Abstract Book
LED-induced microglial activation and rise in Caspase-3 shows reorganization in the retina

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Constant exposure to different wavelengths and intensities of light promoted by Light Emitting Diodes (LEDs), could produce retinal degeneration and neuronal cell death. Accumulated over years, damage induced by chronic phototoxic reactions occurring in the retina has been suggested to be involved in the etiology of many debilitating ocular conditions. We proposed to show how LED light affects our vision by showing the changes in the expression of death and survival factors and microglial activation in LED-induced damage (LID).

To this end, we created a mouse model where the LED exposure of the treated animals matched that of an average office worker. After the treatment, the expression of apoptotic markers (Bax, Casp-3) and a survival factor (Bcl-2) – which can be used to assess cell damage - was determined by qPCR, and primary inflammatory processes were examined by microglia and Casp-3 activation using immunohistochemical markers.

We found that the LED treatment increased levels of apoptotic markers globally. The survival factor expression also significantly increased alongside the two apoptotic markers, which points to potential compensatory mechanisms activating in the animals. Looking at the primary inflammatory processes we found that the microglial and Casp-3 activation increased in the retinas of the treated animals. Overall, our results showed that LED light exposure induces damage and widespread microglial activation within the inner retina, which can be compensated by the increased rate of survival factors. These findings warn against the prolonged use of LED-based light sources.

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Comparative analysis of the topography of electric synapses in the mammalian retina

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Introduction: The retinas of many species show regional specializations that are evident in differences in the processing of visual input from different parts of the visual field. Regional specialization is thought to reflect adaptation to the natural visual environment, optical constraints, and lifestyle of the species. Yet, little is known about regional differences in synaptic circuitry. Here, we were interested in the topographical distribution of connexin-36 (Cx36), the major constituent of electrical synapses in the retina.

Materials and methods: We compared retinas of mice (n = 5), rats (n = 4) and cats (n = 4) to include species with different patterns of regional specializations in the analysis. For incubation with the primary antibodies, we used polyclonal anti-CaR, monoclonal anti-Cx36, polyclonal anti-Prox1, monoclonal anti-parvalbumin and monoclonal anti-calbindin.

We used the density of Prox1-immunoreactive amacrine cells as a marker of regional specialization, with higher cell density signifying more central regions. We used FIJI, Imaris and SPSS software for data analysis.

Results: Double-labelling experiments showed that Prox1 is expressed in all amacrine cells in all three species. Interestingly, large Cx36 plaques were attached to about 8-10% of Prox1-labelled amacrine cells suggesting strong electrical coupling of pairs or small clusters of cell bodies. When analysing the regional changes in the volumetric density of Cx36-immunoreactive plaques, we found it to be tightly correlated to the density of Prox1-expressing amacrine cells in the ON sublamina, whereas no such correlation was evident in the OFF sublamina. These principles applied to retinas of all three species.

Conclusion: The results suggest that the relative contribution of electrical synapses to the ON- and OFF-pathways of the retina change with retinal location, which may contribute to functional ON/OFF asymmetries across the visual field.

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Differential labeling with Parvalbumin antibodies from various origins can be used for clustering retinal ganglion cells

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The retina is composed of the retinal pigment epithelium, photoreceptors, outer nuclear layer, outer plexiform layer, inner nuclear layer, inner plexiform layer, and the ganglion cell layer. In this, the layer that will be focused upon would be the ganglion cell layer. The ganglion cell layer contains at least 8 Parvalbumin (PV) expressing ganglion cells (GC) according to genetic labelings and cell tracing experiments.

The aim of our study is to be able to differentiate the PV GCs in the retina according to their PV immunohistochemical (IHC) labeling. Furthermore, by being able to identify the morphology of the PV expressing GCs, it could be linked to the IHC labeled clusters and also their physiological properties.

The experiment consisted of euthanizing of mice, dissection of the retina, IHC, and confocal microscopy; this was carried out on genetically modified mice that were expressing PV-tdTomato. The most important process being IHC, wherein we used three different antibodies on the PV-promoter-dependent Cre X flox-tdTomato line for PV multiple-labeling. The antibodies were produced in rabbit, mouse, and chicken hosts. We also consistently used rbDL405, msA488, and ckCy5 secondary antibodies for fluorescent labeling before confocal microscopy. For the analysis of the gathered confocal images, Fiji was utilized to gather data on the labeling intensities of the antibodies originating from different animals and produced using different immunogenic domains. To visualize the data gathered it was further processed into a graph using clustering where the principal component (PCA) of the data was used.

The experiment yielded images wherein the PV expressing ganglion cells could be distinguished from other cells present, as well as being able to see different PV expressing GCs. We were able to acquire a differential labeling by using the antibodies. This resulted in at least 4 clusters of cells by using PCA analysis on the expression intensities. Further, the experiment yielded data wherein chicken-PV, PV-tdTomato, as well as rabbit-PV antibodies, had similar labeling intensities.

