The retinal circadian clock is selectively coupled to distinct neuronal clocks to inform behaviour

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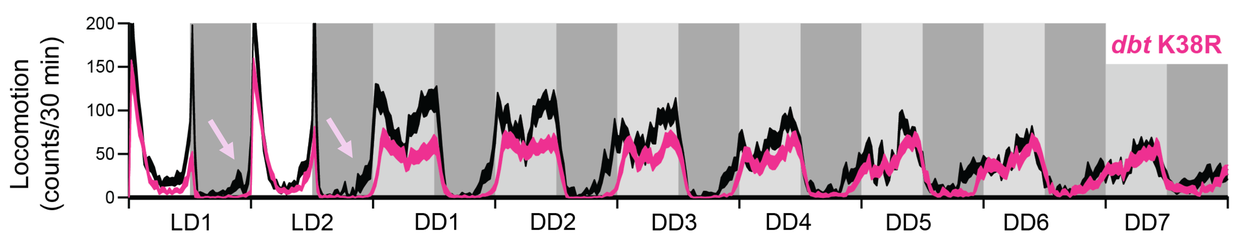
Abstract

Circadian rhythms are physiological and behavioural anticipatory responses to daily changes in the environment, such as day/night cycles. These circadian rhythms are regulated by circadian clocks, transcription negative feedback loops that oscillates with a ~24-hour period. In *Drosophila melanogaster*, circadian rhythms are governed by circadian clocks in a network of 240 neurons across the brain. Importantly, external cues such as light ensures that circadian behaviour (e.g. waking, sleeping) is in phase with daily changes in the environment. One pathway through which light information is transmitted to circadian neurons is through the eye. We found that eliminating the retinal circadian clock leads to a delay in timed waking (morning anticipation) and a short waking period in constant dark conditions, mimicking winter-like activity/sleep behaviours. Interestingly, the loss of the retinal clock disrupts clock oscillations in the dorsal lateral neurons (LNds) and dampens the clock in dorsal neurons (DN1s), two of the seven neuronal clusters that comprise the circadian neuronal network. We interpret these data to suggest that distinct coupling between the retinal clock and different neuronal clocks modulates circadian behaviours to adapt to seasonal changes in the environment.

Introduction

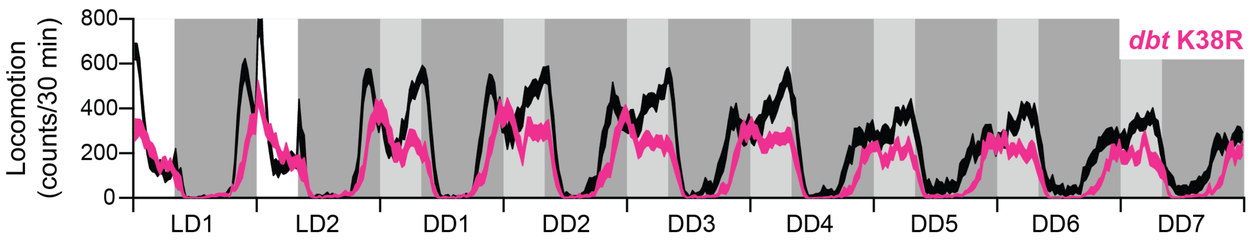
Drosophila circadian behaviour is regulated by circadian clocks that are coupled across the 240 circadian neurons that are arranged into distinct neuronal clusters in the brain. These clocks are composed of the proteins Clock (dCLK) and Cycle (CYC) that form the activator complex, which trigger transcription of hundreds of genes, including period (*per*) and timeless (*tim*). PER/TIM combine to form the core of the repressor complex in the cytoplasm, which is then translocated into the nucleus to repress the dCLK/CYC activator complex. After a delay, the repressor complex is degraded, releasing dCLK/CYC and closing the negative feedback loop. We hypothesize that select clocks across the circadian neuronal network are coupled to regulate specific aspects of circadian behaviour. In this project, we suggest that timed morning anticipation is regulated by retinal clocks that communicate to so-called evening neurons (primarily LNds). We believe that the so-called morning neurons (LNvs) are a bridge that connects signal from the eye to the LNds, likely through the release of the neuropeptide PDF.

