CIRCADIAN PLASTICITY: FROM STRUCTURE TO BEHAVIOR

Lia Frenkel and María Fernanda Ceriani

Laboratorio de Genética del Comportamiento, Fundación Instituto Leloir, Instituto de Investigaciones Bioquímicas de Buenos Aires, CONICET, Av. Patricias Argentinas 435 - Ciudad Autónoma de Buenos Aires, (C1405BWE) Argentina

Over the years it has become clear that the biological clock acts at different levels, ranging from the control of gene expression, protein stability, or subcellular localization of key proteins, to the fine tuning of network properties and modulation of input signals, ultimately ensuring that the organism will be best synchronized to a changing environment at the physiological and behavioral levels. The purpose of this chapter is to discuss the circadian control of clock outputs, spanning the most immediate ones within pacemaker neurons (i.e., membrane excitability, release of neurotransmitters, structural changes) to the circadian modulation of different behaviors (locomotor activity, learning and memory, social interaction), with a focus on the examples that shed light on the surprising degree of plasticity that characterizes the underlying circuits.

INTERNATIONAL REVIEW OF NEUROBIOLOGY, VOL. 99
DOI: 10.1016/B978-0-12-387003-2.00005-7

0074-7742/10 $35.00

I. Introduction

The circadian clock serves as a temporal filter to synchronize gene expression, cell metabolism, physiology, and behavior to the most critical moments in the day, thus contributing to the adaptation of the organism to a changing environment. Although in the last 15 years substantial progress has been made in elucidating the molecular processes that impart this temporal control at the cellular level in *Drosophila* and other organisms (Hardin, 2005), how this translates into rhythmic behavior is less clear (Nitabach and Taghert, 2008). At the cellular level, the circadian clock is based on self-sustaining, cell-autonomous transcriptional negative feedback loops, which ultimately give rise to rhythms in the abundance, phosphorylation state, and nuclear localization of key intracellular proteins, such as PERIOD (PER) and TIMELESS (TIM) (Stanewsky, 2002).

To date several neuronal clusters have been shown to include a molecular oscillator in the *Drosophila* adult brain. The one best understood encompasses the small ventral lateral neurons (sLNvs), which are located anteriorly in the accessory medulla at the level of the esophageal foramen and send projections dorsally and posteriorly toward the superior protocerebrum, ending close to other relevant groups: the dorsal neurons (DNs) clusters 1 and 2 (Helfrich-Forster, 2003). The sLNv cluster comprises five cells, of which four rhythmically release the neuropeptide pigment dispersing factor (PDF) at their dorsal terminals (Park *et al.*, 2000). Relatively close to the sLNvs, there is a second cluster of larger somata known as the large LNvs (lLNvs) that release PDF through ipsi- and contralateral projections in the optic lobe. Other oscillators within the fly brain include the dorsal lateral neurons (LNds), the lateral posterior neurons (LPNs), together with the DN3, which is a group of about 40 neurons relevant for entrainment under light-dark (LD) cycles (Veleri *et al.*, 2003). A thorough description of the different anatomical clusters was provided by Taghert and colleagues (Shafer *et al.*, 2006). Recently, significant progress has been made in the understanding of how different environmental cues (namely, light and temperature) manage to impact on this cellular mechanism (Dubruille and Emery, 2008). This chapter will delve into the circadian control of clock outputs, ranging from the most immediate one within pacemaker neurons to the circadian modulation of different behaviors.

II. Transmitting Molecular Oscillations to Neuronal Networks

A. Circadian Modulation of Membrane Electrical Properties

About a decade ago, the advent of high-density oligonucleotide arrays spanning whole genomes provided an ample picture of the extent of clock-controlled

genes at the transcriptional level, and hence, of the potential diversity of processes that take place at regular intervals throughout the day in a given organism (as diverse as plants (Harmer *et al.*, 2000), flies (Ceriani *et al.*, 2002; Claridge-Chang *et al.*, 2001; Lin *et al.*, 2002; McDonald and Rosbash, 2001; Nagoshi *et al.*, 2010; Ueda *et al.*, 2002), or mammals (Akhtar *et al.*, 2002; Oishi *et al.*, 2003; Panda *et al.*, 2002)). Although cycling transcript levels do not necessarily reflect circadian fluctuations in activity of a given protein, in time it became clear that many aspects of metabolism, physiology, and behavior are structured to anticipate changes accompanying the 24-h cycles imparted by the rotating planet. In fact, clock-controlled transcription is strikingly widespread, and about half of all mammalian genes fluctuate with a 24-h rhythm in at least one tissue (Yan *et al.*, 2008). Although this issue has not been extensively explored in *Drosophila*, very little overlap was reported between cycling genes in the head and body parts (Ceriani *et al.*, 2002), implying that genes might cycle only on those tissues in which they play a circadian relevant function. Moreover, given that the molecular clock is essentially a transcriptional/translational phenomenon, it is not surprising that basic membrane constituents such as ion channels and receptors are regulated at the mRNA level (Ceriani *et al.*, 2002; Claridge-Chang *et al.*, 2001; Kula-Eversole *et al.*, 2010). Claridge-Chang and colleagues reported that genes known to be involved in vesicle recycling and transport oscillate at the transcript level. This observation led them to propose that there is circadian modulation of the synaptic vesicle pool and, hence, that synaptic function and plasticity could change throughout the day (Claridge-Chang *et al.*, 2001). In separate studies, a number of genes involved in neurotransmitter synthesis, transport, and recycling were found to change in a circadian fashion (Ceriani *et al.*, 2002; Ueda *et al.*, 2002), lending further support to the potential modulation of synaptic function exerted by the circadian clock.

One possible way to convey time of day information to locomotor centers and other processing areas in the central brain is to control the firing properties of different circadian clusters, which could be achieved in a straightforward manner through direct transcriptional regulation of ion channels and transporters (Ceriani *et al.*, 2002; Claridge-Chang *et al.*, 2001). Although at the protein level such oscillation has rarely been addressed (except for the Ca^{2+}-dependent, voltage-gated K^+ channel *slowpoke* (Ceriani *et al.*, 2002)), direct measurements of distinct membrane properties support this possibility (Cao and Nitabach, 2008; Park and Griffith, 2006; Sheeba *et al.*, 2008). Park and Griffith (2006) monitored the resting membrane potential and membrane resistance of one of the circadian clusters—the lLNvs—at two different time points under light–dark cycles and constant darkness (LD and DD, respectively). They observed that both the resting potential and membrane resistance fluctuate with the clock and light conditions. Later on, a thorough analysis of basic properties of the same pacemaker cluster indicated that, despite no apparent differences at the morphological level, the lLNvs show two distinct "modes" of spontaneous firing throughout the day, tonic or bursting, and

that a proportion of the sampled neurons could switch between both states during the recording (Sheeba *et al.*, 2008). Holmes and colleagues also showed that spontaneous firing of action potentials rapidly increases in response to light, and such modulation depends on *cryptochrome* (*cry*), the photoreceptor expressed in most circadian cells (Emery *et al.*, 1998, 2000; Stanewsky *et al.*, 1998). In an elegant experiment, Sheeba, Gu, and colleagues showed that, under free-running conditions (DD15; 15 days upon transfer to constant darkness), the spontaneous firing rate maxima occurred during the subjective day and the minima occurred during the subjective night (Sheeba *et al.*, 2008). Accordingly, a larger proportion of lLNvs display burst firing activity during the early subjective day, which progressively decreased by mid-subjective day and was absent later on. Consistent with this behavior the resting membrane potential peaks in the early to mid-subjective day and reaches a minimum at the beginning of the subjective night, underscoring a clear circadian regulation on this intrinsic property of this pacemaker cluster. In parallel, Cao and Nitabach (2008), in a slightly different preparation, also investigated the temporal regulation of membrane excitability. They recorded from the lLNvs, which are the most accessible cluster among the PDF+ cells, and also from the sLNvs, which are the most relevant neurons in terms of rhythmic behavior. Under light–dark cycles they found that the resting membrane potential of the lLNvs becomes most hyperpolarized from dawn to dusk, whereas the firing rate and membrane resistance decreases. In the dark phase the membrane resting potential becomes more depolarized as the night progresses, despite the fact that the firing rate and membrane resistance stay relatively low throughout. As the authors point out, it is unclear why these properties do not change accordingly, and could result from the differential regulation of specific ion channel subtypes (Cao and Nitabach, 2008); alternatively, it could reflect the variability of the recording preparation. As expected, circadian modulation of these membrane properties was lost in a clockless fly (a per^{01} null mutant) and also under free-running conditions (on DD1) in the wild type. The latter is potentially accounted for by the loss of coherent molecular oscillations in this cluster during the first 1–2 days upon transfer to constant darkness (Shafer *et al.*, 2002; Yang and Sehgal, 2001), a potential indication of the cell autonomous nature of this property (Cao and Nitabach, 2008). Interestingly, perhaps as a result of the optimized recording procedure, Cao and Nitabach also detected action potentials of roughly half the amplitude, derived from arborizations within the optic lobe of the contralateral side, opening the possibility that each lLNv processes information received at both sites independently.

In addition, Cao and Nitabach (2008) performed for the first-time recordings from the sLNvs, and reported that these cells are most depolarized near lights on, becoming hyperpolarized as the day proceeds. Toward the end of the night period the resting membrane potential begins to increase, though not as steadily as during the day, and the cells become more depolarized again. As the sLNvs cluster is

responsible for the increase in locomotor activity anticipating lights on, the reported changes in membrane excitability nicely correlate with overt behavior. No clear trend was observed during the 12-h window between transitions (Cao and Nitabach, 2008); whether this reflects a property of the sLNvs or results from an inherently difficult biological preparation is yet to be determined. Whether sLNvs firing rate or membrane resistance is modulated by the circadian clock remains to be explored. No information is yet available for the remainder of the circadian network.