Our results showed that it is possible to differentiate cells according to the labeling intensities of different antibodies for the same protein. In the future, we plan to overlap the resulting IHC-clusters with the morphological clusters paired with an electrophysiological assessment to show how the physiological properties are linked to the PV expression.
Decoding the retinal populational information hidden in burst motives

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Keywords: retina, burst, neural code, data analysis, gap junction

Visual information detected and preprocessed by the retinal circuitry is coded in the action potential (AP) patterns of the retinal output cells, the retinal ganglionic cells (RGCs). This information is then projected through the RGC axons to vision forming and behavior-altering brain areas. The aforementioned AP code, produced by the RGCs, is a complex pattern in order to code for the many distinct visual features detected, such as light intensity, movement, orientation, color, etc... This pattern can relay information summated on an individual cell, or through a network of amacrine cells (AC) and/or directly coupled RGCs this information can be part of a population code.

A neuronal AP "burst" is a group of densely packed AP potential events on the spike-train (ST), however, no precise definition can be given as this relatively higher frequency appears to be cell and activity-dependent. In order to decode the information hidden in the burst motives, we first had to precisely identify and separate these events. Utilizing a loose patch clamp technique, we recorded STs from cells during a constant low light background, quasi spontaneous activity, state and as a response to a full-field light stimulus. We also injected these cells, with a gap junction (GJ) permeable tracer (neurobiotin), in order to identify the cell type based on morphological features. An already established burst detection algorithm (LogISI) was altered to fit our data more precisely. Subsequently, more high throughput techniques, 64 channel and High Definition Multielectrode arrays were used to test our algorithm. Testing the role of the burst motives in population coding, we compared the patterns, using all three of our techniques, before and after the pharmacological blockade of GJs.

We showed that bursting activity is greatly reduced due to pharmacological uncoupling along with RGC correlated activity. Using the transient off alpha cell as an example we show how AC coupled correlated activity is coded in burst motives.

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Relationships and roles of retinal circuit elements and spontaneous activity waves in coding mechanisms of the mammalian retina

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Mammalian RGCs encode visual signals in action potential trains and sent this information towards visual brain centers. However, many RGCs display spontaneous spikes as well even without any light stimulation. Such spontaneous activity occurs in random waves of calcium influxes on in vitro retina under calcium imaging recordings. Due to stochasticity, any form of spontaneous activity appears as background noise in visual information processing.

The spontaneous propagating calcium waves of the RGCs are a well-described phenomenon in newborn postnatal animals. But recently several studies, (including our unpublished works) shed light that similar spontaneous correlating calcium waves persist in the adult retina too. These waves are present when we examined the retina at several levels of experimental approaches (Ca++ imaging, MEA, in silico retina model). We are certain that these correlated waves are not without purpose, they are an essential part of the visual code. We suspect these waves are responsible for the detection of an object’s extension and its’ separation from the background, the detection of the edges.

A computer retina model is a perfect tool to predict such functions in a system. With this application, we are capable to examine this phenomenon in-depth, determine the responsible circuit elements (gap junction, GABAergic inhibition) and their contribution to the exact visual coding. In a model like this, we can specifically turn out certain connection elements (unlike the in vitro physiological experiments) to predict their higher function in the visual coding, based on the loss of function.

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The protective role of PAC-1 receptor in endotoxin induced retinal inflammation

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Background: Lipopolysaccharide (LPS) administration is used as a model of neuroinflammation in the retina. Numerous studies have shown that pituitary adenylate cyclase activating polypeptide (PACAP) has anti-inflammatory and anti-apoptotic actions in the retina. Different analyses have proved that the protective effects are mostly mediated by the PAC-1 receptor.

Methods: We investigated the retinoprotective role of the specific PAC-1 receptor agonist maxadilan in LPS induced inflammation by using different histological (OCT, histology) and molecular biological (cytokine array, western blot) methods. CD-1 IGS wild type mice were used in the experiment. Mice received intraperitoneal injection of LPS (6 mg/kg). The right eye was injected with maxadilan (1 μM) intravitreally and the left eye served as a control (PBS). One group of animals was investigated 24h after LPS injection, the other group was killed 5 weeks later.

Results: Histological analysis showed differences between the maxadilan treated retinas compared to the control LPS injected ones. All the retinal layers were significantly thinner in LPS injected mice compared to the treated animals. After administration of PAC-1 receptor agonist the expression level of several cytokines (such as RANTES, KC) was decreased compared to the control retinas that underwent LPS inflammation. The expression level of several anti-apoptotic factors (such as IL-1, GCSF) was significantly increased in maxadilan injected retinas during inflammation. Western blot experiments showed that intravitreally administered maxadilan increased Akt, GSK-3, ERK and CREB activities, whereas decreased the JNK, p38MAPK levels in LPS injected retinas.

Conclusion: In summary, the PAC-1 receptor has an important retinoprotective role in LPS induced retinal inflammation.

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