The retinal clock regulates morning anticipation behaviour



Circadian locomotor activity was measured for two days in 12 hours of light and 12 hours of dark (LD), followed by seven days in constant darkness (DD). White and light gray represent objective and subjective day respectively, while dark gray represents both objective and subjective night. In flies with no retinal clock (magenta), morning anticipation behaviour (pink arrow) is lost and overall locomotion is reduced in constant darkness, as compared to controls (black)

Morning anticipation is recaptured in a winter-like photoperiod



Circadian locomotor activity was measured for two days in 8 hours of light and 16 hours of dark (LD) to mimic winter photoperiod, followed by seven days in constant darkness (DD). In flies with no retinal clock (magenta), morning anticipation behaviour is partially restored in comparison to control (black line). However, flies lacking a retinal clock do not exhibit the wild-type secondary morning anticipation peak in the longer night. In constant darkness, flies maintain behavioural rhythms similar to flies entrained in equinox; they still lack morning activity locomotion in DD.

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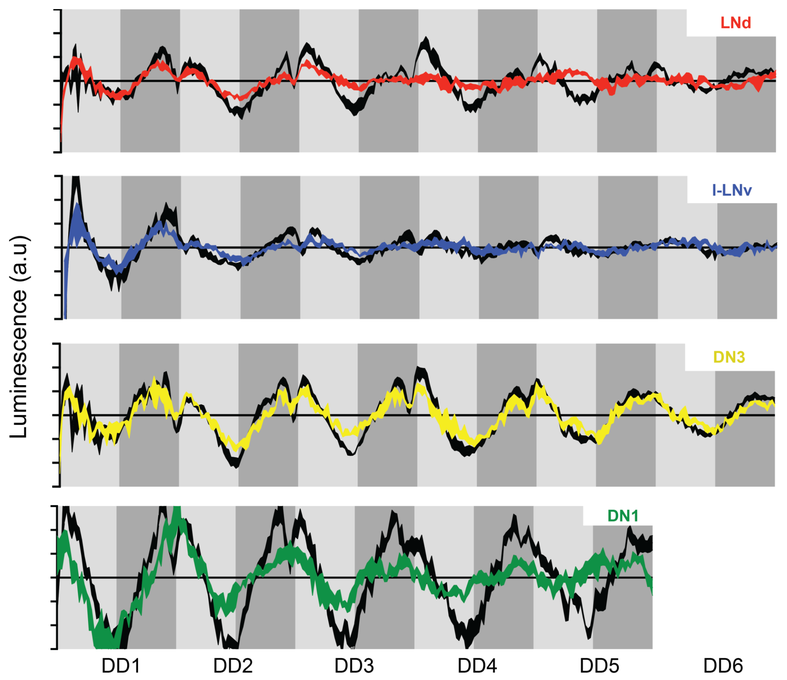
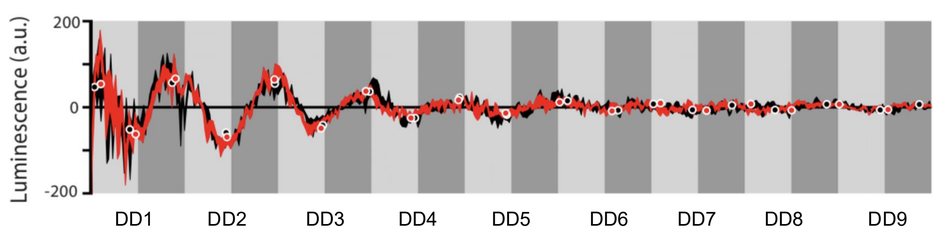
The retinal clock communicates to specific neuronal clusters