The connection between the circadian clock and membrane properties has been explored in different model organisms. Free-running circadian rhythms in membrane conductance and delayed-rectifier K^+ channel current were reported in pacemaker neurons of the molluscan retina (Michel *et al.*, 1993, 1999) and in the suprachiasmatic nuclei (SCNs) in the mammalian brain (De Jeu *et al.*, 1998; Itri *et al.*, 2005, 2010; Kuhlman and McMahon, 2004). *per1* expressing SCN cells exhibit daily changes in membrane potential (Belle *et al.*, 2009) and even cytoplasmic calcium levels have been shown to oscillate in a circadian manner (Colwell, 2000; Pennartz *et al.*, 2002). The conservation of the molecular mechanisms underlying the cell-autonomous biological clock as well as in this subset of physiological properties underscore that very similar mechanisms convey time of day information to prelocomotor centers in evolutionary distant organisms (Welsh and colleagues have recently reviewed this topic (Welsh *et al.*, 2010)).

B. Adjustment in the Release of Signaling Molecules Throughout the Day

Among the properties of circadian pacemaker neurons that are particularly relevant in the control of rhythmic behavior (recently reviewed by Nitabach and Taghert (2008)) is the release of molecules that could trigger specific (i.e., resetting) signaling events in downstream targets. In *Drosophila*, the candidate molecule best suited to play this role is the neuropeptide PDF. Initially, PDF reactive neurons were singled out as potential pacemaker cells as they coexpressed the core component PER and were located in a region that fulfilled the anatomical criteria proposed for circadian pacemakers in insects (Helfrich-Forster, 1995, 1997; Helfrich-Forster and Homberg, 1993). Surprisingly, no oscillations at the mRNA level could be detected by Northern blot analysis (Park and Hall, 1998), opening the possibility that the clock controls other aspects of PDF synthesis. Park and colleagues reevaluated *pdf* expression by *in situ* hybridization and concluded that no temporal regulation of PDF expression by the circadian clock takes place at the mRNA level. Nonetheless, the cyclic expression of PDF is lost within the sLNvs in certain clock mutants, that is, $Clock^{jrk}$ and cyc^{02} (Park *et al.*, 2000). This observation is consistent with the view that, in this cluster, CLOCK and CYCLE are indirect positive regulators of *pdf* expression. Interestingly, upon closer examination they reported changes in PDF immunoreactivity throughout the day in the

axonal projections of the sLNvs at the dorsal protocerebrum, with a peak during the early day or subjective day, and a trough at the beginning of the night, which are lost in two null clock mutants, per^{01} and tim^{01} (Blau and Young, 1999).

The relevance of the PDF neuropeptide in rhythmic control of locomotor behavior became clear as it was reported that a *pdf* null mutant becomes arrhythmic a few days upon transfer to constant conditions (Renn *et al.*, 1999). PDF signaling is mediated by a G-protein coupled receptor, PDFR (Hyun *et al.*, 2005; Lear *et al.*, 2005b; Mertens *et al.*, 2005). In response to exogenous PDF application, increased cAMP levels are observed in most circadian clusters, implying that most of the circadian network is able to respond to PDF, with the notable exception of the lLNvs (Shafer *et al.*, 2008). More recently, the extent of PDFR expression was refined through promoter analysis (Im and Taghert, 2010). Among clock outputs, PDF immunoreactivity at the dorsal projections of the sLNvs has been extensively explored and reported; although to date it is still uncertain whether it reflects circadian changes in peptide synthesis, processing, axonal transport, and/or release. Aberrant PDF levels were found in mutants with impaired neuronal function such as *narrow abdomen* (na^{har}) (Lear *et al.*, 2005a), *slowpoke* (slo^{4}) (Fernández *et al.*, 2007) or *shaw* (Hodge and Stanewsky, 2008), suggesting that membrane excitability directly affects PDF accumulation at the dorsal terminals. Despite extensive reports on the differential PDF immunoreactivity in the axonal terminals of the sLNvs, its relevance in the control of rhythmic locomotor activity has been questioned (Kula *et al.*, 2006). However, in the set of experiments that support their conclusion the control group *pdf*-Gal4 does not show cyclical changes in PDF levels as was repetitively reported (Blanchard *et al.*, 2010; Fernández *et al.*, 2007; Harrisingh *et al.*, 2007; Helfrich-Forster *et al.*, 2000; Lear *et al.*, 2005a; Park *et al.*, 2000), calling for a reexamination of this issue.

Despite the relevance of the PDF neuropeptide, it is possible that additional molecules could be employed by the circuit to convey time of day information to downstream targets. Early on, Kaneko and colleagues reported that expression of the tetanus toxin light chain (TeTxLC), which impairs fast synaptic transmission through cleavage of synaptobrevin, affects circadian behavior if expressed in the PER/TIM+ cells, but not when expressed in the PDF+ cells (Kaneko *et al.*, 2000). In addition, this observation implies that the sLNvs do not release other molecules relevant for circadian control of locomotor behavior, at least through a mechanism sensitive to TeTxLC. In fact, as the authors note, intercellular communication within the network could still be taking place through electrical coupling (gap junctions), thus explaining the rhythmic behavior in flies expressing TeTxLC within the PDF circuit. Evidence of the latter has recently been found in the lLNvs in physiological recordings of acute brain preparations (Cao and Nitabach, 2008), in line with what was previously described in the accessory medulla in the cockroach (Schneider and Stengl, 2006). More recently, complementary analyses were undertaken employing a temperature-sensitive, dominant-

negative version of the *shibire* gene. The *shibire* gene encodes *Drosophila* dynamin. With the shi^{ts1} mutation, one can use temperature shifts to disrupt receptor-mediated endocytosis and vesicle trafficking (reviewed in Kitamoto, 2001). Interestingly, expressing SHI^{ts} in PDF+ cells lengthens the free-running period by almost 3 h at the restrictive temperature (Kilman *et al.*, 2009; Wulbeck *et al.*, 2009). Strikingly, PDF levels still cycle in the axonal terminals at the dorsal protocerebrum, leading Helfrich Forster and colleagues to discount potential effects on PDF transport, and to propose that the long period phenotype—which is lost in the absence of PDFR—derives from PDF acting on the sLNvs (Wulbeck *et al.*, 2009). In a parallel study, Allada and coauthors showed that period lengthening was largely dependent on the LNv cluster because it disappears when broad circadian expression is combined with Gal80-mediated repression exclusively in the PDF+ cells (Kilman *et al.*, 2009). As expected, at the behavioral level, the long period phenotype required PDF; however, at the molecular level, PER entry to the nucleus still showed a delay in the absence of the neuropeptide. These and other observations led them to propose that impaired cell membrane recycling may modulate the clock through an effect on the expression, stability or activity of the clock component CLK, likely through a PKA-signaling pathway (Kilman *et al.*, 2009).

PDF is not the only neurotransmitter of the LNv cells. At the ultrastructural level it was reported that small clear vesicles could be detected near synaptic output sites in the axonal terminals of the sLNvs, in addition to the PDF-containing dense core vesicles (Miskiewicz *et al.*, 2004; Yasuyama and Meinertzhagen, 2010), suggesting that additional fast neurotransmitter(s) could take part in this process. Nassel and colleagues (Johard *et al.*, 2009) have recently shown that the sLNv express small neuropeptide F (sNPF), suggesting that at least part of the effects derived from SHI^{ts} expression could derive from defective release of other neuropeptides; in fact, as PDF not only affects the sLNvs but also additional dorsal clusters (Lin *et al.*, 2004; Peng *et al.*, 2003) an alternative interpretation that accounts for the absence of long period phenotype in $pdfr^{01}$ mutants is that the desynchronization of specific dorsal clusters contributes to the deconsolidated locomotor pattern, thus obscuring the contribution of additional molecules, whose effect on intercellular communication would be most likely subtle (Berni *et al.*, 2008).

Aside from sNPF—whose relevance to circadian biology is yet to be addressed—there is no clear candidate neurotransmitter in the sLNv cells. They were found not to be immunoreactive against several biogenic amines such as serotonin, dopamine, and histamine during the early morning (Hamasaka and Nassel, 2005), which does not rule out a potential accumulation later in the day. In addition, no GABA is expressed in LNv neurons, although they do receive gabaergic inputs relevant in the control of behavior (Chung *et al.*, 2009; Dahdal *et al.*, 2010; Parisky *et al.*, 2008). Less is known about the remaining circadian clusters (Hong *et al.*, 2006), with the notable exception of the work reported by Shafer *et al.*

Table I
Summary of the Neurotransmitters and Neuropeptides Expressed in Different Subsets of Circadian Neurons in the Adult *Drosophila* Brain.

molecule/cluster	DN1	DN2	DN3	sLNv	5th sLNv	lLNv	LNd (6)
PDF[1-3]	–	–	–	+ c	–	+ c	–
sNPF[4]	–	–	–	+	–	–	2(CRY+)/6
NPF[4]	–	–	–	–	–	–	1♀ or 3♂/6
ITP[4]	–	–	–	–	+	–	1(NPF+)/6
chA[4]	–	–	–	–	+	–	2(sNPF+)/6
glutamate[5]	+	–	+	–	–	–	–
NPLP1[6]	+	–	–	–	–	–	–
5HT[7]	–	–	–	–	–	–	–
TH (DA)[5]	–	–	–	–	-	–	–
HA[8-9]	–	–	–	–	–	–	–

Only those pertaining to the LNv cluster were described in the text. +, indicates presence within a subset or all neurons within that specific cluster, which was demonstrated by either direct immunocytochemistry or reporter expression guided by enhancer traps or specific promoter sequences. A lowercase c denotes confirmed cycling. Colocalization with additional circadian relevant markers is indicated in parenthesis. DA, dopamine; HA, histamine; ITP, ion transport peptide; NPLP1, neuropeptide-like precursor 1; 5HT, serotonin; TH, tyrosine hydroxylase; . References: [1]Helfrich-Forster (1995); [2]Renn *et al.* (1999); [3]Park *et al.* (2000); [4]Johard *et al.* (2009); [5]Hamasaka *et al.* (2007); [6]Shafer *et al.* (2006); [7]Hamasaka and Nassel (2006); [8]Hong *et al.* (2006); [9]Hamasaka and Nassel (2008).

(2006) as well as the Nässel laboratory (Hamasaka and Nassel, 2005; Hamasaka *et al.*, 2005, 2007; Johard *et al.*, 2009; Shafer *et al.*, 2006). A summary of potential neurotransmitters expressed in the different clusters is included in Table I.

