Identification of molecular components underlying optical signals applied for spatiotemporal neural activity mapping

(Doctoral thesis)

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1 INTRODUCTION

Over the last years the costs related to different brain disorders lay a tremendous burden on the economy and society (Gustavsson et al., 2011). Without detailed knowledge of the functional properties and dynamics of the nervous system, effective treatments for these diseases cannot be developed. For better understanding brain (dys)functions valid representations of spatiotemporal dynamics of neural activity are essential. For this purpose, two major approaches are used: the electrophysiology based and the optical signal based methods. The electrophysiology based activity mapping takes advantage of the well-characterized electrophysiological signal with exceptionally high temporal resolution (Franke et al., 2012). However, the method also suffers from several important drawbacks, including the limited spatial resolution (Kajikawa and Schroeder, 2011) and severe invasiveness. Spatiotemporal optical imaging approaches offer a solution to overcome this limitation.

The label-free Intrinsic Optical Signal (IOS) is widely used for monitoring spatiotemporal neural activity in extensive networks in vitro (Borbély et al., 2014) and in vivo (Bauer et al., 2014). Due to the simplicity of the method IOS imaging enables non-invasive mapping of neuronal activity of epilepsy patients under intra-operative conditions (Haglund, 2012). In vitro IOS in isolated brain slices is presumed to be almost exclusively caused by changes in light absorption and light scattering (Aitken et al., 1999). Local changes in light scattering due to activity-dependent cell swelling is regarded as the principal component of the afferent stimulation evoked IOS (MacVicar and Hochman, 1991). The proposal that afferent evoked IOS is attributed to neuronal activity induced cell swelling is based on the fact that it could be inhibited by furosemide, a nonspecific inhibitor of the chloride homeostasis regulating transporters, and it was found to be strongly dependent on extracellular [Cl\(^-\)] (MacVicar and Hochman, 1991). In previous studies IOS was hypothesized to reflect glial K\(^+\) clearance via the furosemide sensitive Na\(^+\)/K\(^+\)/Cl\(^-\) cotransporter 1 (NKCC1) (MacVicar and Hochman, 1991). The contribution of other proteins to IOS playing important role in cell swelling like volume-regulated anion channel (VRAC) (Qiu et al., 2014) and astroglial glutamate transporters (Han et al., 2004) have not been examined in detail. The role of the Na\(^+\)/K\(^+\) ATPase (NKA) and K\(_{ir4.1}\) K\(^+\) potassium channel in IOS generation are also undisclosed despite their significant role in astroglial K\(^+\) clearance. Understanding the mechanism of IOS generation may help to better interpret signals used in diagnostics and might support the detailed understanding of cell swelling associated diseases like oedema.

The drawback of the IOS imaging method is that the time scale of the signal is much longer than the time scale of neuronal activity measured by standard field potential approach.
It is still not clear how the long lasting optical signal is coupled to the short term neuronal activity.

Ion selective or voltage-sensitive organic fluorescent or absorbance dyes are widely used to monitor voltage changes associated with the activation of neurons (Ebner and Chen, 1995; Verkhratsky et al., 2012). The time scale of the signals acquired by these dyes is much closer to the time scale of neuronal activity compared to IOS (Ikrar et al., 2013). Voltage-sensitive dye (VSD) imaging is typically considered as the optical counterpart of field potential measurements and is used for spatially expanding the one-dimensional field potential (Hill and Greenfield, 2013). Since the number of applicable electrodes is limited, in this work I used voltage-sensitive dye imaging to overcome the spatial limitation of field potential recording as an intermediate layer between IOS and field potential. NK3630 dye was used to follow the spatial spreading of neuronal activity.

The contribution of different processes in the generation of the VSD signal is widely debated. Kojima and coworkers differentiated a fast (~< 10 ms) and a slow component (~>10 ms) of the signals of the NK3630 and NK3041 dye. They considered the slow component as the depolarization response of the astrocytes (Kojima et al., 1999). Conversely, Konnerth and coworkers associated this slow component with astroglial potassium uptake (Konnerth et al., 1987). Thus the question arises whether VSD imaging can be safely applied as a spatial extension of the field potential.

