



# A Proposed Role For Melatonin: Circadian Regulation in *Drosophila melanogaster*

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## Background

### Circadian Clock

Molecular circadian systems govern organism-wide responses to environmental stimuli, entraining peripheral functions to a core biological clock<sup>[2]</sup>. The circadian clock of *Drosophila melanogaster*, with a conserved feedback loop mechanism, has seen use as a model for mammalian systems. The core circadian feedback loop of *D. melanogaster* corresponds to the rhythmic cycling of four proteins: *PER*, *TIM*, *CLK*, and *CYC*<sup>[1][2]</sup>.

### CREB

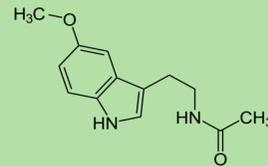
The fly ortholog of mammalian CREB, *dCREB2*, has been shown to affect levels of *PER* protein, and proposed to be a key element of the *PER* negative feedback loop<sup>[9]</sup>. On a larger scale, *dCREB2* has been implicated in regulating the temporal aspect of processes such as sleep and memory formation.<sup>[10][11]</sup>

### Melatonin

In mammals, melatonin has been shown to affect rhythmic clock phase by a G-protein signaling pathway. In fact, regular doses of melatonin are taken by those who struggle falling asleep or suffering from jetlag.

## Question

What role, if any, does melatonin play in the circadian system of *Drosophila melanogaster*?