There is evidence that synaptic interactions involving neuropeptides play critical roles in the maintenance and synchronization of circadian rhythms in the mammalian brain. In fact, vasoactive intestinal peptide (VIP) appears to play a similar role to PDF in the mammalian brain, synchronizing SCN neurons, modulating molecular oscillations within individual oscillators, and acting downstream of light cues in entrainment (reviewed in (Vosko *et al.*, 2007).

C. Daily Changes in Neural Structure: Another Output of the Clock

Over the years it has become increasingly evident that the biological clock acts on different layers within a cell, not only controlling the levels and activity of given proteins but also modulating the structural properties of the cell itself. An example of this structural remodeling can be found in the visual system of different species,

which undergoes circadian morphological changes. Pyza's laboratory found circadian changes in the size and shape of fly interneurons within the first optic neuropil both in *Musca* (Pyza and Meinertzhagen, 1996) and *Drosophila* (Pyza and Meinertzhagen, 1999). By quantifying the axon caliber of L1 and L2 monopolar cells, the authors found that axons of adult flies swell and shrink rhythmically both under LD and DD and that axon size correlates with the crepuscular pattern of locomotor activity. Axons swell at the beginning of both day and night, and shrink throughout the interval in between (Pyza and Meinertzhagen, 1999). As axon girth is associated with conduction velocity (Matsumoto and Tasaki, 1977), the fact that axon size correlates with the crepuscular pattern of locomotor activity might be functionally linked to a need for increased speed in the processing of visual cues. In fact, in L2 neurons not only the size of the axon is changing but also the nuclei and dendrites (revised in Pyza and Gorska-Andrzejak (2008)). Pyza and coworkers recently reported that the size of the L2 dendritic tree and spine morphology change daily, and is regulated by a circadian clock, increasing at the beginning of the day and decreasing afterward; this pattern persists under DD, in agreement with changes in axonal girth; no rhythmic changes were observed in dendritic tree size under LL (Weber *et al.*, 2009). Moreover, while exploring dendritic size in *per* mutants they were not able to find a temporal pattern of dendritic changes either under LD or in constant conditions (DD and LL), leading them to conclude that the structural plasticity in the L2 dendritic tree is exclusively circadian-driven (a pure clock output) as it cannot be masked by light (in contrast to what happens to rhythmic locomotor activity). In addition, they found that spines in per^{01} flies were shorter than in wild type under LD, in line with what was reported in other examples of circadian structural plasticity (see below within this section (p. 116)). However, given that these analyses were performed in the same null mutant background, developmental defects cannot be ruled out. To address the source of oscillations that govern this type of plasticity they restored *per* expression in different clusters. Neither *per* expression in lateral neurons nor in lateral neurons and retina photoreceptors appears to completely rescue circadian plasticity, suggesting that additional clock cells could be involved, for example, glial cells (Pyza and Gorska-Andrzejak, 2008). In fact, Pyza and colleagues had already suggested that *Musca* homologues of *Drosophila* PER+ glial cells could contribute to the regulation of structural plasticity (Pyza and Gorska-Andrzejak, 2004). The relevance of glial cells in the regulation of this circadian remodeling has been extensively reviewed (Jackson, 2010). Weber *et al.* (2009) also examined structural plasticity in the L2 dendritic tree in *cry baby* (cry^{b}) mutants, which have nonfunctional peripheral circadian oscillators (so far confirmed in specific organs (Ivanchenko *et al.*, 2001; Krishnan *et al.*, 2001; Myers *et al.*, 2003)) but operative central pacemakers (Krishnan *et al.*, 2001) and found circadian changes in dendritic tree size in LD and DD, but not in LL. This result provides evidence that circadian structural plasticity in L2 dendrites is dependent on the central clock,

perhaps within the lLNv because they extend their projections to the optic neuropils (Helfrich-Forster, 1997, 2003). Interestingly, a recent report from the Strauss laboratory shows that structural plasticity in the lamina, measured in cartridge cross-sections as the circumference of the membrane surrounding the photoreceptor terminals, the extension of epithelial glial cells (capitate projections) and the lamina volume, is controlled by the circadian clock but also depends on phototransduction mechanisms (Barth *et al.*, 2010). Additionally, PER expression restricted to photoreceptor cells in the null per^{01} mutant rescues volumetric plasticity of the lamina in both DD and LL (Barth *et al.*, 2010). Moreover, such restricted expression is enough to recover the day/night differences in optomotor responses that can be found in the wild-type strain. Thus, Strauss and colleagues concluded that the circadian system would optimally adapt the visual system to the ambient light environment, as a functional circadian clock in photoreceptor cells R1–6 is sufficient to control photoreceptor terminal plasticity, thereby regulating the changes in sensitivity of optomotor behavior (Barth *et al.*, 2010).

In sum, the visual system of *Drosophila* undergoes circadian-driven structural changes in the photoreceptor–interneuron L2 synapses; interestingly, oscillators controlling pre- (Barth *et al.*, 2010) and postsynaptic (Weber *et al.*, 2009) changes appear not necessarily to be the same; although at the postsynaptic level dendritic plasticity depends mostly on the central clock, presynaptic photoreceptor plasticity is driven by its own clock and modulated by light input. Thus, rhythmic structural plasticity in the visual system is a striking example of the complexity underlying this phenomenon.

A while ago our laboratory showed evidence that remodeling in the axonal terminals of the PDF circuit may be a mechanism complementary to that of rhythmic PDF release for the synchronization between circadian clusters (Lin *et al.*, 2004; Peng *et al.*, 2003). The measurement of the complexity of the arborizations within the dorsal protocerebrum at different *zeitgeber* times revealed the existence of daily plastic changes in the axonal termini (Fernández *et al.*, 2008). The degree of axonal arborization was quantified and showed that these projections are more complex during early morning than during early night, which coincides with peak and trough levels of PDF immunoreactivity at these terminals. Such remodeling is not light-driven as it can be found in animals that were kept under constant darkness for 2 days. In addition, this circadian structural plasticity seems to persist over days because it is not restricted to newly eclosed flies (even 2-week-old flies showed differential complexity in the dorsal PDF projections), and there is no sexual dimorphism. To address the involvement of the circadian clock in this axonal remodeling, the number of PDF branches was quantified in the early morning and night in per^{01} and tim^{01} strains. per^{01} and tim^{01}, which display constitutively lower and higher PDF levels respectively (Park *et al.*, 2000), show no changes in axonal complexity throughout the day (Fernández *et al.*, 2008).

Interestingly, a detailed analysis evidenced a contrasting phenotype with regard to the length and degree of arborization of these termini: short and hyperbranched in tim^{01}, and long and poorly developed in per^{01}. In that work it was proposed that structural plasticity could be a mechanism for transmitting clock information on a time-dependent manner, leading to a circadian-regulated variation in the establishment of synapses (Fernández *et al.*, 2008).

Another example of circadian-dependent neuronal plasticity was found in an identified motoneuron (MN5) that innervates two longitudinal indirect flight muscles in *Drosophila* (Mehnert *et al.*, 2007). When comparing the size of the boutons of MN5 terminals from flies entrained to a 12:12-h LD cycle, they reported that mean bouton size is bigger in the middle of the day than in the middle of the night, with a peak at ZT3 and a trough at ZT15 (ZT stands for *zeitgeber* time; ZT0/−12 indicates lights on/off). In spite of a decrement both during the subjective day and night, the difference in size at these two time points is preserved in animals that were kept under constant darkness, reminiscent of a circadian regulation (Mehnert *et al.*, 2007). No changes throughout the day were observed in various *tim* and *per* mutants, again indicative of a clock-controlled phenomenon. Their analysis uncovered another phenotype in these arrhythmic mutants: while *tim* alleles cause hyperbranching of the axonal projections, per^{01} displays significantly fewer branches compared to wild-type flies, as previously discussed. Interestingly, the double per^{01};tim^{01} mutant has a normal branching pattern but no circadian-dependent neuronal plasticity in the size of the boutons. Moreover, enduring plasticity was reported in 1-month-old flies but not in older ones, in line with the characteristic age dependency of neuronal plasticity. Later on, Mehnert and Cantera showed that rhythmic changes in the size of the MN5 terminal synaptic boutons depends on peripheral functional clocks because it remains intact in decapitated flies (Mehnert and Cantera, 2008). The authors suggested that the clock governing MN5 bouton plasticity lies within the PER+ glial cells in the thoracic ganglia, as they did not find PER or TIM expression either in flight motorneurons or in muscles. Surprisingly, rhythmic changes in the MN5 terminals do not seem to depend on locomotor activity itself because neither morning suppression nor evening increment of synaptic activity for 4 h (by paralyzing the flies and forcing locomotor activity, respectively) affect the underlying rhythm (Mehnert *et al.*, 2007; Mehnert and Cantera, 2008). However, synaptic activity disruption for longer bouts could lead to a different conclusion as is the case in zebrafish hypocretin neurons (Appelbaum *et al.*, 2010), in line with a previous report from Shaw and colleagues stating that, in *Drosophila*, a 6-h window of sleep deprivation is needed to see changes in synaptic markers (Donlea *et al.*, 2009). Hypocretin neurons in zebrafish larvae also exhibit circadian regulation of structural plasticity, measured by time-lapse imaging in living animals across day (Appelbaum *et al.*, 2010). An increased number of active synapses is found during the day in projections to the hindbrain and pineal

gland, although the phase of such synaptic plasticity is not the same in both areas. A brief (3 h) period of sleep deprivation does not impinge on this form of circadian plasticity. However, a longer period of forced locomotor activity (6 h) disrupts the rhythm by increasing the number of active synapses near the pineal gland during the subjective night (Appelbaum *et al.*, 2010). The latter result suggests that, in addition to the circadian-driven fluctuation, there is also homeostatic control of the number of active synapses of hypocretin neurons. A similar mechanism could be in place in the fly MN5, where longer periods of paralysis and sleep deprivation could impinge upon synaptic bouton size. Daily changes in the size and distribution of synaptic vesicles in the MN5 terminals could provide a functional correlate of the structural plasticity (Ruiz *et al.*, 2010). Appelbaum *et al.* (2010) offered a possible mechanism responsible of this plasticity, whereby the circadian expression of neuronal pentraxin (NPTX2b) in the hypothalamus would command its secretion in the axon terminals promoting AMPA receptor clustering in postsynaptic neurons and, consequently, lead to the configuration of new synapses. Interestingly, there are two pentraxin-like molecules (*furrowed* and b6) in *Drosophila*, which have not been studied in great detail; although no connection to the clock has been established, that is, they do not appear to be particularly enriched in the PDF+ cells (Kula-Eversole *et al.*, 2010).

