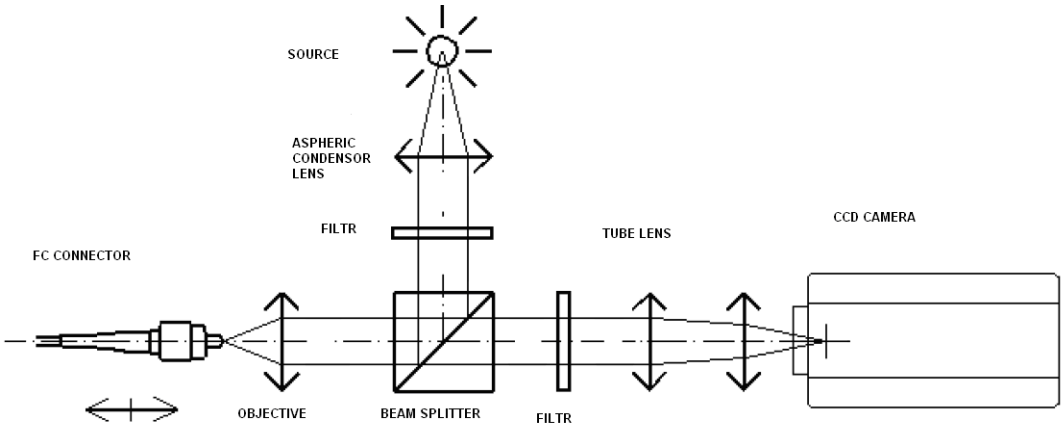

Figure 13

3.2. Light source

LEDs as a light source present many advantages such as lower energy consumption and longer lifetime. Primarily, we are taking advantage of the possibility to switch in between the different light wavelengths also for the fluorescent measurements. We set the light source with 3 LEDs with different wave length of the emitted light. The main problem for the construction of such light source is to direct all three sources into one light beam. We would like to reach this goal with diachronic mirrors. We have designed the preliminary basic setup containing 4 LED chips of certain wavelength and beam combiners (diachronic mirror) (Paska, 2009).

