Project 1:

TITLE OF THE COLLABORATIVE PROJECT

CIRCADIAN CONTROL OF OUTER RETINAL INHIBITION

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1. PARTNERS INVOLVED IN THE COLLABORATION

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2. DESCRIPTION OF THE PROJECT

Background

In the outer retina, horizontal cells feedback to photoreceptors. This inhibitory interaction forms the basis for the center surround organization of the bipolar cells, a feature that is preserved in all vertebrates. The main purpose of the inhibitory interaction is to reduce redundancy both in space and time such that only relevant information (i.e. deviations from the mean) is send to the brain (Srinivasan *et al.*, 1982). This poses an interesting dilemma. For spatial redundancy reduction the inhibitory systems needs to be fast such that no motion blur occurs. The bipolar cell surround should not lag the center when a light spot is moving across the retina. However, the essence of temporal redundancy reduction is the slowness of the inhibition. To complicate matters even further, the signal to noise ratio of the signals to be processed and the kinetics of photoreceptors change dramatically during light-dark adaptation and during the circadian phases. For instance, it is widely known that negative feedback from horizontal cells to cones is down regulated in the dark-adapted retina (Raynauld, 1972; Weiler & Wagner, 1984; Djamgoz *et al.*, 1988). This requires a very complicated and plastic inhibitory synapse.

The goal of this project is to identify the basic organization and plastic mechanisms functioning during light-dark adaptation and during the circadian phases of the inhibitory synapse between horizontal cells and cones in the mammalian retina.

The synaptic mechanism underlying the inhibition between horizontal cells and cones is a matter of intense debate. We recently published a study showing that horizontal cells in the goldfish and zebrafish retina feed back to cones via a combination of an ultrafast connexins hemichannel-based mechanism, and a slow pannexin 1/ATP base system (Vroman & Kamermans, 2015). In fish the mechanism works as follows:

Horizontal cells affect the neurotransmitter release of cones by modulating their calcium current. This modulation consists of two processes each with different kinetic properties; a slow one and a fast one. The latter is very fast indeed (< 1 ms), inconsistent with the inevitable delay imposed when cells are separated by a synaptic gap. Instead, connexin hemichannels on the horizontal cell membrane allow current to flow into the horizontal cell, increasing the negative charge of the intercellular space and increasing calcium flow into the cones, locally depolarizing it. This direct ("ephaptic") connection makes the synapse among the fastest of all known inhibitory synapses.

The slow component depends on another channel called pannexin 1. Pannexin 1 is also present in the synapse and is known to mediate release of ATP. We showed that depolarized horizontal cells release ATP, an effect that could be blocked by addition of a specific pannexin 1 antagonist, probenecid. ATP when hydrolyzed produces phosphates and H^+ ions (protons), which acidify the intercellular space and buffer it against pH changes. Immunolabelling showed that an ATP hydrolase was present on the exterior surface of the horizontal cell, and that blocking this enzyme reduces the slow component of the feedback response. Artificial alkalization of the synaptic space had the same effect. The ATP that is released via pannexin 1 is thus hydrolyzed and leads to slight

acidification of the synaptic cleft, which inhibits pre-synaptic calcium channels and inhibits the neurotransmitter release of photoreceptors.

A direct demonstration of such interactions in the mammalian retina is still lacking. This is mainly due to the fact that mice are highly rod dominated (~97% rods) and that extensive electrophysiological examination is almost unfeasible due to their small size. So far, the studies addressing negative feedback in the mouse system have therefore relied on fluorescent imaging techniques. These techniques are too slow to access the ultrafast ephaptic mechanism and not sensitive enough to evaluate the subtle feedback-induced changes in the Ca-current in cones.

Planned research

In this proposal we want to elucidate the mechanism of horizontal cell to cone feedback mechanism in a cone-dominated mammalian system: Arvicanthis. Arvicanthis is a diurnal rodent with a retina that consists of 30% cones (Bobu *et al.*, 2006). Our working hypothesis is that the feedback mechanism in Arvicanthis has highly similar properties to that found in fish. We do however expect major quantitative differences because of the vastly different visual environment zebrafish and Arvicanthis experience.