Although the mechanisms that underlie circadian plasticity (or plasticities?) are still elusive, one possibility is that they share components with the ones involved in circuit formation and establishment. During development, gradients of cell surface and secreted molecules (synaptotrophins) target axons and dendrites to approximately the correct brain region, and then synaptic transmission trigger events that either increase or decrease the likelihood that synapses would be maintained (reviewed in Cline and Haas (2008)). In that regard, our laboratory reported that a hypomorph mutant in a transmembrane receptor crucial for proper axon guidance during embryogenesis (*roundabout*^{hypomorph}, *robo*^{hy}) exhibits an unexpected earlier entrance of PER to the nucleus and, concomitantly, a short period in locomotor activity (Berni *et al.*, 2008). It is noteworthy, that the loss of rhythmicity of *pdf*^{01} null flies (in a *robo* wild type background) is rescued in the heterozygous *robo*^{hy} (*robo*^{hy}/+), suggesting that both molecules can affect the coherent output from the circadian network, and thus impinge upon behavioral rhythmicity. As ROBO is expressed in most neuropils in the adult brain (Berni *et al.*, 2008), it is likely playing a role in the maintenance of synaptic connections throughout life. Thus, if ROBO mediates development/stabilization of synaptic contacts (i.e., through the modulation of N-cadherin homophilic interactions (Rhee *et al.*, 2002, 2007), it follows that a possible outcome of reduced ROBO levels would be a less stable/more plastic (hence, more robust) circadian network, with higher ability to cope with the loss of synchronizing signals. Such a scenario would offer an explanation for the increased rhythmicity of *robo*^{hy}/+;*pdf*^{01} flies. However, it has yet to be demonstrated that ROBO plays a role in the circadian structural remodeling of

Table II
Summary of Neuronal Structures That Undergo Circadian Structural Plasticity in Adult *Drosophila*.

Structure	Genotype	LD	DD	LL
L1 and L2– axon[1]	wt	+	+	ns
	tim^{01}	–	–	ns
	per^{01}	–	–	ns
	cry^{b}	ns	ns	ns
L2– dendrites[2]	wt	+	+	–
	tim^{01}	ns	ns	ns
	per^{01}	–	–	–
	cry^{b}	+	+	–
lamina volume[3]	wt	+	+	+
	tim^{01}	+	–	–
	per^{01}	+	–	–
	cry^{b}	ns	ns	ns
MN5[4]	wt	+	+	ns
	tim^{01}	–	–	ns
	per^{01}	–	–	ns
	cry^{b}	ns	ns	ns
sLNv [5]	wt	+	+	ns
	tim^{01}	–	ns	ns
	per^{01}	–	ns	ns
	cry^{b}	ns	ns	ns

L1 and L2 refer to lamina interneurons, MN5 to a motoneuron that innervates two longitudinal indirect flight muscles, and sLNv to the small ventral lateral neurons. +, represents occurrence of circadian structural plasticity; – , indicates the absence the phenomena and ns, not studied. References: [1]Pyza and Meinertzhagen (1999); [2]Weber *et al.* (2009); [3]Barth *et al.* (2010); [4]Mehnert *et al.* (2007); [5]Fernández *et al.* (2008).

the PDF circuit. A summary of the structural plasticity described in adult flies is included in Table II.

So far, there is evidence supporting the hypothesis that the mechanism triggering morphological plasticity might act nonautonomously, as appears to be the case in the MN5 (Mehnert and Cantera, 2008), as well as cell autonomously. Such examples include the sLNvs (although additional experiments are required to more directly address this issue) and the hypocretin terminals. In this case, circadian plasticity would depend to some extent on the secretion of a clock-regulated protein that promotes postsynaptic receptor clustering. However, in the latter case, the postsynaptic terminal could play a major role as terminals from specific neurons projecting to different targets cycle with different phases (Appelbaum *et al.*, 2010).

Circadian plasticity refers to changes in communication efficacy throughout the day, probably through the regulation of synaptic assembly. Hence, it could involve both pre- and postsynaptic adjustments; nonetheless, it is likely initiated by the presynaptic neuron. Supporting this notion: (1) during development, axon guidance and branching is independent of a functional synapse. In contrast, it depends on the highly regulated expression of attraction and rejection molecules (i.e., ROBO/SLIT) but not on the synaptic communication with the target. (2) Remodeling takes place within the axon terminals of the neuronal pacemakers, as it is the case of the sLNvs, a clear example of presynaptic initiation. Regarding the circadian structural changes in L2 and MN5, it has been suggested that the glial clock plays a preponderant role in commanding synaptic remodeling. Although retrograde signaling from muscles to motoneurons was demonstrated during larval development (Keshishian and Kim, 2004; Marques and Zhang, 2006), circadian plasticity in MN5 refers to changes in the presynaptic site. On the contrary, L2 daily remodeling is the only example that discloses synaptic plasticity at the postsynaptic site.

Daily changes in neuronal architecture were reported in the mammalian major circadian pacemaker, the SCN. Vasointestinal peptide (VIP) but not arginine-vasopressin (AVP) immunoreactive neurons in the SCN experience ultrastructural reorganization in the neuronal-glia network (Becquet *et al.*, 2008) and at glutamatergic and nonglutamatergic synaptic terminals (Girardet *et al.*, 2010). As these changes are light-driven, the authors suggested that the changes have a role in photic synchronization (reviewed in Bosler *et al.* (2009)). But this kind of plasticity is not restricted to the SCN, as dendritic architecture and spine density of rat neurons from medial prefrontal cortex increase in the dark period, when the animals are active, and decrease during day time, when they rest. When the authors analyzed several parameters that account for the morphology of pyramidal neurons located in layer III of the infralimbic cortex (which receives direct input from the SCN), they found greater changes in basilar dendrites compared to apical ones. Stress during the active period abolishes these daily differences in morphology (Perez-Cruz *et al.*, 2009). In line with these results, a very recent report shows that a circadian disruption protocol (in which mice were kept in 20-h LD cycles 10-h light:10-h dark) not only disrupt temperature and metabolic hormonal level rhythms but also decrease apical, but not basal, dendrite size and morphology complexity of layer III medial prefrontal cortex neurons. Moreover, at the behavioral level, circadian disruption is not correlated with a difficulty in learning but with a reduced capability of re-learning, suggesting reduced behavioral plasticity (Karatsoreos *et al.*, 2011).

Thus, circadian remodeling of synaptic terminals appears to be widespread (and not only restricted to the projections of circadian pacemakers) and could in itself encode timing information relevant for the coherent control of a variety of behaviors.

III. From Oscillating Networks to Coherent Circadian Behaviors

A. Peripheral Clocks Control Behavioral Responses Independently From the Central Clock

An increasing body of evidence suggests that multiple modes of sensory processes are modulated by the endogenous clock (Allada and Chung, 2010; Glossop and Hardin, 2002). These studies show that peripheral circadian oscillators are crucial for sustaining these rhythms. First, regulation of olfactory responses in the antennae by a circadian clock was demonstrated (Krishnan *et al.*, 1999; Tanoue *et al.*, 2004), and reported to operate independently from the central clock neurons that govern rhythmic eclosion and locomotor activity patterns during adulthood (the PDF+ sLNvs). Soon after, Guo and colleagues showed a circadian rhythm in attractive and repulsive olfactory responses that depends neither on PDF nor on the presence of the LNvs (Zhou *et al.*, 2005). When looking for the cellular and molecular mechanisms that drive this olfactory rhythm, it was reported that spontaneous spike amplitude fluctuate throughout the day in certain sensillae, probably due to rhythmically controlled abundance of a particular G-coupled protein kinase that controls odorant receptor-dependent ion channel activity/composition and localization (Krishnan *et al.*, 2008; Tanoue *et al.*, 2008). In line with previous reports, Krishnan *et al.* showed an increased sensory processing during either midnight or subjective midnight (ZT17 and CT17), which coincides with the peak performance in the olfactory responses (Zhou *et al.*, 2005).

More recently, Chatterjee *et al.* (2010) elegantly demonstrated that the major chemosensory organ in *Drosophila*, the proboscis, harbors a circadian oscillator that controls gustatory physiology and regulates appetitive behavior independently of the central brain (Chatterjee *et al.*, 2010). These results are in line with those of Barth *et al.* (2010), whose work focused on photoreceptor cells and the control of optomotor turning response. Both reports suggest that behaviorally relevant adaptive processing of visual and gustatory information is performed, in part, at the input level (Barth *et al.*, 2010).

B. The Locomotor Activity Pattern of *Drosophila melanogaster*: An Extensively Studied Circadian Behavioral Output

Drosophila constitutes an excellent animal model for learning about the neuronal clock that drives rhythmic behaviors. The very first observation of a rhythmic behavior in flies was the time of emergence of the adult from the pupal case. Since the 1960s and 1970s eclosion rhythms have been used to reveal mutations in clock genes (as an example, see Konopka and Benzer, 1971). The eventual development

of devices capable of automated recording of locomotor activity patterns of individual adult flies opened the research to a second, and by far, best understood circadian-driven behavior that, in contrast to eclosion that takes place only once in a lifetime, can be analyzed during the entire adult life.