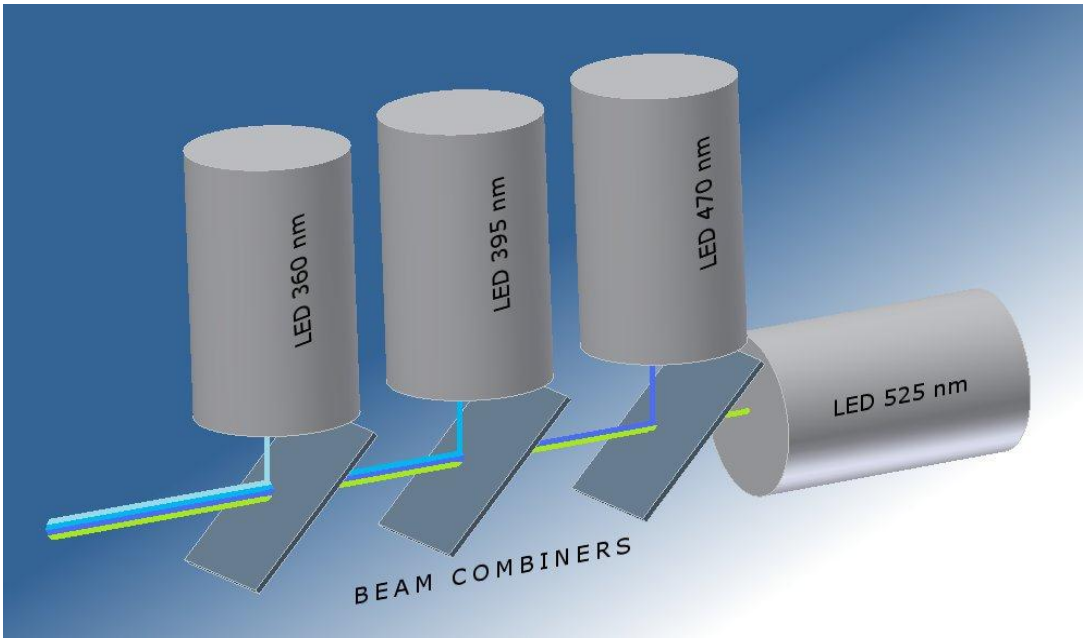

Figure 14 schematic drawing of the LEDdiodes

4. DISCUSSION

4.1. Methods:

4.1.1. Slice preparation:

Studies that are made with help of brain slices provide valuable data on the basic features of neurons and synapses. In vitro placement of the tissue allows us the other relevant information and make possible to change the conditions and apply pharmacologic agents. By altering the ionic composition of the superfusion media or by the addition of pharmacological agents it is possible to capture naturalistic network activity even in such brain slice preparations.

For the maintenance of the live rat slices and subsequent optical and electrical recording the submerged type of chamber has been used in our experiments. Submerged type of chamber brings some specific advantages in the physiological studies slightly similar to conditions in real life compared to interface chamber where the slice lies on the fluid (ACSF) and is surrounded by gaseous atmosphere. Submerged type of chamber further enables optical imaging of the slice because it allows the usage of water immersion microscopy.

Experiments with tissue slices represent primarily conditions where the different structures and even distinct cells are nicely seen, avoiding certain sources of noise, but only with the disadvantage that oxygen supply and connectivity is changed in a simplified manner (Davies et al., 2007, MacVicar et al., 1993). The slices are cut, so like this we are loosing connections to the other structure, but not only the connectivity but also the surface of the slice is corrupted. Different conditions such as higher temperature during preparation have impact on the slice at the level of synapses. In ice cold preparations reversible cytoskeleton changes can appear and the inhibition of ATPas occurs. Slices prepared at room

temperature can suffer from metabolic stress and can lose some synapses, but via warmer preparation, we can avoid the excessive spine loss occurring at colder temperatures due to Na+, Na+ ATPase inhibition. (Bourne et al., 2007)

We have to keep in mind that there is also an unequal distribution of vulnerable regions within hippocampus with increased vulnerability to hypoxia-ischemia in CA1 than in CA3 region both in vivo and in vitro (Kreisman et al., 2000).

One of the most prominent disadvantages of the submerged type of chamber is the insufficient oxygen supply. Furthermore the oxygen is transferred into the tissue via diffusion which might not be adequate and doesn't respect the demand of the tissue compared to the situation in vivo where the instantaneous demand is covered via increased blood flow. (Turner et al, 2007)

The way to increase the diffusion is the temperature increase or the speed of the bubbled ACSF up to 7ml/min (Hajos et al., 2009).

4.1.2. Light source and optical detection

Our results confirm the impact of the surrounding light on the final optical signal detected. One of the biggest easily avoidable sources of noise is the intensity of the light source. There is a transition curve that accompanies change of the intensity of the light source. The results illustrate those characteristic measured during increasing respectively decreasing the intensity. This problem can be easily eliminated if we switch on the light source and start the measurement sufficient time period after the last time of the detection or intensity change. The stabilizing the intensity appears within 40 minutes. This fact makes uneasy mainly electrophysiological measurements and we tend to have the slice illuminated for minimal time possible because of the toxicity of the light. This finding is very important for further measurements because we can easily confuse this source of noise

and the movement of the slice. To get rid of such complications, we proposed LED diode as a light source.

4.2. Results in vitro experiments, the origin of the optical signals:

The present experiments we are demonstrating that the optical methods are useful in the studies of processes in the CNS applying tissue slices. A series of experiments have been performed to demonstrate that we can detect changing optical properties of the nervous tissue in connection to its specific activity with a slight delay from electrical signal in range of 0.5s. (Grinvald, 1982)

4.2.1. Stimulation intensity

Imaging of optical signals revealed the fact neuronal activity can be detected via changes in transmittance of the light through the tissue by causing swelling of the cells. Evaluating these changes provide advantageous method for imaging neuronal activity (Grinvald, 1986). When applying different stimulation intensity, we can conclude that this increasing intensity of stimulation causes subsequent increase in the optical signal. The more neuronal connections we activate the bigger the changes are. This is in accordance with the previous studies where increase in LT and cellular swelling were graded with increasing frequencies of stimulation in optic nerve (MacVicar, 2002). The cellular swelling occurs as a consequence of ionic flow during action potential. The crucial event for these changes is Na-K-2Cl co-transport. In cell tissue culture, high extracellular potassium causes astrocytic swelling as a consequence of KCl uptake and water movements (Walz, 1984).

