Identification of a circadian output circuit for rest:activity rhythms in Drosophila

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The circadian system is composed of clock neurons, which contain molecular clocks, input pathways, which synchronize these clocks to external signals such as light, and output pathways, which couple clock cells to overt behaviors. Though much is known about the core clock neurons and the underlying molecular clock, little is known about the downstream neuronal populations that comprise the output pathway. Through a screen for circadian-relevant neurons in the Drosophila brain, we identify here specific subsets of cells of the pars intercerebralis (PI), a functional homologue of the mammalian hypothalamus, as necessary components of the circadian output pathway for rest:activity rhythms. Temporally and spatially restricted activation of PI neurons induces behavioral arrythmicity without affecting the molecular clock, and genetic deletion of PI neurons also renders flies arrhythmic. Notably, the circadian relevant PI neurons are distinct from those expressing the insulin-like peptide, dilp2, which are known to be involved in sleep and metabolic functions in the fly, suggesting that molecularly-distinct subsets of PI neurons couple to different physiological outputs. We further use GFP Reconstitution Across Synaptic Partners (GRASP) to trace a circuit that extends from the master pacemaker clock cells, through dorsal clock neurons, and finally to cells of the PI, thus identifying an anatomical substrate through which the PI could receive circadian signals. Finally, we use single cell RNA sequencing of PI neurons to identify the corticotropin releasing factor (CRF) homologue, DH44, as a potential signaling molecule through which the PI may communicate with downstream locomotor control areas, and demonstrate that RNAi-mediated knockdown of DH44 degrades rest:activity rhythms. Together these studies establish the PI as an integral component of the Drosophila circadian output pathway for rest:activity rhythms, delineate an anatomical circuit that underlies the circadian control of these PI neurons, and pinpoint a specific output molecule that is expressed by PI neurons and is necessary for the full display of locomotor rhythms.

Trainee Award Eligible