Crepuscular animals such as *Drosophila* show a bimodal pattern of activity (two peaks per cycle under light–dark conditions): around dawn and dusk activity increases, and at noon and during the night activity is reduced. In the laboratory, the complex natural illumination spanning changes in light intensity and wavelength content throughout the day are replaced by a square pulse of light that is either on (usually for 12-h nonstop) or off. Under this simplistic condition, at a constant temperature (20–25°C) and 12-h of light alternated with 12-h of darkness (LD 12:12), individually housed male flies display a bimodal activity pattern. While looking for sexual dimorphism in the pattern of rhythmic locomotor activity of several wild-type strains, Helfrich-Forster (2000) found that males tend to be active before lights-on and reached their peak of activity around lights-on; their activity decreases continuously and stays low for several hours until approximately 2 h before lights-off. After this, activity is reduced until the next day. On the contrary, females (both virgin and mated) do not anticipate the lights-on transition with an increase in locomotor activity. They reach their peak about 1-h after lights-on and remain active until later than males. While analyzing the phase of the female's evening activity bout, Helfrich-Forster found strain differences in the degree of sex-dependency, leading her to propose that the phase of the morning peak has a stronger dependency on sex than the evening one. After transferring the flies to constant darkness (DD), strain differences in activity pattern emerge more prominently than sex-specific ones. Half of the flies changed the organization of activity from a bimodal to a unimodal display. In these flies, the morning peak merged with the evening peak to form a band of activity. In persistently bimodal flies, the morning peak becomes weaker and the phase relationship between peaks of activity becomes smaller. The examination of locomotor patterns after prolonged time under constant darkness (25–40 days) uncovered a proportion of flies showing spontaneous changes in period that correlated with changes in phase relationship between morning and evening peaks; only a few showed sudden internal desynchronization. The author proposed that this pattern of activity could represent another example of hierarchical organization between oscillators: one behaving as the pacemaker and the other one as the slave or driven (reminiscent of the "morning" and "evening" oscillator model put forward by Pittendrigh and Daan (Pittendrigh, 1976)). Thus, differential regulation of morning and evening peaks and spontaneous splitting and disorganization of locomotor patterns would suggest the existence of two oscillators with a pacemaker–slave relationship that control circadian locomotor behavior (Helfrich-Forster, 2000).

The major role played by the LNvs in locomotor rhythms was first inferred by analyzing the performance of the *disconnected* mutant, which lacks this cluster

(Helfrich-Forster, 1998). Soon after, it was shown that pdf^{01} flies, lacking the main output from the LNvs (the PDF neuropeptide), become largely arrhythmic once they are transferred to DD (Renn *et al.*, 1999), lending further support to the crucial role of the LNvs. In addition, PER overexpression in the LNvs disrupts locomotor rhythmicity despite protein oscillations in specific dorsal clusters (Blanchardon *et al.*, 2001). In order to further dissect the neural basis of rhythmic locomotor activity two groups made use of different genetic strategies to elucidate which neurons control the morning and evening activity peaks. Rouyer and colleagues reinstated PER function in a subset of clock neurons in an otherwise *per* null mutant, employing different Gal4 drivers (Grima *et al.*, 2004). They focused on *pdf*-Gal4 (expressed in the LNvs), Mz520-Gal4 (LNvs), *cry*-Gal4 (DN1-3; LNds; LNvs), C929-Gal4 (lLNvs among other nonclock neurons), and Mai179-Gal4 (neurosecretory cells including sLNvs and the non PDF+ 5^{th} sLNv, occasionally one lLNv and some LNds). Later, they evaluated the locomotor activity under LD and DD in a per^{01} null background carrying different drivers and UAS-*period* transgenes, and found morning but not evening anticipation in *pdf>per* and Mz520>*per* flies, but no anticipation at all in c929>*per* animals. These results indicated that PER expression in the sLNvs is sufficient to rescue the control of morning anticipation. Moreover, as Mai179>*per* flies anticipate both morning and evening transitions but *pdf>per*, Mz520>*per* and Mz520>*per* flies do not, they concluded that PER expression in the LNds is enough to restore the evening peak. Moreover, PER expression in the sLNvs supported robust PER cycling and rhythmic behavior in *pdf>per* and Mz520>*per* but not in c929>*per* flies, indicating that the PDF+ sLNvs are an autonomous oscillator capable of driving rhythmic behavior in the absence of any other functional clock. In parallel, a similar strategy (specific drivers controlling a proapoptotic transgene expression or rescue of PER function) enabled Rosbash and colleagues to arrive to basically the same conclusions: the LNvs would embody the morning oscillator and the DN1, LNds and the 5^{th} sLNv would form the evening one. Their approach allowed them to conclude that (i) such oscillators are self-sustained, (ii) the LNvs are necessary and sufficient for anticipating the lights-on transition and are not responsible for the light-off response, and (iii) the LNvs are necessary and sufficient for sustaining rhythmicity in DD (Stoleru *et al.*, 2004). In addition, the leading role of the LNvs in the control of rhythmic behavior was evidenced from the observation of the progressively defective PER oscillations (in terms of amplitude and synchronization within the cluster) in the sLNvs and LNds clusters in pdf^{01} mutants after increasing bouts in constant darkness. From this work Taghert and colleagues concluded that PDF is required to coordinate the phase of the molecular rhythms within the sLNvs, and set the phase of the LNds (Lin *et al.*, 2004).

To more specifically address the relationship between the different clusters, Stoleru *et al.* shortened the period of molecular oscillations through the overexpression of a TIM kinase (SHAGGY, SGG) in different clock clusters, and

examined its impact on locomotor activity under DD. Restricting SGG overexpression to the PDF circuit not only leads to an advance in the evening activity but also to an increased pace of the molecular oscillations—measured as *tim* RNA level—in neuronal clusters, including the ones responsible for the evening activity bout (LNds and DN1s). Surprisingly, *tim* oscillations are not affected in the lLNVs despite SHAGGY overexpression within this subset of neurons. In contrast, the period observed in the lLNvs is locked to that of the DN2s, and both structures are unresponsive to SHAGGY overexpression. Guiding SHAGGY overexpression to the DN1s, LNds and the 5th sLNv clusters does not affect the free-running period (which is determined mainly by the sLNvs) but shortens the length of the subjective day. All in all, the authors suggest that the evening phase within each cycle is a reflection of the endogenous rhythm of the evening oscillator but the period of the cycle correlates with the morning clock (Stoleru *et al.*, 2005).

The circadian clock of animals in the wild is faced with a variety of input signals at once, which might change the hierarchy between the oscillators described under laboratory conditions. Thus, more complex and/or subtler interactions among the different clusters could be uncovered under different environmental conditions. As an example, rest–activity cycles and PER and TIM oscillations can be driven by temperature cycles in LL (Glaser and Stanewsky, 2005; Matsumoto *et al.*, 1998; Yoshii *et al.*, 2005) despite the fact that constant light disrupts molecular and behavioral rhythms at a given temperature. The study of circadian locomotor rhythmicity under constant light in *cry^{b}* mutants, together with other evidence, led Rouyer and colleagues to ascribe a novel effect of light—through differential CRY degradation—, which would allow the control of rhythmic locomotor behavior to either the morning or evening oscillators, depending on ambient conditions. Thus, aside from its ability to synchronize to the environment, light controls the behavioral output from the morning and evening oscillators in an opposite fashion (Picot *et al.*, 2007). In a parallel study also exploring the response to constant light, Rosbash and colleagues concluded that the circadian network switches control between the morning and evening oscillators depending on day length, and further suggested that this switch could define the adjustment to a changing photoperiod (Stoleru *et al.*, 2007). An even more flexible interpretation of morning and evening oscillators was put forward from experiments carried out under dim light; Helfrich-Forster and colleagues found that under these conditions only four cells support PER cycling; two LNds behave as the morning oscillator, and the 5th sLNv and one additional LNd constitute the evening one; these observations prompted them to conclude that the morning/evening oscillator function may not be restricted to certain anatomically defined groups of clock neurons, but instead depends on the environmental conditions (Rieger *et al.*, 2009).

How environmental conditions affect the mutual relationship between clock clusters is still not understood; for example, experiments carried out under temperature cycles indicated that the LNvs seem to be preferentially light-entrainable,

whereas the DNs and LPNs seem to be primarily temperature-entrainable (Miyasako *et al.*, 2007). Not only responsiveness to environmental conditions appears to differ among clock clusters, but also the mechanism leading to this response. Although light synchronization through CRY is mainly a cell-autonomous process, temperature seems to require signaling from specific sensory structures, the chordotonal organs, to the brain (Sehadova *et al.*, 2009). However, a highly overlapped set of cycling genes could be detected upon entrainment to light–dark or temperature cycles underscoring that irrespective of the *zeitgeber* there is a rather defined set of cycling genes, that is, clock outputs (Boothroyd *et al.*, 2007).

Although the underlying mechanism is not fully understood yet, behavioral output demonstrates a synergic effect of light and temperature cycles in entrainment. When flies are exposed to both *zeitgebers* the rhythm of locomotor activity is more robust than after synchronization to each *zeitgeber* by itself (Yoshii *et al.*, 2009). This result suggests that an even more complex set of conditions would modify the fine tuning of the rhythm, ensuring a better adjustment to the particular environment. A summary of the best known circadian outputs from the sLNvs are depicted in Fig. 1.

In sum, these results underscore the high degree of plasticity displayed by the connectivity within the circadian network, which would enable it to accommodate to variable environmental input signals and result in coherent behavior. The mechanisms underlying how biological clocks ensure rhythmic rest–activity cycles have been the object of extensive review and are beyond the scope of the present work (see Allada and Chung (2010); Nitabach and Taghert (2008); Peschel and Helfrich-Forster (2011)).

