Environmental chemicals’ effect on an in vitro model with a synchronized circadian rhythm

**Project type**
Master degree project, 30 - 45 credits

**Location**
Department of Organismal Biology, Physiology and Environmental Toxicology program
EBC, Uppsala University

**Contact**
Send application (CV and why you are interested in the project) to Caroline Agrillo
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**Project description**
The circadian clock affects most living things: from humans and other animals, to plants and microbes. In humans it is mainly mediated by the light-dark cycle and regulates processes such as hormone release via gene expression throughout the day (Oster et al. 2017). Almost half of our genes are thought to be under circadian control, pointing out the importance for this internal clock (Ndikung et al. 2020). The relevance for the circadian clock has been studied in fields such as progress and treatment of diseases like metabolic disorders, cancers, cardiovascular diseases and sleeping disorders (Ruan et al. 2021, Takeda and Maemura 2016, Zhang et al. 2021).

The importance of the circadian clock has been discussed not only in disease development but also in the context of xenobiotic response in an organism. Several recent studies have proposed that the body’s response to pharmaceuticals and environmental chemicals may potentially differ depending on the time of the day for the exposure. The proposed reason for this is the phase of the circadian clock which the body and its tissues are at (Nahmias and Androulakis 2021, Dallmann, Okyar and Lévi 2016). For example, biotransformation capacity after administration of dioxin in vivo has been shown to be different depending on in which phase of the circadian clock it was administered. In this context, wild type mice with normal function of the circadian clock had an oscillating expression of Cyp3a11, a gene under the control of the clock-gene Bmal1. When the expression was high, so was biotransformation and vice versa (Lin, 2019).

Animals are used for biological and medical research in general, and toxicological testing in particular. However, for scientific and ethical reasons, there is an increased demand for alternative methods, e.g., cellular in vitro models. In a typical in vitro system, the cells follow a circadian rhythm but not in synchrony. This differs from the whole organism where the circadian rhythm is synchronized in a tissue-specific manner (Ndikung et al. 2020). This raises the question whether in vitro testing of chemicals in non-synchronized cell cultures is relevant enough to explain real life scenarios. Synchronizing the circadian rhythm of cell culture may improve the physiological and regulatory relevance of in vitro systems.

**Student project**
The aim of the project is to investigate if neurotoxic effects of chemicals in vitro differ between cells with synchronized and asynchronized circadian rhythm, and if the effects vary between different time points of the circadian rhythm.

The student will synchronize an in vitro system used for neurotoxicity testing to a circadian rhythm and subsequently expose the cells to non-persistent chemicals at different time points, followed by analysis of circadian synchrony and neurotoxicity. In the lab the student will learn cell culture techniques (thawing, feeding, plating, exposing), DNA/RNA extraction, cDNA conversion, PCR, qPCR, acetylcholine esterase assay and gel electrophoresis. Data will be analyzed and presented by the student using e.g., R, and in this part of the project the student will have the possibility to deepen their understanding about statistics and data interpretation.
References


Lin, Yanke, Shuai Wang, Ziyue Zhou, Lianxia Guo, Fangjun Yu, and Baojian Wu. “Bmal1 Regulates Circadian Expression of Cytochrome P450 3a11 and Drug Metabolism in Mice.” *Communications Biology* 2, no. 1 (October 16, 2019): 1–11. [https://doi.org/10.1038/s42003-019-0607-z](https://doi.org/10.1038/s42003-019-0607-z).


Takeda, Norihiko, and Koji Maemura. “Circadian Clock and the Onset of Cardiovascular Events.” *Hypertension Research* 39, no. 6 (June 2016): 383–90. [https://doi.org/10.1038/hr.2016.9](https://doi.org/10.1038/hr.2016.9).