4.2.2. TTX

We used TTX to distinguish from the non-neural origin of the signal. TTX block voltage-gated sodium channels on the surface of the neuronal membrane with very tight binding site. Sodium movement is like that effectively shut down and the action potential along the nerve membrane is

not spread anywhere. The results showed that the signal was reduced to app. 5% of the previous value, so the signal wasn't blocked entirely. We assume that TTX didn't fill all the binding sites and that some Na/K channels were activated. The other explanation for this event could be the fact that the electrical pulse itself influences the cellular volume. Waltz also suggested that TTX block electrically induced LT changes but not high K indused IOS increase, for these changes Na-K-2Cl co-transport is responsible (Walz et al., 1984), for the remaining LT change Na-K-2Cl can be responsible. This signal was sufficiently reduced to neglect these and we could focus on the exact mechanism and the possible pharmacological reduction of IOS.

4.2.3. APV/CNQX

To distinguish between the synaptic transmission and non-synaptic activity of the nervous tissue, we blocked glutamate receptors using a selective NMDA receptor blocker APV (R-2-amino-5-phosphonopentanoate) and further AMPA/kainate receptor blocker CNQX (6-cyano 7 nitroquinoxaline-2,3-dione). In this case the IOS signal during stimulation was blocked to the half values of the previous signal. This indicates that the signal is mainly of synaptic origin. The other questions remain unrevealed such as if all Glu binding sites were occupied with APV, CNQX since these substances can compete over the binging site. Anyhow, it has been proved that the signal is strongly glutamate dependent. It goes in accordance with MacVicar who blocked the LT with kynurenic acid which is non-competitive glutamate receptor antagonist and confirms that postsynaptic activation is necessary for the generation of LT (MacVicar, 1991). MacVicar managed to block the change in LT with was probably because of the fact they use noncompetitive glutamate receptor antagonist. Interestingly glutamate antagonist were not able to block anoxic depolarization (increase in LT due to lack of oxygen) (Jarvis, 2001).

4.2.4. TBOA

Physiological interactions between astrocytes and neurons are coupled and there is no rigorous generalization of both cell type and their role in IOS generation.

Removal of neurotransmitter out of the extracellular is coordingated by many signaling pathways and this is necessary for the normal functioning of neural structures. (Agulhon, 2008) In principle, some of the slow optical signals may come from glia, rather than neurons. Previous studies showed that uptake of K into astrocytes was probably responsible for the cellular swelling and high K (MacVicar, 2002, Ransom,1985). That's why we were interested in the evaluation of glial contribution to the IOS signal. In the previous experiment, we concluded that the LT signal is strongly dependent on the synaptic glutamatergic transmition in hippocampus. Glial cells are responsible for the removal of Glu from the synaptic cleft. In our set of experiments we examined two conditions. Firstly we added blocker of glial glutamate transport blocker TBOA to prevent reuptake of Glu and this increased the IOS to 159% ± 19.62%. This data points to the fact that the accumulation of glutamate in the synaptic cleft due to lack of Glu glial cleaning leads to a slight increase of the signal. Therefore we concomitantly blocked Glu receptors using APV and CNQX in the next set of the experiments. This reduced the optical signal during stimulation down to 76.74% ± 7.48%. We assume that this occurred as a result of increase in extracellular glutamate, so the IOS increased independly of the fact glutamatergic transmission was abolished.