C. Circadian Modulation of Short-Term Memory in *Drosophila*

The projections of the sLNvs branch near the calyx of the mushroom bodies (Helfrich-Forster, 1997), which are essential centers for memory processing in insects (Dubnau *et al.*, 2001; Heisenberg, 1998; Heisenberg *et al.*, 1985); such a neuroanatomical proximity led to propose a functional relationship between these systems, even though the presence of PDF receptors in the calyx had not yet been demonstrated. Whether other clock clusters contact the mushroom bodies has yet to be explored. In 2009, Lyons and Roman showed circadian modulation of short-term memory (Lyons and Roman, 2009). Employing the T-maze device developed by Tully and Quinn (Tully and Quinn, 1985) young adult flies receive an electric shock while exposed to an air current transporting a first odor but not when exposed to a second odor. During testing, animals are placed at a decision point where they should move toward one of the odors. Memory is expressed by an avoidance behavior to the shock-associated odor 3 min after training. Lyons and Roman assayed two kinds of training protocols, a strong one (12 shocks, 1-min odor presentation) and a weak one (1 shock, 10-s odor presentation) at various circadian

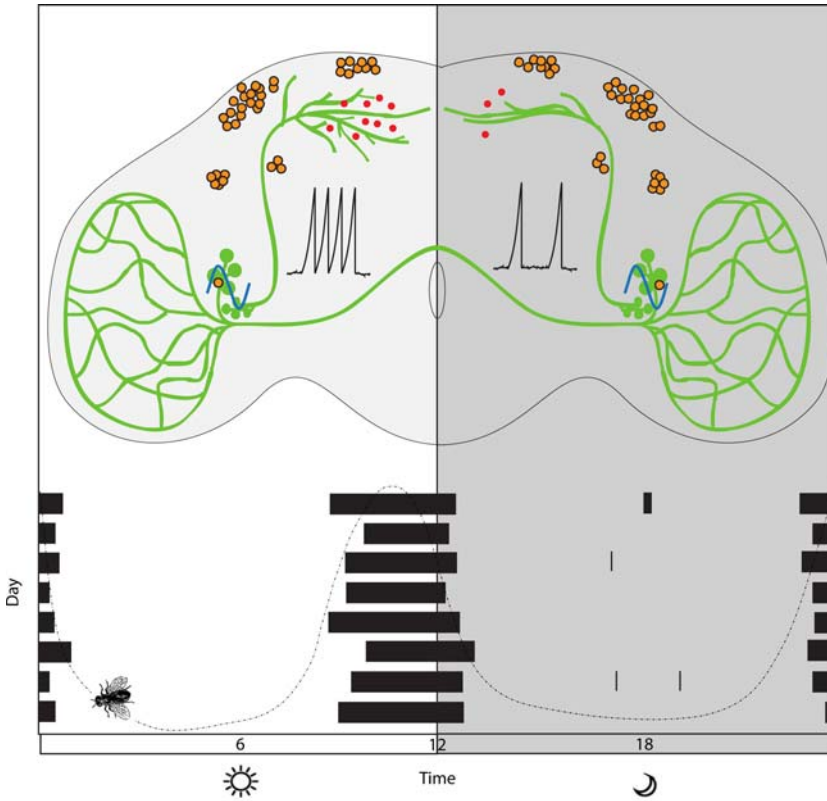

FIG. 1. From oscillating networks to coherent behavior. Schematic diagram displaying examples of circadian outputs in the adult *Drosophila* brain. The clock provides multiple layers of organization to provide a coherent response to the surrounding environment. Among them, electrical properties (indicated as action potentials) as well as the morphology of axonal terminals within PDF+ neurons (in green) are affected by the clock; in addition, variations in transmitter release (red circles) and complex behaviors (such as locomotor activity) are also examples of outputs modulated by the clock that change rhythmically accompanying the daily cycles. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this book.)

times, to find that the weaker stimulus uncovers a larger circadian effect. This would indicate that a "bad time for learning" could be overcome with a stronger training, suggesting that the time-of-day effect would contribute to improving rather than worsening memory formation, as (1) animals are capable of learning throughout the day and (2) both per^{01} and tim^{01} mutants, as well as animals exposed to constant-light (another means to disrupt the molecular clock) display memory levels similar to the worst time points in wild-type strains. In order to assess whether the rhythm on performance depends on modulation of sensory processing along the day, rhythms in shock and odor avoidance behaviors were characterized. Under the conditions

used in their study they did not find any rhythm, suggesting a central clock involvement on circadian modulation of memory. But a circadian rhythm in the olfactory attractive and repulsive responses dependent on the antennal oscillator was described (Zhou *et al.*, 2005) with a peak response at CT17. Despite the coincidence between the time points for best olfaction responses (CT17) and memory performance (CT13 and CT17), the authors discounted a major antennal peripheral clock contribution on memory performance because *cry* mutant flies (cry^b), which have nonfunctional peripheral antennal circadian oscillators but operative central pacemakers, displayed circadian rhythms in memory.

Although not excluding the possibility that the circadian clock also affects memory recall, Lyons and Roman suggested that the circadian clock is regulating the strength of the association during memory formation probably due to a modulation of the availability of second messengers or other molecules taking part of the signaling cascade. In support of this possibility a diurnal rhythm of short-term synaptic plasticity was found in GABAergic synapses of the SCN (Gompf and Allen, 2004).

D. Circadian Modulation on Long-Term Memory

Although not yet studied in flies, evidence from other animal models suggests that long-term memory is also modulated by the circadian clock (Barbosa and Albuquerque, 2008; Decker *et al.*, 2007; Fernandez *et al.*, 2003; Gerstner and Yin, 2010; Hauber and Bareiss, 2001; Lyons *et al.*, 2005). For example, the long-term sensitization of the siphon withdrawal reflex in *Aplysia californica*—a non-associative form of learning—is influenced by the circadian clock. Moreover, authors demonstrated that the effect seems to depend on the time of training rather than the time of testing, suggesting that the circadian clock might play an important role in the acquisition/consolidation to form long-term memories *in vivo* (Fernandez *et al.*, 2003). A similar result was obtained with an associative form of learning in the cockroach *Leucophaea maderae* (Decker *et al.*, 2007), indicating that the circadian imposition is not linked to the type of learning but to the cellular mechanisms underlying them. In vertebrates—specifically birds and mammals—, the literature is ambiguous regarding a time-of-day effect, probably due to the diversity of paradigms and experimental procedures employed; nonetheless, a clear light-phase effect on memory was repeatedly reported (Chen and Wolpaw, 1995; Hauber and Bareiss, 2001; Moura *et al.*, 2009; Reijmers *et al.*, 2001; Valentinuzzi *et al.*, 1997, 2004). The mechanism underlying such phenomena is still not clear, although it was proposed that the dynamics of hippocampal clock gene expression imprints a temporal structure on memory processing and shapes, at the same time, the efficacy of behavioral learning (Jilg *et al.*, 2010; Wang *et al.*, 2009). Moreover, data coming from reports on hippocampus long-term potentiation (LTP), a well-established model for studying activity-dependent changes in

synaptic strength, reveal a circadian action on neuronal responses that relies on the endogenous clock (Chaudhury *et al.*, 2005; Harris and Teyler, 1983). The current hypothesis states that there is an independent circadian pacemaker that controls time-of-day-dependent changes in hippocampal plasticity, and that the arousal state or sleep *per se* are not necessary for those circadian changes in LTP (Chaudhury *et al.*, 2005). A charming hypothesis is that consolidation—the ability to store memories, which requires synaptic plasticity—changes in response to circadian structural remodeling in the memory centers. In fact, structural plasticity was proposed as a mechanism that could trigger circadian changes in the number of active synapses (Fernández *et al.*, 2008). But it is not restricted solely to pacemaker cells because dendritic architecture and spine density of pyramidal neurons in the rat infralimbic cortex display daily rhythms (Perez-Cruz *et al.*, 2009). As the infralimbic cortex is involved in higher order cognitive functions (Bach *et al.*, 2008; DeSteno and Schmauss, 2008; Vertes, 2006; Wall *et al.*, 2004), it is legitimate to expect that memory consolidation would be modulated by the endogenous clock through, among others, changes in the degree of structural plasticity in memory centers. This potential mechanism would give alternative explanations to, for example, the susceptibility of certain types of memories to the disruption of the clock by constant illumination (Ma *et al.*, 2007), rapid changes in light/dark cycle (Loh *et al.*, 2010) or prolonged 20-h light/dark cycles (Karatsoreos *et al.*, 2011).

E. Circadian Rhythms and Social Interactions

Honey bees display complex social interactions that provide the opportunity to study the relationship between endogenous rhythms and social contact. When studying the molecular oscillations on clock genes in worker bees that switch between nursing (around the clock activity) and foraging (fine-tuned circadian activity), strong oscillations were found only in foragers (Bloch *et al.*, 2001). Furthermore, the lack of oscillation in the nurses is due to their contact with the brood demonstrating a social imposition to the central clock in individual bees (Shemesh *et al.*, 2010).

In flies, such a social interaction was studied at the behavioral level: while the addition of arrhythmic animals (per^{01}) to a group of wild-type flies disperses the phase of the host flies, introducing per^{S} mutants advances it. This effect is dependent on the proportion of visitors, the time-of-day when the animals are inserted, and on a functional olfactory system, as anosmic hosts do not change their behavior when arrhythmic flies are introduced (Levine *et al.*, 2002). In a 2D arena, Fujii *et al.* (2007) described a novel rhythmic locomotor output based on the observation of the social interaction between a male and female fly, termed "close proximity." Close proximity is defined as the percentage of time spent within

≤5 mm of each other. They established that these pairs display an activity pattern that stays rhythmic over days, linked to courtship and copulation. Opposite to the classical crepuscular activity and nocturnal inactivity exhibited by isolated male flies under LD and DD, the rhythmic "close proximity" behavior, reliant mainly on male activity, is elevated during most of the subjective night and morning and minimal at the approximate time of anticipated dusk. This rhythm is not entrainable, as it depends on the continuous presence of the female and is conditional to a functional male central circadian clock. The authors suggested that external cues perceived by the olfactory and other sensory systems feed into the male central pacemakers to cause a shift in the circadian activity. This would imply that a female presence acts as a peripheral stimulus that resets the central pacemaker only in males, as the female activity pattern is not influenced by the presence of a second animal (Fujii *et al.*, 2007). It is yet to be determined whether the relative hierarchy of the different clock clusters is modified by the presence of a social cue. More recent experiments suggest that functional LNvs are essential for male rhythms and lack of PDF disrupts it. In addition, DN1s are required to synchronize the trough at dusk in male proximity behavior (Fujii and Amrein, 2010). The important role of DN1s is supported by another report suggesting that neurons responsible for close proximity rhythms are among the neuropeptide F negative (NPF–) LNds and DN1s, as *npf*-Gal4 directed ablation does not impair this rhythm (Hamasaka *et al.*, 2010). However, Ishida and colleagues also found that disruption of the evening, but not the morning, oscillator caused arrhythmic male proximity behavior, ruling out that the LNvs would be the primary determinants. The former proposed that under the influence of PDF, the DN1 cluster would signal whether the male fly should generate a sex drive or, in contrast, an isolated rhythmic locomotor response (Fujii and Amrein, 2010). On the contrary, Hamasaka *et al.* suggested that, similarly to the notion that morning and evening cells act differently depending on the environmental conditions (Rieger *et al.*, 2009), the NPF–DN1s could also change their behavioral pattern from isolated locomotor rhythms to courtship rhythms triggered by the presence of the female (Hamasaka *et al.*, 2010).

