

Project 7:

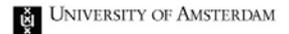
TITLE OF THE COLLABORATIVE PROJECT

**DOES A POORLY-FUNCTIONING CIRCADIAN CLOCK
CONSTITUTE A RISK FACTOR FOR (GENETICALLY
DETERMINED) RETINAL DISEASE?**

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1. PARTNERS INVOLVED IN THE COLLABORATION

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2. DESCRIPTION OF THE PROJECT

Intrinsic, \approx 24h rhythms are a fundamental hallmark of every cellular and physiological process throughout the body. These daily rhythms are programmed by molecular clocks widely distributed in mammalian tissues and coordinated by a hypothalamic master clock. Circadian clocks keep time by using complex transcriptional/post-translational feedback loops involving transcription factors encoded by “clock genes” (mainly *Bmal1*, *Clock*, *Per1*, *Per2*, *Cry1*, *Cry2*) and which transmit their cycling patterns to their target genes, hence to regulate gene expression programs. Genome-wide transcriptome profiling studies have uncovered a wide array (about 43% of all protein encoding genes) of ubiquitous and tissue-specific genes under circadian control, in agreement with the need, for different organs, to fulfill distinct temporally controlled tasks (Zhang et al., 2014). Thus, circadian rhythmicity has strong adaptive value and it is now established that chronic disturbance of these timed mechanisms leads to increased morbidity and reduced lifespan (West and Bechtold, 2015)

Daily rhythmicity is a central hallmark of vision. The clock contained within the retina plays a crucial function in adapting retinal physiology and visual function to the day/night cycle by controlling the expression of photopigments and phototransduction related genes, visual sensitivity and processing of light. It also regulates processes that are directly linked to retinal survival such as nocturnal release of the cytoprotective melatonin, photoreceptor outer segment phagocytosis by the underlying pigmented epithelium (RPE), and phototoxicity (McMahon et al., 2014, for review).

At the same time, hundreds of genetic retinal disease genes have been recently identified (www.retnet.org). These disease (genes) primarily affect the photoreceptor and/or the RPE cells, typically resulting in altered RNA and protein expression profiles. Multiple, potentially pathogenic, molecular consequences occur downstream until apoptosis and cell death sets in, and vision is lost. Together, this process may involve thousands of genes. Since the retinal circadian clock affects expression of approximately half of the cellular genes, we hypothesize that a disturbed retinal clock may influence the expression of disease genes (downstream) and, hence, influence the time of onset, severity and progression of a vast array of genetic retinal diseases.

Little is known in the literature concerning possible links between clock function and genetic retinal disease. Examples are a limited study about the influence, on the clock, in the *Crx*-related degeneration (Rovsing and Møller 2014), and a study by Tosini and coworkers (2007) who investigated circadian rhythms in RCS and wild-type rats. They found that degeneration of the photoreceptors affected the expression level and patterns of many clock genes. Nevertheless, the overall retinal clock function appeared not to be disturbed, since circadian rhythms in the retinal dopaminergic and melatonergic systems were normal.

However, in the present project we want to investigate *the reversed* hypothesis: a poorly-functioning circadian clock might constitute a risk factor for disorders affecting the retina. For example, in man, mutations in the *Rds* gene result in, so far unexplained, extremely variable retinal phenotypes, even within families carrying the same mutations. This variability may be in part due to light damage (Hsu et al. 2015) but a role of a partly or chronically disturbed circadian rhythm has not been excluded.

To illustrate and validate our hypothesis, the PhD student will investigate the molecular and pathophysiological effects of clock and/or retinal disease gene inactivation, in relevant knock-out mice.

We will use the *Bmal1* knock out which has a severely inactivated retinal clock (Storch et al., 2007), the *Rd2* (*Rds; Prph2^{Rd2}*) mutant as a well established model of retina degeneration (Schalken et al. 1990), and double mutants of these mice.

Photoreceptor impairment will be evaluated by behavioral analysis of light response (entrainment to the light/dark cycle, masking) (Strasbourg) and by longitudinally, non-invasive assessment of retinal structure and function using optical coherence tomography (OCT) and electroretinography (ERG) (infrastructure modernized in 2015; in Amsterdam).

Eyes from double mutant and control animals (WT and single mutants) will be harvested at regular intervals before and along the process of retinal degeneration and will be submitted to biochemical/immunohistochemical/histological analysis and staining (Strasbourg). They will also be submitted to molecular analyses (photoreceptor and RPE specific cellular transcriptome studies) to uncover altered pathways and (new) clock influenced disease genes (Amsterdam).

We expect no, or hardly any, retinal degeneration in the *Bmal1* knock-out mice, although subcellular molecular pathways and ERG signals may be altered compared to mice with the same background. The *Rd2* mouse has a dominant spontaneous mutation in the *Rds/Prph* gene and was characterized by slow degeneration of the outer nuclear layer of the retina beginning at 5 weeks, loss of retinal rod cells by 7-10 months, loss of cone cells and all visual cell structures by 12 months of age, loss of some pigment epithelial cells and increased density of Müller cells accompanied by fibrillary tangles (www.jax.org).

We expect the double knock-out to have an (statistically significant) earlier onset of disease, a specific molecular pathology and a more severe phenotype than the single knockouts and wild type controls. Each mice category to be investigated will include 6-10 animals with the appropriate genotype and various measurements described will be performed in 4, 12, 24, 38 and 52 weeks old mice

The results of this project should give new insight into the functional and molecular link between the circadian clock and retina physiology/health. In the context of the modern lifestyle, with its uncontrolled use of artificial light, shift work or jet lag, it is expected that this project will also allow finding out whether circadian misalignment also targets retinal function and disease.

References:

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3. DESCRIPTION OF THE EXPECTED MOBILITY TRACK

- Oct. 1st 2016 to April 30th 2017: 7 mo: Unistra: breedings, behavioral characterization, initial eye sampling and staining
- May 1st 2017 to October 31st 2018: 18 mo: UvA/NIN: longitudinal study of visual deficits (OCT, ERG), transcriptome analysis
- Nov. 1st 2018 to Sep. 30th 2019: 11mo: Unistra: final data analysis, confirmation of gene expression alterations, redaction of the thesis manuscript

4. SPECIFIC REQUIREMENTS FOR THE CANDIDATE

Master degree (or equivalent) in Science (M.S.) mandatory.

Education in cellular and molecular biology, and/or neuroscience.