UNIVERSITY OF PÉCS

Doctoral School of Biology and Sportbiology

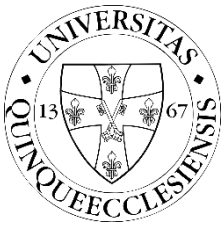
Mechanisms affecting the temporal characteristics of retinal ganglion cell light responses: a study of transiency and oscillatory activity in the mammalian retina

PhD thesis

Ganczer Alma

Supervisor:

Dr. Völgyi Béla



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1. Introduction

Visual information is a paramount contributor to the human sensory perception. In the retina, even a single photon of light can initiate a signal cascade culminating in the firing of action potentials towards the brain, allowing us to navigate our surroundings using visual cues collected from the environment. As an easily accessible extension of our nervous system, the retina has become an ideal experimental model for studying both sight and neural physiology. Owing to its neatly organized structure, the mammalian retina can be divided into six separate layers that contain five defining classes of retinal neurons: photoreceptor cells (PR) to carry out the crucial step of phototransduction, bipolar cells (BC) to pass the information along, ganglion cells (GC) to generate the final sensory output, and finally, horizontal (HC) and amacrine cells (AC) to regulate the network. As a consequence, the retinal circuitry is complex and yet to be fully defined.

GCs contribute to the innermost retinal layer, the ganglion cell layer (GCL). They can be divided primarily into ON, OFF or ON-OFF cells according to their physiology, considering whether a cell starts firing at the on- and/or offset of the light stimuli. GCs can also be distinguished by their function; e.g. cells reacting to movement in a certain direction (direction selective retinal ganglion cell, DS RGC). This diversity allows the retina to separately code for different aspects of the visual scene, including (but not exclusively) movement, contrast, colour, or background luminosity. In addition, RGCs also differ in their response kinetics: some cells are quick to respond,

others react to stimuli with longer delay in their spiking activity. The same can be said about the time it takes a cell to stop firing once active: some RGCs (or RGC light responses, separately) are considered transient, meaning that they quickly cease generating action potentials (APs) after a short burst, while sustained responses are maintained for longer periods of time and often follow a plateau phase in their decline. The exact mechanisms determining RGC transiency (the speed of response decay) are currently unknown.

RGCs are also known to exhibit patterns of gamma oscillation, just like other neurons in the central nervous system. This oscillatory activity (OA) has been observed in various experimental models and is suspected to affect escape reaction in frogs when confronted with a potential predator. The function of OA and the mechanisms responsible for generating synchronized OA in the retina are not yet fully understood.

2. Aims

First and foremost, our goal was to gain a better understanding of how RGC response transiency is determined. While numerous attempts were made by other scholars to uncover the mechanism underlying the transient/sustained dichotomy, so far, no consensus has been reached. At the same time, we hoped to obtain valuable insight regarding the OA that was undeniably present in many of our recordings. Thus, my objectives for the present work were:

1. To establish a reliable method for measuring and quantifying transiency

2. To study the distribution of transient and sustained responses and reliably separate the two groups

3. To uncover the defining mechanism(s) responsible for the development of transient and sustained light responses

4. To identify the defining mechanism(s) controlling synchronized OA in RGCs.

3. Materials and methods

3.1 Extracellular recordings

RGC light responses were gathered from both extracellular single-cell (carbon microelectrodes, Kation Scientific LCC, Minneapolis, MN, USA) and multi electrode array (MEA; MultiChannel Systems MCS Gmbh, Reutlingen, Germany) recordings carried out on isolated murine (*Mus musculus*) retinas.

3.2 Pharmacology

All control recordings (and experiments without pharmacological treatment) were done using mammalian retina ringer solution (NaCl 125mM/dm^3, KCl 3mM/dm^3, $CaCl_2$ 2mM/dm^3, NaH_2PO_4 1.25mM/dm^3, $MgCl_2$ 1mM/dm^3, $NaHCO_3$ 25mM/dm^3, glucose 10mM/dm^3). Picrotoxin (PTX, 50μM) was used to selectively block GABA-erg synaptic transmission and meclofenmic acid (MFA, 50μM) was applied to block gap junctions. L-2amino-4-phosphonobutyric acid (L-AP4, APB, 50μM) was used as a metabotropic glutamate receptor agonist in select experiments.

