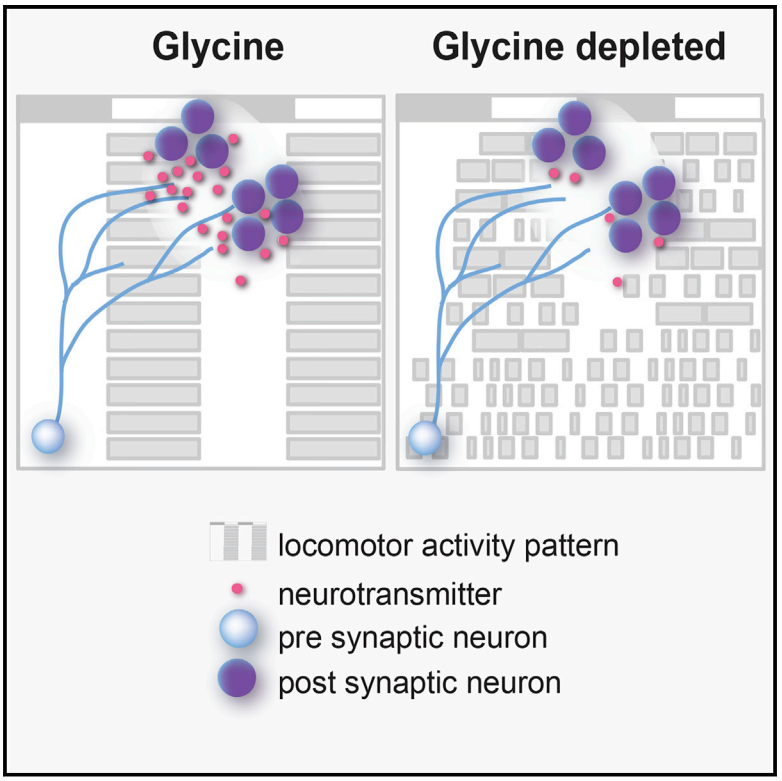
Article

Cell Reports

Organization of Circadian Behavior Relies on Glycinergic Transmission

Graphical Abstract



Authors

Lia Frenkel, Nara I. Muraro, Andrea N. Beltrán González, ..., Cristina Marino-Busjle, Daniel J. Calvo, M. Fernanda Ceriani

Correspondence

fceriani@leloir.org.ar

In Brief

Frenkel et al. discovered that glycine, a fast inhibitory neurotransmitter, plays a key role in the organization of daily patterns of locomotor activity in *Drosophila.* Thus, circadian changes in tonic inhibition provide a time-of-day switch that rapidly turns off specific targets to keep the circadian network in tune.

Highlights

- LNvs are glycinergic neurons
- Glycine is an inhibitory neurotransmitter in the adult fly brain
- Glycine depletion renders the circadian network susceptible to environmental changes
- Glycine provides coherence to the circadian network

Frenkel et al., 2017, Cell Reports *19*, 72–85
April 4, 2017
http://dx.doi.org/10.1016/j.celrep.2017.03.034

OPEN ACCESS
CellPress

Cell Reports
Article

Organization of Circadian Behavior Relies on Glycinergic Transmission

Lia Frenkel,[1] Nara I. Muraro,[1,6] Andrea N. Beltrán González,[2] María S. Marcora,[3] Guillermo Bernabó,[1] Christiane Hermann-Luibl,[4] Juan I. Romero,[1] Charlotte Helfrich-Förster,[4] Eduardo M. Castaño,[3] Cristina Marino-Busjle,[5] Daniel J. Calvo,[2] and M. Fernanda Ceriani[1,7,*]