It has been suggested that after ischemic injury, extracellular glutamate concentration rises and during cerebral ischemia actually reaches levels capable of inducing neuronal death. Using glu-transport blockers such as TBOA indicated that this significant rise in glu occurs as a consequence of reduces uptake and increased vesicular and nonvascular release of glutamate (Jabaudon et al., 1999). On the contrary acute disruption of the

activity of glutamate transporters results in the accumulation of extracellular glutamate which can further lead to cellular death.(Jabaudon et al., 2000)

4.2.5. Furosemide

The aim of furosemide incubation was to determine the dependency of ECS decrease on the IOS. ECS is important for the volume transmission, cellular signals and also changing resistance of the extracellular space. Furosemide thanks to increasing extracellular space through action on Na-K-2Cl cotransporter results in decrease of the excitability of the nervous tissues and has been used as a anticonvulsant drug in humans (Haglund, 2005). Our main hypothesis consisted in the fact that the stimulation increases light transmittance of the tissue will be further suppressed with the application of furosemide. Indeed, the high K induced IOS ware depressed by furosemide and bumetanide, antagonists for Na-K-2Cl cotransporter (MacVicar, 2002). The results were surprising compared to what we expected. The perfusion with furosemide results in increased signal although in the study of Holthof lead to the decrease in LT in rat neocortical slices (Holthof, 1996).

To better understand mechanism of furosemide action ion-selective electrode should be used. Underlying changes in K+ and volume of ECS can clarify processes responsible for IOS changes.

Furosemide has dose dependent effect on GABA A receptor even in micromolar concentrations and thus in our case when 0.2 mM concentration was used we should assume that this might suppress GABA inhibition. Korpi et al. applied furosemide (5uM) has show selective antagonism at GABA A receptors. Moreover, there is also a different expression of GABA receptors in the hippocampus during development, which might have played role in our experiments as well because we have used animals in age group 20-30 days which is rather larger age group which could explain the differences in the furosemide effect on the tissue. In future experiments we will use bumetanide which lacks GABA A activity.

4.3. Hardware and software

In addition to the experimental aims, the other part of the study was to design software and devices enabling recording and evaluation of IOS in combination with the electrophysiological signals. These signals are usually recorded using two different environments, one for optical data and second one for electrical data. In our case, we designed software enabling just one environment which in principle facilitates usage and detection of both signals. One environment accomplishes our aim of the highly accurate synchronous recordings of both electrical and optical signal.

A software component of the setup is presented by the Vision Brain program. The software was designed using C# programming language and Microsoft .NET framework 3.5. because of its object orientation, wide range of class libraries and easy portability among different computers running Microsoft Windows operating systems.

To make this detection system available in terms of cost the hardware components National Instruments multifunction low-cost card M Series were used for recordings of electrophysiological data. QImaging Retiga 2000R camera was used for recording video data because of its high resolution (12bit images with max resolution 1600 x 1200 pixels, 16bit a/c convertor) and low noise (chapter 3.3.1). The synchronization is realized using camera's trigger output and input signals that are connected to the card's digital outputs and inputs. The quality of the measured data (12bit images with max resolution 1600 x 1200 pixels, 16bit a/c convertor) enabled by used hardware is sufficient for our purposes, but can be increased using higher models of digitalization card or camera. Also just minimal requirements are requested for PC's computational resources; this includes 1GB RAM, dual core processor and sufficient capacity to store the captured data. This all makes the system available especially in terms of cost.

To enable utility of the software for other types of experiments, where synchronization of electrical and optical data is required (e.g. long term video-EEG monitoring), it is enables communication with another types of

camera such as standard web cameras. When compared to the other systems and the other imaging methods, our system is user friendly and rather inexpensive.

5. CONCLUSION

Intrinsic optical signals are one of the possible modalities for imaging of the activity of nervous tissue.

We characterized the generation of changing optical properties with set of experiments using tissue slices to prove that the signal is of neuronal origin and we were able to detect and influence pharmacologically ion channels, synapses, glial reuptake and mitochondrial fluorescence signal. We defined and revealed the different sources of noise and reveled one of the biggest sources of noise, which was the noise of the own light source. Don’t understand

We designed the experimental setup for in vivo experiments where we would use these obtained data in the whole animal the attachment of the optic fiber and LED diode light source.

Specialized software VisionBrain for in vivo detection enabling detection of both optical and electrical signals using our specialized software has been developed.

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