3.3 Data analysis

APs were sorted using Spike2 (Cambridge Electronics Design Ltd., Cambdrige, UK), which also allows for the manual sorting of electric activity originating from different RGCs. The timestamps for APs were used to create perstimulus time histograms (PSTHs) that show the number of APs recorded under a given time period (bin=10ms in the present work) as a function of time. Statistical tests were carried out using IBM SPSS Statistic (IBM, Armonk, NY, USA) and Origin (OriginLab, Northampton, MA, USA).

4. Results

4.1. Measuring transiency

By comparing four different methods of calculation, we identified the one dubbed PSTHτ (or PSTHtau) as the most ideal for measuring and quantifying transiency. PSTHτ calculates transiency by identifying the time it takes for the peak firing activity of the cell to decrease to 1/e (e referring to the mathematical constant). As a result, transiency values are interpreted as values of time (defined by milliseconds or seconds), with lower values representing transient and higher values representing sustained responses. Transiency values obtained through this method are independent of the experimental arrangement and can potentially be compared to the transiency of slow or graded potentials of BCs.

4.2. The distribution of transiency

We found that – unlike or expectations – transient and sustained responses do not seem to form two distinct categories. PSTHτ values cover a wide spectrum of transiency and do not provide representative peaks for separate groups. Instead, according to both calculated values

and the curve visible on the histogram, a third category is present that does not seem to exhibit clear transient and sustained features and applies to a large number of our recorded responses. In addition, this wide-scale distribution was present in both ON and OFF responses, with ON-OFF cells sometimes presenting different response kinetics for the ON and OFF components of their light response.

4.3 Mechanisms determining transiency

Our data shows that both ON and OFF RGCs are capable of generating a variety of both transient and sustained responses, suggesting that post-synaptic glutamate receptor distribution (mGluR6 solely for ON BCs and AMPA/Kainate receptors for OFF BCs) cannot be the determining factor behind RGC transiency. A switch between parallel retinal pathways does not necessarily result in a change in transiency: when changing the stimulus intensity from scotopic to photopic, only ON responses changed their PSTHτ values significantly ($p<0.05$) while OFF responses displayed no statistically relevant changes in their transiency. Under scotopic conditions, however, only the rod BC remains active (using mGluR6), which also supports our previous statement that post-synaptic BC glutamate receptors have little to no effect on RGC transiency.

When PTX treatment was applied, a large number of RGCs exhibited changes in their transiency, often resulting in a full sustained-to-transient switch. By interfering with GABA-erg inhibition in the inner retina, we could effectively change the temporal kinetics of the light response while the number of action potentials fired also increased. PTX treatment also resulted in an appearance of transient OFF

responses that we could not register under control circumstances. Sustained ON control responses, however, have often been truncated by an inhibitory effect shortly after reaching peak firing intensity, showing that functional GABA-erg signalling could potentially play a role in elongating RGC firing activity in response to light stimuli. RGC transiency was also significantly affected by MFA treatment, showing that gap junctions are also crucial in deciding whether a response will be transient or sustained.

4.4 Oscillatory activity of RGCs

In our experiments, we have recorded synchronised OA in RGCs averaging around 24Hz in frequency. Strangely, the OA was only observable in ON responses, even in the case of ON-OFF GCs. The oscillations originally posed a problem in the analysis of transiency as they resulted in repeating local minimum and maximum values in the histogram. On further analysis, we found that PTX was able to minimize oscillation in RGC firing, while the application of MFA seems to affect the synchronization of OA as a whole across the retina.

5. Summary

To reflect on the points addressed in aims section:

1. We have tested and chosen the PSTHτ as a method for measuring transiency and feel that it is suitable for quantifying RGC light responses
2. We found that transient and sustained RGC responses cannot, in fact, be separated into two distinct categories and introduced a third, intermediate category

3. We conclude that transiency is determined by multiple signal streams converging and interacting on the level of RGCs and not by a single mechanisms of action
4. We present evidence that OA is dependent on intact GABA-erg signalling but gap junctions are crucial for the cross-retinal synchronization of said oscillation.

6. List of publications

6.1. Publications related to the present work:

Ganczer A., Balogh M., Albert L., Debertin G., Kovács-Öller T., Völgyi B (2017): Transiency of retinal ganglion cell action potential responses determined by PSTH time constant. *PLoS One*, 12(9):e0183436. doi:10.1371/journal.pone.0183436.