2 AIMS OF THE STUDY

The main aim of this work was to examine the molecular mechanisms underlying IOS and VSD signal generation and the comparison of the optical signals with the field potential response to gain a deeper insight into how neuronal activity is coupled to the optical signals. The main objectives were the following:

1. What is the relation between the IOS detected by the fast imaging and IOS documented in the literature? What kind of neuronal activity (presynaptic or postsynaptic) is followed by the optical signals?
2. What is the spreading pattern and the temporal dynamics of the optical signals? How is the spreading pattern and the spreading velocity of the optical signals related to that of the field potential? Can the differently activated regions be discriminated by the parameters of the optical signals?
3. IOS is proposed to reflect the astroglial swelling via KCl uptake and VSD signal has been shown to be associated with astroglial K\(^+\) and glutamate
uptake. The question then arises, what is the specific contribution to optical signals of those proteins that play role in cell swelling and potassium uptake, like the astroglial glutamate transporter (EAAT2), NKCC1, Kir4.1, NKA and anion channels?

3 METHODS

To answer these questions the hippocampus brain slice was chosen as a model, because its synaptic connections are well known. To evoke simultaneously measured field potential and optical signals, Schaffer collaterals were triggered by voltage stimuli in the stratum radiatum of the CA3 region. Optical signals were detected by a 464-element photodiode array (PDA) detector having temporal resolution of 0.6 ms, making it achievable to align the optical signals with the simultaneously measured field potential recordings. Field potential was recorded at a single location in the stratum pyramidal of the CA1 region, while the optical signals covered the whole CA1 CA3 and dentate gyrus regions. To examine the molecular mechanisms underlying optical signals the potential target proteins were blocked by their inhibitors and changes in the parameters of the optical signals were compared to the changes of the field potential response.

Experimental data was analysed by first comparing the parameters of a given optical signal and the parameters of the simultaneously measured field potential to the signals measured under control conditions (without inhibitor application). Than the parameters of the field potential measured in the CA1 were compared to the optical signal parameters measured on the photodiode near the field potential recording electrode and in the whole CA1. In the next step parameters of the optical signals were compared across different regions. Finally the changes in IOS and VSD signals were compared to each other in the CA1, CA3 and CG regions.

Activation of astrocytes were studied by fluorescence confocal imaging of an intracellular Ca\(^{2+}\) sensitive dye.

4 RESULTS

Major findings obtained by this study are summarized as follows:

i) to evoke IOS with acceptable signal-to-noise ratio at least four times fewer and eight times shorter stimuli is enough than the stimulus protocol used in
previous studies (MacVicar and Hochman, 1991; Buchheim et al., 2005). Since the stimulation protocol in this study is shorter the detected IOS reached its peak at least two times earlier and declines seven times faster than in previous studies (MacVicar and Hochman, 1991; Buchheim et al., 2005).

ii) Despite their different time-scales, the IOS and VSD amplitudes are linearly correlated to the field potential amplitude. Tetrodotoxin blockade of voltage-gated Na$^+$ channels abolished, while inhibition of GABA$_A$ receptor mediated inhibitory signaling by picrotoxin substantially increased the amplitude of both optical signals, suggesting the dependency of the optical signals on neuronal activity.

iii) Major part of the optical signals were inhibited by ionotrophic glutamate receptor antagonists indicating that a substantial portion (~70 %) of the optical signals is initiated by glutamatergic neuronal activity.

iv) Despite their different time-scales, the spatial spreading of both IOS and VSD signals corresponds to the activated region and both signals discriminate between the differently activated (ortho- and antidromic) areas.

v) The estimated spreading velocity of the VSD signal is 300±80 μm/ms which is in agreement with the data found in the literature for action potential spreading (Schmidt-Hieber et al., 2008). VSD signal spreads three orders of magnitude faster than IOS (0.23±0.05 μm/ms).