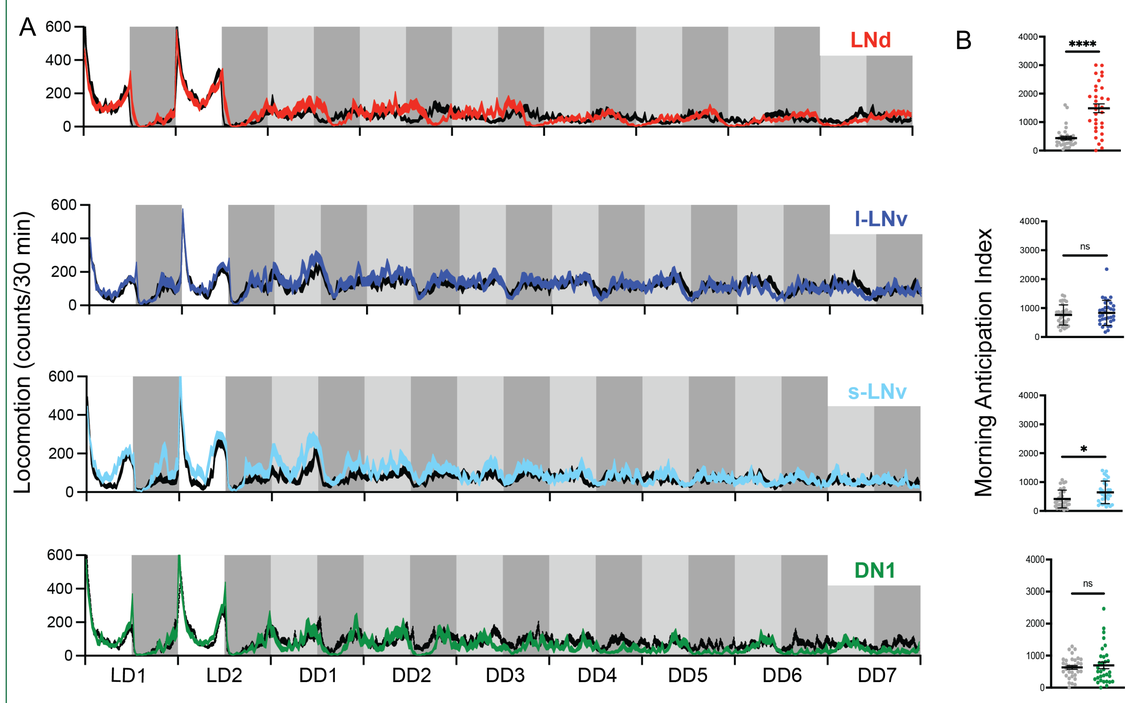
In flies lacking retinal clocks (coloured lines), LNd clocks are disrupted (red), DN1 clocks lose amplitude (green), but the l-LNv (blue) and DN3 (yellow) clocks remain unchanged, as compared to control flies with a functional retinal clock (black line). Thus, disruption of the retinal clock disrupts subsets of circadian neurons, suggesting preferred transcriptional coupling between distinct clocks in the brain.

Circadian clock of LNv neurons is not coupled to LNd clock



Flies in which the LNv clock (red) is eliminated do not affect LNd clock oscillations compared to controls flies (black). Thus, the LNv clocks and LNd clocks are not coupled. This suggests that the retinal clock is unlikely to alter LNd clocks through disruption of LNv clocks.

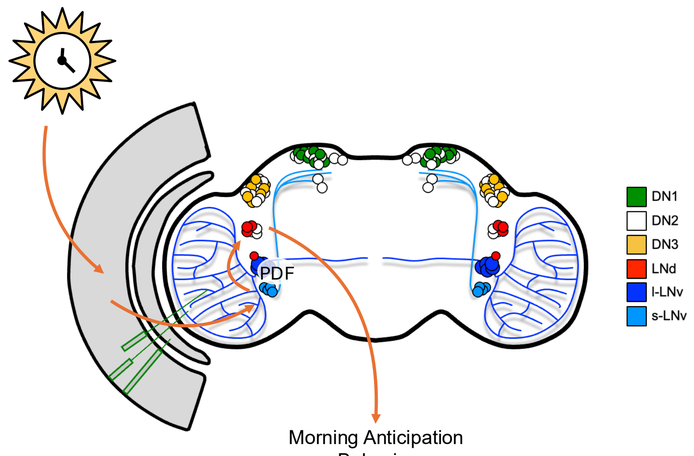
Restoration of PDF signal to LNds restores morning anticipation



A. Circadian locomotor activity measured for two days in 12 hours of light and 12 hours of dark (LD) followed by seven days in constant darkness (DD). Tethered PDF is selectively expressed in the indicated neurons to mimic PDF signaling in a *pdf*^{01} background (coloured lines). Tethered scrambled PDF peptide (non-functional) was similarly restored as controls (black lines), mimicking a lack of PDF signaling.

B. Morning anticipation behaviour is quantified in flies expressing tethered PDF or tethered scrambled peptide. PDF signal restoration in LNds (red) show restoration of morning anticipation behaviour compared to controls. Thus, morning anticipation behaviour is regulated by LNd response to PDF signaling.

Current Model



Communication pathway from the retinal clock to the LNd clock. The retinal clock communicates to LNvs (light blue neurons), which in turn communicates to the clock of the LNds (red neurons) through PDF signaling, without incorporating clock information from the LNvs.