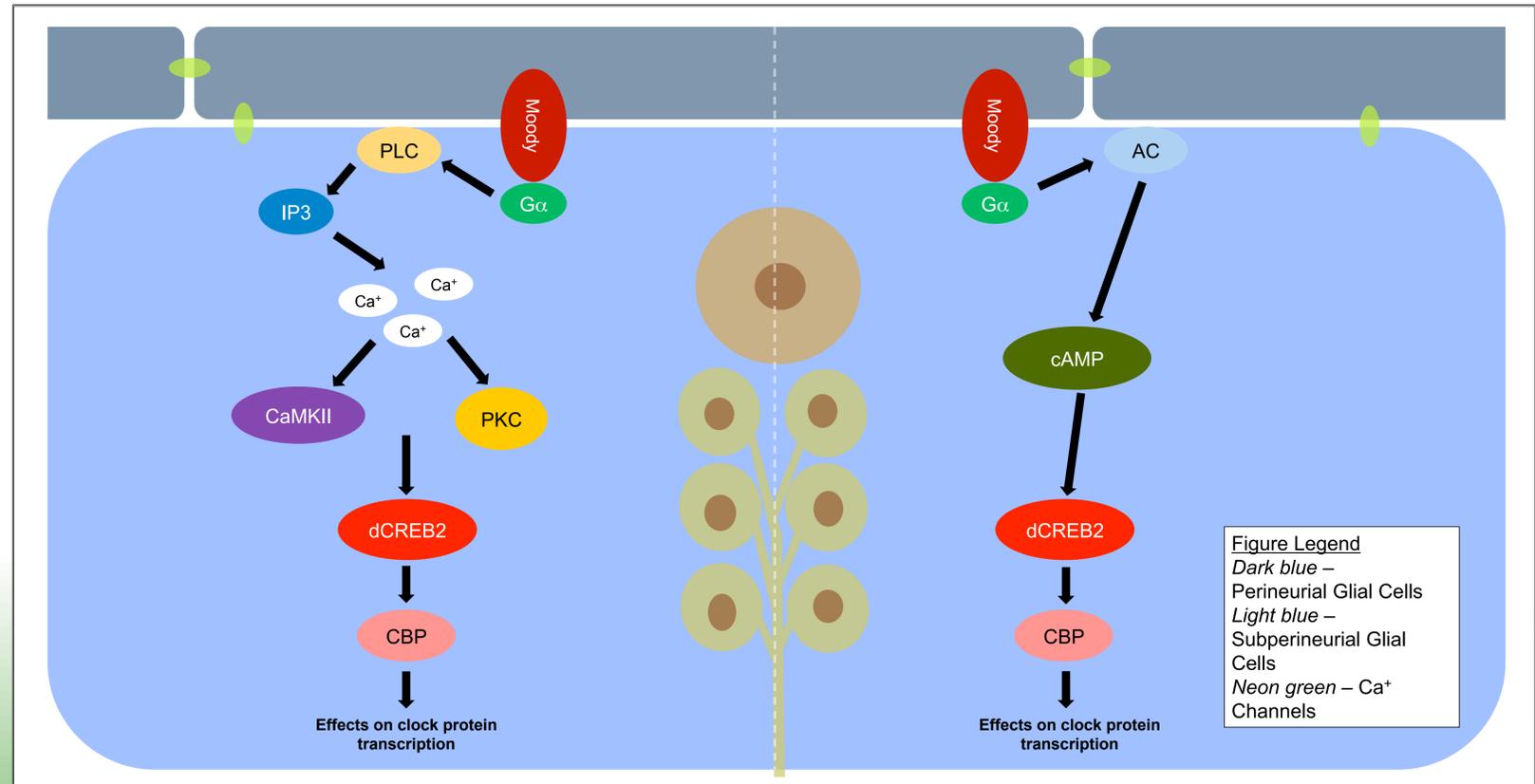
## Hypothesis

Melatonin acts on a G-protein melatonin membrane receptor, Moody, which is present in the blood-brain barrier of *Drosophila*.

## Identifying a Receptor

Melatonin is synthesized in flies, implying the. This lead us to identify and characterize a G-protein receptor in *Drosophila*. Multiple lines of evidence converged on **Moody**:

- Orphan G-protein receptor O77270 clustered with human melatonin receptors via phylogenetic analysis<sup>[4]</sup>
- O77270 previously grouped with NPY receptors, providing possible link to clock function<sup>[4]</sup>
- O77270 identified as Moody via UniProt, ortholog of mammalian melatonin receptors via FlyBase.org
- Moody is present in the glial cells of the *Drosophila* blood-brain barrier, and regulates permeability and nutrient access<sup>[5][6]</sup>



## Melatonin Signaling in *Drosophila*

Melatonin affects CREB-mediated clock gene expression via G-protein signaling and either a (1) Ca<sup>2+</sup> and PKC or (2) cAMP pathway.

## Defining a Mechanism

The signaling cascade that Moody initiates in *Drosophila* is unknown. We were able to turn to mammalian models due to the conserved nature of G-protein cascades and proteins including Moody and CREB.

- Melatonin can affect rhythmic expression of *Per1* and *Cry1* in rat models<sup>[7][8]</sup>
- Melatonin induces CREB phosphorylation in rat models<sup>[12]</sup>
- *Drosophila* WNT pathway activates CREB via Ca<sup>+</sup> influx<sup>[3]</sup>
- CBP inhibits dCLK/CYC transcriptional regulation, and physically interacts with dCLK<sup>[2]</sup>

The nature of melatonin's affect on circadian clock is tissue-specific in mammalian models, and this variability leaves open a role for *Drosophila* homologs.

## Implications

Teasing out the mechanism of a potential role for melatonin in *Drosophila* would provide a new model to address topics such as:

- Endogenous melatonin regulation on circadian systems
- Applications for treatment of SAD and Bipolar Disorder<sup>[8]</sup>
- Daily rhythmicity in learning and memory formation<sup>[10][11]</sup>
- Antioxidative effects of melatonin treatment

The presence of Moody implies an intact melatonin signaling pathway in the brain of *Drosophila melanogaster*. Further investigation is required to define a role.

### Methods for Future Study

Visualizing Moody – *in situ* hybridization<sup>[13]</sup>

Melatonin and CREB regulation – stain for pCREB2<sup>[12]</sup>

Assess gene regulation function – qPCR on *per*, *clk*, *tim*