M2 project: Functional imaging of clock neurons during a circadian cycle

A brain circadian clock controls sleep-wake rhythms and integrates light signals to adapt the behavior to the daily and seasonal changes of the environment. Drosophila melanogaster has been instrumental in understanding the largely conserved molecular mechanisms of circadian oscillators as well as neuronal circuitry principles that control circadian behavior (Dubowy and Sehgal, 2017). Fruit flies are mostly diurnal and display a bimodal activity pattern in light-dark (LD) conditions, with a morning activity bout, a siesta that is more pronounced in males, an evening activity bout and a night sleep.

The Drosophila brain clock relies on about 150 clock neurons that show 24h oscillations of clock proteins and are organized in half a dozen anatomically defined subsets with specific contributions to the building of the sleep-wake behavior, in particular morning and evening cells (Chatterjee and Rouyer, 2016; Chatterjee et al., 2018). Different morning and evening neuronal oscillators build the bimodal activity but how they interact to do so remains poorly understood and many neurons of the network do not have yet an identified function. Experiments based on light-sheet microscopy and genetically encoded calcium sensors expressed in the clock neurons have shown that different clock neuron subsets display differently-phased calcium oscillations through the day (Liang et al., 2017; Liang et al., 2016). However, how photoreceptive inputs modify its activity along the day-night cycle remains unknown.

A collaborative work (Brainscopes project, Université Paris-Saclay) allowed us to mount a light-sheet fluorescence microscope (LSFM), which has been successfully used to record clock neuron activity by calcium imaging (GCaMP) in the dissected brain. Based on the acquired experience, we will modify the setup to accommodate a living fly and perform long term (24h) recordings within a light and temperature-controlled chamber. The first experiments will aim at detecting circadian calcium oscillations in a wild-type fly exposed to standard (white light, 12h:12h) light-dark cycles. Then, we will investigate how light inputs shape the oscillations in the different clock neurons by modifying the light conditions (intensity, wavelength, duration) and using different genotypes that affect either the visual system or the cryptochrome brain photoreceptor (Saint-Charles et al., 2016; Yoshii et al., 2016; Alejevski et al., 2019).

The cellular heterogeneity within the neuronal groups requires a single cell resolution to understand the operating mode of the circadian network. This point is a key one since neuronal groups as small as 5-10 cells contain several cellular types, based on peptide/neurotransmitter expression, supporting functional heterogeneity. Obtaining single cell resolution with live imaging would thus be an important step forward in the field. To improve calcium imaging resolution, we will include an adaptive optics (AO) loop to correct optical aberrations generated by tissue inhomogeneity (ANR InovAO in collaboration with ESPCI/LPEM and the Imagine optic company) (Hubert et al., 2019).

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