[1]Laboratorio de Genética del Comportamiento, Fundación Instituto Leloir (FIL) IIBBA-CONICET, 1405BWE Buenos Aires, Argentina
[2]Laboratorio de Neurobiología Celular y Molecular, INGEBI-CONICET, C1428ADN Buenos Aires, Argentina
[3]Laboratorio de Amiloidosis y Neurodegeneración, FIL-IIBBA-CONICET, 1405BWE Buenos Aires, Argentina
[4]Department of Neurobiology and Genetics, Theodor-Boveri Institute, Biocenter, University of Wuerzburg, Wuerzburg 97070, Germany
[5]Laboratorio de Bioinformática Estructural, FIL-IIBBA-CONICET, 1405BWE Buenos Aires, Argentina
[6]Present address: Biomedicine Research Institute of Buenos Aires (IBioBA) MPSP-CONICET, C1425FQD Buenos Aires, Argentina
[7]Lead Contact
*Correspondence: fceriani@leloir.org.ar
http://dx.doi.org/10.1016/j.celrep.2017.03.034

SUMMARY

The small ventral lateral neurons (sLNvs) constitute a central circadian pacemaker in the *Drosophila* brain. They organize daily locomotor activity, partly through the release of the neuropeptide pigment-dispersing factor (PDF), coordinating the action of the remaining clusters required for network synchronization. Despite extensive efforts, the basic principles underlying communication among circadian clusters remain obscure. We identified classical neurotransmitters released by sLNvs through disruption of specific transporters. Adult-specific RNAi-mediated downregulation of the glycine transporter or impairment of glycine synthesis in LNv neurons increased period length by nearly an hour without affecting rhythmicity of locomotor activity. Electrophysiological recordings showed that glycine reduces spiking frequency in circadian neurons. Interestingly, downregulation of glycine receptor subunits in specific sLNv targets impaired rhythmicity, revealing involvement of glycine in information processing within the network. These data identify glycinergic inhibition of specific targets as a cue that contributes to the synchronization of the circadian network.

INTRODUCTION

The ~24-hr rhythm imposed by the rotation of the Earth around its own axis gives rise to the perpetual repetition of day/night cycles. On Earth, the circadian clock evolved under the pressure to anticipate this timing sequence. In *Drosophila*, the central clock comprises ~150 neurons, organized in ventral (i.e., the small and large lateral neurons ventral [sLNvs and lLNvs, respectively] and dorsal [lateral neurons dorsal, lateral posterior neurons, and dorsal neurons 1–3 (LNds, LPNs, and DN1–3 s, respectively)]) clusters (Shafer et al., 2006). Much is known about the molecular ~24-hr self-sustained oscillations taking place within clock neurons (Ozkaya and Rosato, 2012), but how these heterogeneous clocks communicate to each other is almost an enigma. Some neuropeptides are known to play a crucial role; among them, pigment-dispersing factor (PDF) released from sLNvs is essential, as it sets the phase of several other circadian clocks in the brain (Shafer and Yao, 2014).

But regarding fast classical neurotransmission from central pacemakers, even less is known: a screen for biogenic amines in the sLNvs did not provide indication for the presence of dopamine, serotonin, or histamine (Hamasaka and Nässel, 2006), although serotonin and dopamine modulate circadian entrainment by affecting light sensitivity (Hirsh et al., 2010; Yuan et al., 2005), and aminergic systems affect locomotor activity downstream of pacemaker neurons (Chen et al., 2013). Aside from input- and output-related components, less than 10% of the core pacemakers have been ascribed a classical neurotransmitter; in each hemisphere, two DN1as express the vesicular glutamate transporter vGlut (Collins et al., 2012; Daniels et al., 2008; Hamasaka et al., 2007) and four LNds the vesicular acetylcholine transporter vAChT (Beckwith and Ceriani, 2015a; Johard et al., 2009).

In an attempt to solve the puzzle of how cellular clocks interact with each other, we developed a simple method to uncover the nature of fast neuronal communication. We initially tested the efficiency of this approach in the LNvs. The well-documented hierarchy of the sLNvs in the temporal organization of locomotor activity makes them particularly appealing (Renn et al., 1999; Stoleru et al., 2005). Moreover, the presence of typical output synapses with active zones accompanied by small clear vesicles (Yasuyama and Meinertzhagen, 2010) and the perturbing effect of blocking fast synaptic transmission (Kilman et al., 2009; Umezaki et al., 2011; Wülbeck et al., 2009) are two pieces of evidence supporting the anatomical and functional presence of classical neurotransmitters within LNvs neurons. Along this work, we identified glycine as the neurotransmitter employed by the