The aim of this research proposal is: 1) Establish Arvicanthis as a model animal for studying the diurnal mammalian retina, 2) Establish the mechanism of feedback from horizontal cells to cones in a mammalian system, and 3) Establish the mechanism by which feedback from horizontal cells to cones is modulated during light-dark and circadian cycles.

We will analyze the nature of the feedback pathway from HCs to cones in Arvicanthis using whole cell voltage clamp recording techniques in the isolated retina (Kamermans - Amsterdam). We will test pharmacologically whether the various components of the feedback pathway identified in fish are present in Arvicanthis and quantify their contribution to the feedback signal in different circadian phases (day, night, constant dark). In addition we will localize key components of the feedback mechanism using molecular biological and immunohistochemical techniques (Hicks - Strasbourg) during the same time points. Unpublished results in zebrafish show that Cx55.5, the connexin forming the hemichannels in zebrafish, is strongly regulated during day and night, suggesting that the balance between the various feedback systems might be very different between the day versus the night. Whether this is a true circadian modulation is not known but will be tested in this project. Since Arvicanthis is a novel rodent model, not much is known yet about its molecular biology. Therefore, we recently started to sequence the genome of Arvicanthis (USIAS: Kamermans, Hicks, Baas) which will be of great value to identify the various components of the feedback system in Arvicanthis.

Collaboration

The collaboration between Hicks and Kamermans in this project is of great additional value. Hicks established the Arvicanthis model in France and has extensive experience with this animal model. Furthermore, he is highly experienced with immunecytochemical, histological and molecular approaches to identify proteins in the retina. Kamermans has extensive experience with the circuit analysis of the retina and the use for state of the art electrophysiological approaches. Combined this generates a team fully capable to addressing the ambitious goal of this project.

References

Bobu C, Craft CM, Masson-Pevet M & Hicks D. (2006). Photoreceptor organization and rhythmic phagocytosis in the nile rat Arvicanthis ansorgei: a novel diurnal rodent model for the study of cone pathophysiology. *Invest Ophthalmol Vis Sci* **47,** 3109-3118.

Djamgoz MBA, Downing JEG, Kirsch M, Prince DJ & Wagner H-J. (1988). Light-dependent plasticity of horizontal cell functioning in cyprinid fish retina: effects of background illumination of moderate intensity. *J Neurocytol* **17,** 701-710.

Raynauld JP. (1972). Goldfish retina: sign of the rod input in opponent color ganglion cells. *Science* **177,** 84-85.

Srinivasan MV, Laughlin SB & Dubs A. (1982). Predictive coding: a fresh view of inhibition in the retina. *ProcRSocLond, B, BiolSci* **216,** 427-459.

Vroman R & Kamermans M. (2015). Feedback-induced glutamate spillover enhances negative feedback from horizontal cells to cones. *J Physiol* **593,** 2927-2940.

Weiler R & Wagner H-J. (1984). Light-dependent change of cone-horizontal cell interactions in carp retina. *Brain Research* **298,** 1-9.

3. DESCRIPTION OF THE EXPECTED MOBILITY TRACK

- Oct. 1st 2016 to Sept. 30th 2017: 12 mo: Strasbourg
- Oct. 1st 2017 to Sept. 30th 2018: 12 mo: Amsterdam
- Oct. 1st 2018 to March 30th 2019: 6 mo: Strasbourg
- April 1st 2019 to Sept. 30th 2019: 6 mo: Amsterdam

4. SPECIFIC REQUIREMENTS FOR THE CANDIDATE

Master of Science (M.S.) in **Neuroscience** mandatory.

Candidates should have good basic training in neuroscience, including if possible vision. It would be an advantage to have experience in cellular, molecular and physiological techniques including microscopy, immunochemistry, PCR and electrophysiology. Candidates should be fluent in English.