vi) Glutamate clearance by the astroglial glutamate transporter EAAT2 is a major player in both IOS and VSD.

vii) EAAT2 contributes to VSD generation in very short time scale (< 5 ms)

viii) Region specific contribution of astrocyte activity to VSD in the order of CA3 > CA1 correlates with the EAAT2 localization pattern.

ix) Activity of neuronal K$^+$/Cl$^-$ cotransporter, but not the previously hypothesized glial Na$^+$/K$^+$/Cl$^-$ cotransporter contributes to IOS development.

x) Astroglial K$^+$ clearance through K$_{ir4.1}$ channel and Na$^+$/K$^+$ ATPase is only a minor contributor to IOS opposing previous hypotheses, although it has significant contribution to the VSD signal with similar region specificity as EAAT2.

xi) Inhibition of anion channels decoupled field potential and VSD signal from IOS, indicating the importance of these channels and the chloride ion gradient in IOS generation.
xii) Ca$^{2+}$ signal measurements confirmed the activation of astrocytes by the stimulation of Schaeffer collaterals. Since the P2Y$_1$ receptor antagonist did not decouple IOS and the field potential, the activation of astrocytes via P2Y$_1$ does not contribute to IOS generation.

5 DISCUSSION

Based on the experimental results I propose the following mechanism of afferent evoked IOS generation: first, neurons in the pyramidal layer are activated leading to swelling of their soma, followed by swelling of glial cells in the dendritic region. This is suggested by the facts that IOS first appears in the str. pyramidale and also by the TTX- and Glu receptor inhibition sensitivity of the signal. Glial activity related IOS appears as the consequence of neuronal activation by the release of glutamate that activates EAAT2 and glial Mg$^{2+}$--independent NMDA receptors. Glutamate also depolarizes neurons inducing elevated extracellular KCl concentration via the activity of neuronal KCC2 cotransporter. Cell swelling induced by Glu or KCl uptake activates VRAC which is responsible for the decay phase of the signal. Components taking part in the IOS generation and the difference between the spatiotemporal IOS pattern of the ortho- and antidromically activated areas imply that IOS reflects the intimate communication between neurons and glial cells. Contrary to previous hypotheses KCC2 does not affect IOS by increasing the extracellular potassium concentration rather it modulates IOS by elevating extracellular chloride concentration and indirectly acts on the bicarbonate transporter and affects chloride ion gradient.

Contrary to the general view of IOS that attributes signal genesis mostly to glial cell swelling, a significant neuronal component was identified which is mediated by the K$^+$/Cl$^-$ cotransporter KCC2. Surprisingly, the findings also suggest that the interpretation of VSD signals as spatially extended field potentials may not be relevant since the VSD transients contain a significant astrogial component that is not present in the field potential response. EAAT2 was shown to contribute not only to the slow components of the VSD generation but also to the very short time scale (< 5 ms) fast component opposing previous hypothesis. K$_{ir4.1}$ channel as well as the astrogial Na$^+$/$K^+$ ATPase were identified as minor contributors to VSD since they affect only the slow component of the VSD signal. Therefore, neither VSD imaging can be considered as a predominantly neuronal signal nor IOS should be associated with primarily glial processes. Instead, both optical signals convey astrogial responses to neuronal activity. These findings cast light on neuro-glia communication as the origin of spatiotemporal optical signals within the brain.
This work aimed the better understanding of the detailed mechanism underlying the spatiotemporal imaging techniques. Further investigating the components of these signals and further revealing the detailed mechanism may give us deeper insights into the spatial and temporal mechanisms of neuronal tissue function and might help to improve diagnostics in the future.

6 REFERENCES


7 PUBLICATIONS RELATED TO THE TOPIC OF THE DOCTORAL THESIS


8 PUBLICATIONS NOT RELATED TO THE TOPIC OF THE DOCTORAL THESIS


9 CONFERENCE ABSTRACTS

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