IV. Conclusions

How do molecular clocks manage to transmit time of day information to physiology and behavior? Although the molecular mechanisms are yet to be defined, the notion that the biological clock directly controls output at different levels, from the most immediate one within the nucleus (i.e., gene expression) and cellular properties (i.e., axonal girth, synaptic strength) to a variety of behaviors has

consolidated over the years. Perhaps one of the most striking discoveries is the degree of plasticity within the circadian network *per se*, as different neuronal clusters dictate the pace of overt behavior in response to a changing environment.

Do the different cues that synchronize the clock impinge upon different clusters changing their relative weight in the final output? No definite answer is possible today, although initial observations included in this review point in that direction. A striking example of network plasticity is the stomatogastric ganglion of crustaceans, where a small number of neurons exhibit a broad range of outputs depending on which input they receive (Marder and Bucher, 2007). In brief, this ganglion produces several rhythmic outputs that trigger different motor programs finally leading to the processing of different types of food; neuromodulatory molecules can reconfigure circuit dynamics by altering synaptic strength- and voltage-dependent conductances. Moreover, individual neurons can switch among different functional circuits. It would be tempting to speculate that the circadian network operates in a similar fashion, where distinct *zeitgebers* could reconfigure circuit dynamics by uniquely affecting each neuronal cluster (or even specific neurons). Certainly, in this scenario the role of PDF should not be underestimated.

How pervasive is the circadian control of brain functions? An interaction between the PDF circuit and arousal has already been established (Lebestky *et al.*, 2009; Shang *et al.*, 2008). Thus, it is appealing to propose that the clock would exert its modulatory effects not only in complex brain functions such as arousal but also in motivational states, behavioral flexibility, and time awareness among others.

Acknowledgments

We thank Nara Muraro and Ana Depetris-Chauvin for critical comments on the manuscript and Axel Gorostiza for help in assembling the figure. L.F. holds a postoctoral fellowship and M.F.C. is a member of the Argentine Research Council (CONICET). This work was supported by a grant from the ANPCyT, Argentina (PICT2006-1249) and by a FIRCA-NIH grant (1R03TW008342) to M.F.C.

References

Akhtar, R.A., Reddy, A.B., Maywood, E.S., Clayton, J.D., King, V.M., Smith, A.G., Gant, T.W., Hastings, M.H., and Kyriacou, C.P. (2002). Circadian cycling of the mouse liver transcriptome, as revealed by cDNA microarray, is driven by the suprachiasmatic nucleus. *Curr. Biol.* **12**, 540–550.

Allada, R., and Chung, B.Y. (2010). Circadian organization of behavior and physiology in Drosophila. *Annu. Rev. Physiol.* **72**, 605–624.

Appelbaum, L., Wang, G., Yokogawa, T., Skariah, G.M., Smith, S.J., Mourrain, P., and Mignot, E. (2010). Circadian and homeostatic regulation of structural synaptic plasticity in hypocretin neurons. *Neuron* **68**, 87–98.

Bach, M.E., Simpson, E.H., Kahn, L., Marshall, J.J., Kandel, E.R., and Kellendonk, C. (2008). Transient and selective overexpression of D2 receptors in the striatum causes persistent deficits in conditional associative learning. *Proc. Natl. Acad. Sci. U S A* **105**, 16027–16032.

Barbosa, F.F., and Albuquerque, F.S. (2008). Effect of the time-of-day of training on explicit memory. *Braz. J. Med. Biol. Res.* **41**, 477–481.

Barth, M., Schultze, M., Schuster, C.M., and Strauss, R. (2010). Circadian plasticity in photoreceptor cells controls visual coding efficiency in Drosophila melanogaster. *PLoS One* **5**, e9217.

Becquet, D., Girardet, C., Guillaumond, F., Francois-Bellan, A.M., and Bosler, O. (2008). Ultrastructural plasticity in the rat suprachiasmatic nucleus. Possible involvement in clock entrainment. *Glia* **56**, 294–305.

Belle, M.D., Diekman, C.O., Forger, D.B., and Piggins, H.D. (2009). Daily electrical silencing in the mammalian circadian clock. *Science* **326**, 281–284.

Berni, J., Beckwith, E.J., Fernandez, M.P., and Ceriani, M.F. (2008). The axon-guidance roundabout gene alters the pace of the Drosophila circadian clock. *Eur. J. Neurosci.* **27**, 396–407.

Blanchard, F.J., Collins, B., Cyran, S.A., Hancock, D.H., Taylor, M.V., and Blau, J. (2010). The transcription factor Mef2 is required for normal circadian behavior in Drosophila. *J. Neurosci.* **30**, 5855–5865.

Blanchardon, E., Grima, B., Klarsfeld, A., Chelot, E., Hardin, P.E., Preat, T., and Rouyer, F. (2001). Defining the role of Drosophila lateral neurons in the control of circadian rhythms in motor activity and eclosion by targeted genetic ablation and PERIOD protein overexpression. *Eur. J. Neurosci.* **13**, 871–888.

Blau, J., and Young, M.W. (1999). Cycling vrille expression is required for a functional Drosophila clock. *Cell* **99**, 661–671.

Bloch, G., Toma, D.P., and Robinson, G.E. (2001). Behavioral rhythmicity, age, division of labor and period expression in the honey bee brain. *J. Biol. Rhythms* **16**, 444–456.

Boothroyd, C.E., Wijnen, H., Naef, F., Saez, L., and Young, M.W. (2007). Integration of light and temperature in the regulation of circadian gene expression in Drosophila. *PLoS Genet.* **3**, e54.

Bosler, O., Girardet, C., Sage-Ciocca, D., Jacomy, H., Francois-Bellan, A.M., and Becquet, D. (2009). Mechanisms of structural plasticity associated with photic synchronization of the circadian clock within the suprachiasmatic nucleus. *J. Soc. Biol.* **203**, 49–63.

Cao, G., and Nitabach, M.N. (2008). Circadian control of membrane excitability in Drosophila melanogaster lateral ventral clock neurons. *J. Neurosci.* **28**, 6493–6501.

Ceriani, M.F., Hogenesch, J.B., Yanovsky, M., Panda, S., Straume, M., and Kay, S.A. (2002). Genome-wide expression analysis in Drosophila reveals genes controlling circadian behavior. *J. Neurosci.* **22**, 9305–9319.

Claridge-Chang, A., Wijnen, H., Naef, F., Boothroyd, C., Rajewsky, N., and Young, M.W. (2001). Circadian regulation of gene expression systems in the Drosophila head. *Neuron* **32**, 657–671.

Cline, H., and Haas, K. (2008). The regulation of dendritic arbor development and plasticity by glutamatergic synaptic input: a review of the synaptotrophic hypothesis. *J. Physiol.* **586**, 1509–1517.

Chatterjee, A., Tanoue, S., Houl, J.H., and Hardin, P.E. (2010). Regulation of gustatory physiology and appetitive behavior by the Drosophila circadian clock. *Curr. Biol.* **20**, 300–309.

Chaudhury, D., Wang, L.M., and Colwell, C.S. (2005). Circadian regulation of hippocampal long-term potentiation. *J. Biol. Rhythms* **20**, 225–236.

Chen, X.Y., and Wolpaw, J.R. (1995). Operantly conditioned plasticity and circadian rhythm in rat H-reflex are independent phenomena. *Neurosci. Lett.* **195**, 109–112.

Chung, B.Y., Kilman, V.L., Keath, J.R., Pitman, J.L., and Allada, R. (2009). The GABA(A) receptor RDL acts in peptidergic PDF neurons to promote sleep in Drosophila. *Curr. Biol.* **19**, 386–390.

Colwell, C.S. (2000). Circadian modulation of calcium levels in cells in the suprachiasmatic nucleus. *Eur. J. Neurosci.* **12**, 571–576.

Dahdal, D., Reeves, D.C., Ruben, M., Akabas, M.H., and Blau, J. (2010). Drosophila pacemaker neurons require g protein signaling and GABAergic inputs to generate twenty-four hour behavioral rhythms. *Neuron* **68**, 964–977.

De Jeu, M., Hermes, M., and Pennartz, C. (1998). Circadian modulation of membrane properties in slices of rat suprachiasmatic nucleus. *Neuroreport* **9**, 3725–3729.

Decker, S., McConnaughey, S., and Page, T.L. (2007). Circadian regulation of insect olfactory learning. *Proc. Natl. Acad. Sci. U S A* **104**, 15905–15910.

DeSteno, D.A., and Schmauss, C. (2008). Induction of early growth response gene 2 expression in the forebrain of mice performing an attention-set-shifting task. *Neuroscience* **152**, 417–428.

Donlea, J.M., Ramanan, N., and Shaw, P.J. (2009). Use-dependent plasticity in clock neurons regulates sleep need in Drosophila. *Science* **324**, 105–108.

Dubnau, J., Grady, L., Kitamoto, T., and Tully, T. (2001). Disruption of neurotransmission in Drosophila mushroom body blocks retrieval but not acquisition of memory. *Nature* **411**, 476–480.

Dubruille, R., and Emery, P. (2008). A plastic clock: how circadian rhythms respond to environmental cues in Drosophila. *Mol. Neurobiol.* **38**, 129–145.

Emery, P., So, W.V., Kaneko, M., Hall, J.C., and Rosbash, M. (1998). CRY, a Drosophila clock and light-regulated cryptochrome, is a major contributor to circadian rhythm resetting and photosensitivity. *Cell* **95**, 669–679.