Tengölics Á.J., Szarka G., **Ganczer A.,** et al. (2019): Response Latency Tuning by Retinal Circuits Modulates Signal Efficiency. *Scientific Reports*, 9(1):15110. doi:10.1038/s41598-019-51756-y

6.2. Conference abstracts related to the present work:

Ganczer A., Szarka G., Völgyi B. (2019): Gap Junction Coupled Amacrine Cell Networks Mediate Synchronized Oscillatory Spiking in Mouse Retinal Ganglion Cells. *European Retina Meeting (ERM 2019).*

Ganczer A., Szarka G., Völgyi B. (2019): Inhibiting Inner Retinal Signal Transmission Results in Sustained-to-Transient Switch in RGC Light Responses. *A Magyar Idegtudományi Társaság konferenciája (MITT 2019).*

Ganczer A., Balogh M., Völgyi B. (2017): Temporal Response Features of Retinal Ganglion Cells are Mostly Determined in the Inner Retina. *Federation of European Neuroscience Societies konferenciája (FENS 2017).*

Ganczer A., Tengölics Á.J., Völgyi B. (2017): Response Transiency of Retinal Ganglion Cells is Maintained when Input Dominance Switches Between Parallel Signalling Streams. *European Retina Meeting (ERM 2017).*

Tengölics Á.J., **Ganczer A**., Balogh, M., Völgyi, B. (2017): Ganglion cell responses impose a postdictive processing of Visual Signals in the brain. *Federation of European Neuroscience Societies konferenciája (FENS 2017).*

Ganczer A., Balogh M., Atlasz T., Völgyi B. (2016): Transient and Sustained Response Characteristics of Ganglion Cells are Determined in the Inner Retina. *IBRO Workshop (IBRO 2016)*.

6. 3. Publications unrelated to the present work

Kovács-Öller T., Szarka G., Tengölics Á.J., et al. (2020): Spatial Expression Pattern of the Major Ca2+-Buffer Proteins in Mouse Retinal Ganglion Cells. *Cells*, 9(4):792. doi:10.3390/cells9040792

Kovács-Öller T., Szarka G., Ganczer A., Tengölics Á., Balogh B., Völgyi B. (2019): Expression of Ca2+-Binding Buffer Proteins in the Human and Mouse Retinal Neurons. *International Journal of Molecular Sciences*, 20(9):2229. doi:10.3390/ijms20092229

Kovács-Öller T., Debertin G., Balogh M., et al. (2017): Connexin36 Expression in the Mammalian Retina: A Multiple-Species Comparison. *Frontiers in Cellular Neuroscience*, 11:65. doi:10.3389/fncel.2017.00065

Szalai R., Ganczer A., Magyari L., Matyas P., Bene J., Melegh B. (2015): Interethnic differences of cytochrome P450 gene polymorphisms may influence outcome of taxane therapy in Roma and Hungarian populations. Drug Metabolism and Pharmacokinetics, 30(6):453-456. doi:10.1016/j.dmpk.2015.08.001

Nagy A., Sipeky C., Szalai R., et al. (2015): Marked differences in frequencies of statin therapy relevant SLCO1B1 variants and haplotypes between Roma and Hungarian populations. *BMC Genetics*, 16:108. doi:10.1186/s12863-015-0262-4

Sipeky C., Weber A., Melegh B.I., et al. (2015): Interethnic variability of CYP4F2 (V433M) in admixed population of Roma and Hungarians. *Environmental Toxicology and Pharmacology*, 40(1):280-283. doi:10.1016/j.etap.2015.05.008

Sipeky C., Matyas P., Melegh M., et al. (2014): Lower carrier rate of GJB2 W24X ancestral Indian mutation in Roma samples from

Hungary: implication for public health intervention. *Molecular Biology Reports*, 41(9):6105-6110. doi:10.1007/s11033-014-3488-8

6.4. Conference abstracts unrelated to the present work

Tengölics Á., Albert L., Varga D., Ganczer A., Balogh M., Völgyi B.: A retinális ganglionsejt válaszok befolyásának vizsgálata a vizuális jelek posztdiktív agyi feldolgozásában, *I. Móra Nemzetközi Interdiszciplináris Konferencia* (2017)

Tengölics Á., Ganczer A., Balogh M., Völgyi B.: Ganglion cell responses impose a postdictive processing of Visual Signals in the brain, *Federation of European Neuroscience Societies konferenciája* (FENS 2017).

7. Bibliometric data (2021)

Cumulative impact factor of the publications related to the thesis: 6.74

Number of independent citations: 2