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Title: Encoding visual features by retinal ganglion cell oscillatory activity

We planned (specific aim i) to determine the types of retinal ganglion cell (RGC) oscillations in the mammalian retina. We found that spiking oscillations occure for most ON polarity RGCs but hardly any oscillations for the OFF cells (a few cells displayed ~50 Hz oscillations). Another surprising finding was the fact that all observed oscillations fell into the 25-27 Hz frequency range with no apparent subtype specific distinction. In many recordings the oscillatory spiking appears coherent for the ON polarity ganglion cell responses. The relative high occurrence of coherent spiking indicates that not only the same physiological types, but various RGC subtypes can synchronize their spike oscillations as well. This reveals an unexpected heterotypic (among different cells) signal correlation across the RGC. In pharmacological tests we also examined if RGC signal oscillations are due to gap junction (GJ) signaling (specific aim 2). We found that RGC spike oscillations were diminished/deleted by GJ blockers but only altered when GABA receptor blockade was applied. Some of the latter pharmacological data was used for two publications (Ganczer et al. 2022; Szarka et al. 2024). We also tried to use Thy1-GCaMP3 and PV-GCaMP6 mice to perform Ca^{++}-imaging to detect spike signal oscillations. Although, the signal oscillations could not be clearly detected in these tests, the attempts brought us to another experimental line in which we targeted OFF alpha RGCs. The fluorescent indicator allowed us to reconstruct and morphologically identify target RGCs. These reconstructions were utilized in many poster presentations and also some research papers (Szarka et al. 2023; Szarka et al. 2024).

We also planned (specific aim iv) to combine our functional investigations with an existing retinal model (PetaVision), which was created by our collaborator (Dr. Garrett Kenyon's lab; National Laboratory, Los Alamos, USA). We were able to detect travelling spontaneous activity waves (TSAWs) of RGCs in both the live retina and the model. This is a new finding because the previously described cholinerg, glutamaterg and gap junction mediated activity waves were observed only in the developing retina and showed that all three forms halt following eye opening. In contrast, TSAWs occur in the healthy, adult retina. We described the TSAW kinetic features and provided pharmacological description to dissect the neuronal circuitry that underlies them. We demonstrated that TSAWs largely depend on the subset of GJs that are created between RGCs and neighboring amacrine cell interneurons. These latter results were first predicted by the model as it allowed us to turn on and off circuit elements at will and to examine how the RGC output signal is altered. Our subsequent pharmacological experiments supported these model predictions. Some of these data were utilized in poster presentations and research papers (Tengölics et al. 2019; Völgyi et al. 2019; Ganczer et al. 2022) We also had an attemt to publish the full characterisation of TSAWs with mixed results (several turns with iScience editors and final rejection); we need to run some further supporting tests to be able to publish these data in a prestigeous journal.

While detecting signal oscillations we also examined other RGC signal kinetic features. One of these examined response transience. We examined the effects of GJ coupling on the kinetic features of RGC responses (Ganczer et al. 2022). We found that RGC full-field light responses cover a wide range of transiency, transiency is determined by inner retinal circuits and RGC GJ are critical elements. By using imaging and pharmacology (GJ and/or GABA inhibition blockade) we showed OFF alpha RGC GJs serve to homogenize temporal properties (delay, decay, amplitude) of neighboring cells. We also show that OFF alpha ganglion RGC GJs equalize light response kinetic features in order to send a population signal towards the brain. This work has recently been published (Szarka et al. 2024). Besides the regular somatic recordings, in certain GMO mouse lines we are able to record dendritic Ca^{++} -transients as well, which is a next line of research (potentially funded by a next grant).

Our patch-clamp RGC electrical recordings were combined with tracer injections that allowed for the morphological investigation of the recorded neurons and also for subsequent IHC labeling. This allowed us to describe the relation of excitatory bipolar cell chemical synapses and amacrine cell GJs to OFF alpha RGCs, one of our major targets in the mammalian retina. We collected a sizeable histology data showing the topographical distribution of Cx36 GJs in 3 model animals and the dendritic Cx36 distributions in human RGCs; data were published it in research papers (Kántor et al. 2018; Telkes et al. 2019; Fusz et al. 2021). In addition we described how extrinsic and intrinsic factors (such as the level of ambient light, postnatal changes or circadian rhythmicity) alter the expression of connexin proteins in the retina (Kovacs-Oller et al. 2023a). Another line of work showed that Ca^{++} -buffer proteins are useful neurochemical markers of RGCs. This latter work resulted a research paper (Kovacs-Oller et al. 2019) and an editorial summary (Volgyi 2020). A further work described serotonin as a potent GJ permeant tracer that can be intracellularly injected into cells to reveal the full soma/dendritic morphologies of both the injected and the electrically coupled cells. We describe the method and also show its feasibility to label the coupled OFF alpha ganglion cell array. This data has been published (Szarka et al. 2023). Finally, our morphological investigations also revealed that transient OFF alpha RGCs form electrically coupled domains where intradomain asymmetry and orientation preferences of the cells are shared. In contrast, the same features might vary considerably among domains. This project has been presented in the form of a poster in the European Retina Meeting 2023 (September 2017-2020; Tubingen), the manuscript has been written and ready to submit soon (Futacsi et al. 2024).

Besides the healthy retina we gathered data from pathological retinal samples. This line of research describes a previously unnoticed role of retinal inhibition that can potentially be utilized for therapeutic purposes to rescue the retinal tissue throughout pathological changes (Wang et al. 2020), show a potentially curative effect of imatinibe in the retina by controlling the formation/deformation of the perycite mosaic (Kovacs-Oller et al. 2020b), summarizes the role of GJs in degenerative processes of the retina (Szarka et al. 2021), describes how LED lights induce degenerative changes in the retina (Balogh et al. 2021) and describes how traumatic brain injury affects the retinal tissue (Kovacs-Oller et al. 2023b).

Finally, the work on RGC GJs lead us to the conclusion that GJs play a pivotal role in visual coding. To show that approach motion sensing, the primary function of transient OFF alpha RGCs strongly depend on GJ connections we performed behavioral tests on mice. The blockade of GJs (MFA delivered with eyedrops) in the free moving mice caused the disruption of the escape behavior, a reflex initiated by the above mentioned OFF alpha ganglion cells. This strongly indicates that the OFF alpha RGC GJs have a crucial role in the encoding of approach stimuli and also the initiation of the escape behavior. We also investigated the retinal circuitry that lies behind this phenomenon. We found that OFF alpha RGC GJs form an array with neighbor alpha cells and a set of nearby amacrine cells that is crucial to signal the approaching motion of objects. The basis of the mechanism is a GJ mediated priming signal initiated in the RGC that lies in the focus of the stimulus. This signal spreads to neighboring RGCs though GJs. Lately we started in vivo testing to see how this latter signal reaches postsynaptic neuron targets in the superior colliculus (SC). This last line of research on encoding approaching motion by the retina/SC signaling route will be the subject of our next grant proposal.