Title: Circadian Rhythms in Murine Ocular Tissues including Sclera are affected by Neurobasal A Medium Preincubation, Mouse Strain, but not Sex

Authors:

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Abstract

Motivation: Our understanding of ocular clocks has been profoundly advanced by the development of real-time recording of bioluminescence of PER2::LUC knock-in mouse explants. However, the effect of sex, mouse strain and culturing conditions on ocular clocks remains unknown. We also do not know whether the sclera contains a circadian oscillator.

Aim: We aimed to reveal the effect of sex, mouse strain and preincubation media on ocular clocks. We also aimed to reveal whether the sclera contains a circadian oscillator by using scraped posterior eye cups (PEC) as a proxy.

Methods: Retinas, corneas, intact and scraped PECs were obtained from male and female PER2::LUC knock-in mice maintained on either a pigmented C57BL/6J or albino RjOrl:SWISS background. PER2::LUC bioluminescence rhythms in ocular tissues were measured using a Lumicycle®.

Results: We compared PER2::LUC bioluminescence rhythms between ocular tissues and found that all ocular tissues oscillated, including the scraped PEC (i.e. sclera). The rhythms in scraped PECs had lower amplitudes, longer periods and distinct acrophases compared to other ocular tissues. Ocular tissues of RjOrl:SWISS mice oscillated with higher amplitudes compared to the ones of C57BL/6J, with corneal rhythms being most affected by mouse strain. A 24h preincubation with Neurobasal A medium enhanced rhythms of ocular tissues, whereas sex differences were not detected for these rhythms.

Conclusions: PER2::LUC bioluminescence rhythms in murine ocular tissues are affected by Neurobasal A medium preincubation, mouse strain but not sex. The discovery of circadian rhythms in the sclera is important considering the role of the sclera in glaucoma.