Emery, P., Stanewsky, R., Hall, J.C., and Rosbash, M. (2000). A unique circadian-rhythm photoreceptor. *Nature* **404**, 456–457.

Fernández, M.P., Berni, J., and Ceriani, M.F. (2008). Circadian remodeling of neuronal circuits involved in rhythmic behavior. *PLoS Biol.* **6**, e69.

Fernández, M.P., Chu, J., Villella, A., Atkinson, N., Kay, S.A., and Ceriani, M.F. (2007). Impaired clock output by altered connectivity in the circadian network. *Proc. Natl. Acad. Sci. U S A* **104**, 5650–5655.

Fernandez, R.I., Lyons, L.C., Levenson, J., Khabour, O., and Eskin, A. (2003). Circadian modulation of long-term sensitization in Aplysia. *Proc. Natl. Acad. Sci. U S A* **100**, 14415–14420.

Fujii, S., and Amrein, H. (2010). Ventral lateral and DN1 clock neurons mediate distinct properties of male sex drive rhythm in Drosophila. *Proc. Natl. Acad. Sci. U S A* **107**, 10590–10595.

Fujii, S., Krishnan, P., Hardin, P., and Amrein, H. (2007). Nocturnal male sex drive in Drosophila. *Curr. Biol.* **17**, 244–251.

Gerstner, J.R., and Yin, J.C. (2010). Circadian rhythms and memory formation. *Nat. Rev. Neurosci.* **11**, 577–588.

Girardet, C., Blanchard, M.P., Ferracci, G., Leveque, C., Moreno, M., Francois-Bellan, A.M., Becquet, D., and Bosler, O. (2010). Daily changes in synaptic innervation of VIP neurons in the rat suprachiasmatic nucleus: contribution of glutamatergic afferents. *Eur. J. Neurosci.* **31**, 359–370.

Glaser, F.T., and Stanewsky, R. (2005). Temperature synchronization of the Drosophila circadian clock. *Curr. Biol.* **15**, 1352–13563.

Glossop, N.R., and Hardin, P.E. (2002). Central and peripheral circadian oscillator mechanisms in flies and mammals. *J. Cell Sci.* **115**, 3369–3377.

Gompf, H.S., and Allen, C.N. (2004). GABAergic synapses of the suprachiasmatic nucleus exhibit a diurnal rhythm of short-term synaptic plasticity. *Eur. J. Neurosci.* **19**, 2791–2798.

Grima, B., Chelot, E., Xia, R., and Rouyer, F. (2004). Morning and evening peaks of activity rely on different clock neurons of the Drosophila brain. *Nature* **431**, 869–873.

Hamasaka, Y., and Nassel, D.R. (2005). Mapping of serotonin, dopamine, and histamine in relation to different clock neurons in the brain of Drosophila. *J. Comp. Neurol.* **494**, 314–330.

Hamasaka, Y., Rieger, D., Parmentier, M.L., Grau, Y., Helfrich-Forster, C., and Nassel, D.R. (2007). Glutamate and its metabotropic receptor in Drosophila clock neuron circuits. *J. Comp. Neurol.* **505**, 32–45.

Hamasaka, Y., Suzuki, T., Hanai, S., and Ishida, N. (2010). Evening circadian oscillator as the primary determinant of rhythmic motivation for Drosophila courtship behavior. *Genes Cells* **15**, 1240–1248.

Hamasaka, Y., Wegener, C., and Nassel, D.R. (2005). GABA modulates Drosophila circadian clock neurons via GABAB receptors and decreases in calcium. *J. Neurobiol.* **65**, 225–240.

Hardin, P.E. (2005). The circadian timekeeping system of Drosophila. *Curr. Biol.* **15**, R714–R722.

Harmer, S.L., Hogenesch, J.B., Straume, M., Chang, H.S., Han, B., Zhu, T., Wang, X., Kreps, J.A., and Kay, S.A. (2000). Orchestrated transcription of key pathways in Arabidopsis by the circadian clock. *Science* **290**, 2110–2113.

Harris, K.M., and Teyler, T.J. (1983). Age differences in a circadian influence on hippocampal LTP. *Brain Res.* **261**, 69–73.

Harrisingh, M.C., Wu, Y., Lnenicka, G.A., and Nitabach, M.N. (2007). Intracellular Ca^{2+} regulates free-running circadian clock oscillation in vivo. *J. Neurosci.* **27**, 12489–12499.

Hauber, W., and Bareiss, A. (2001). Facilitative effects of an adenosine A1/A2 receptor blockade on spatial memory performance of rats: selective enhancement of reference memory retention during the light period. *Behav. Brain Res.* **118**, 43–52.

Heisenberg, M. (1998). What do the mushroom bodies do for the insect brain? an introduction. *Learn Mem.* **5**, 1–10.

Heisenberg, M., Borst, A., Wagner, S., and Byers, D. (1985). Drosophila mushroom body mutants are deficient in olfactory learning. *J. Neurogenet.* **2**, 1–30.

Helfrich-Forster, C. (1995). The period clock gene is expressed in central nervous system neurons which also produce a neuropeptide that reveals the projections of circadian pacemaker cells within the brain of Drosophila melanogaster. *Proc. Natl. Acad. Sci. U S A* **92**, 612–616.

Helfrich-Forster, C. (1997). Development of pigment-dispersing hormone-immunoreactive neurons in the nervous system of Drosophila melanogaster. *J. Comp. Neurol.* **380**, 335–354.

Helfrich-Forster, C. (1998). Robust circadian rhythmicity of Drosophila melanogaster requires the presence of lateral neurons: a brain-behavioral study of disconnected mutants. *J. Comp. Physiol. [A]* **182**, 435–453.

Helfrich-Forster, C. (2000). Differential control of morning and evening components in the activity rhythm of Drosophila melanogaster—sex-specific differences suggest a different quality of activity. *J. Biol. Rhythms* **15**, 135–154.

Helfrich-Forster, C. (2003). The neuroarchitecture of the circadian clock in the brain of Drosophila melanogaster. *Microsc. Res. Tech.* **62**, 94–102.

Helfrich-Forster, C., and Homberg, U. (1993). Pigment-dispersing hormone-immunoreactive neurons in the nervous system of wild-type Drosophila melanogaster and of several mutants with altered circadian rhythmicity. *J. Comp. Neurol.* **337**, 177–190.

Helfrich-Forster, C., Tauber, M., Park, J.H., Muhlig-Versen, M., Schneuwly, S., and Hofbauer, A. (2000). Ectopic expression of the neuropeptide pigment-dispersing factor alters behavioral rhythms in Drosophila melanogaster. *J. Neurosci.* **20**, 3339–3353.

Hodge, J.J., and Stanewsky, R. (2008). Function of the Shaw potassium channel within the Drosophila circadian clock. *PLoS One* **3**, e2274.

Hong, S.T., Bang, S., Paik, D., Kang, J., Hwang, S., Jeon, K., Chun, B., Hyun, S., Lee, Y., and Kim, J. (2006). Histamine and its receptors modulate temperature-preference behaviors in Drosophila. *J. Neurosci.* **26**, 7245–7256.

Hyun, S., Lee, Y., Hong, S.T., Bang, S., Paik, D., Kang, J., Shin, J., Lee, J., Jeon, K., and Hwang, S et al., (2005). Drosophila GPCR Han is a receptor for the circadian clock neuropeptide PDF. *Neuron* **48**, 267–278.

Im, S.H., and Taghert, P.H. (2010). PDF receptor expression reveals direct interactions between circadian oscillators in Drosophila. *J. Comp. Neurol.* **518**, 1925–1945.

Itri, J.N., Michel, S., Vansteensel, M.J., Meijer, J.H., and Colwell, C.S. (2005). Fast delayed rectifier potassium current is required for circadian neural activity. *Nat. Neurosci.* **8**, 650–656.

Itri, J.N., Vosko, A.M., Schroeder, A., Dragich, J.M., Michel, S., and Colwell, C.S. (2010). Circadian regulation of a-type potassium currents in the suprachiasmatic nucleus. *J. Neurophysiol.* **103**, 632–640.

Ivanchenko, M., Stanewsky, R., and Giebultowicz, J.M. (2001). Circadian photoreception in Drosophila: functions of cryptochrome in peripheral and central clocks. *J. Biol. Rhythms* **16**, 205–215.

Jackson, F.R. (2011). Glial cell modulation of circadian rhythms. *Glia.* **59**, 1341–1350.

Jilg, A., Lesny, S., Peruzki, N., Schwegler, H., Selbach, O., Dehghani, F., and Stehle, J.H. (2010). Temporal dynamics of mouse hippocampal clock gene expression support memory processing. *Hippocampus* **20**, 377–388.

Johard, H.A., Yoishii, T., Dircksen, H., Cusumano, P., Rouyer, F., Helfrich-Forster, C., and Nassel, D. R. (2009). Peptidergic clock neurons in Drosophila: ion transport peptide and short neuropeptide F in subsets of dorsal and ventral lateral neurons. *J. Comp. Neurol.* **516**, 59–73.

Kaneko, M., Park, J.H., Cheng, Y., Hardin, P.E., and Hall, J.C. (2000). Disruption of synaptic transmission or clock-gene-product oscillations in circadian pacemaker cells of Drosophila cause abnormal behavioral rhythms. *J. Neurobiol.* **43**, 207–233.

Karatsoreos, I.N., Bhagat, S., Bloss, E.B., Morrison, J.H., and McEwen, B.S. (2011). Disruption of circadian clocks has ramifications for metabolism, brain, and behavior. *Proc. Natl. Acad. Sci. U S A* **108**, 1657–1662.

Keshishian, H., and Kim, Y.S. (2004). Orchestrating development and function: retrograde BMP signaling in the Drosophila nervous system. *Trends Neurosci.* **27**, 143–147.

Kilman, V.L., Zhang, L., Meissner, R.A., Burg, E., and Allada, R. (2009). Perturbing dynamin reveals potent effects on the Drosophila circadian clock. *PLoS One* **4**, e5235.

Kitamoto, T. (2001). Conditional modification of behavior in Drosophila by targeted expression of a temperature-sensitive shibire allele in defined neurons. *J Neurobiol.* **47**, 81–92.

Konopka, R.J., and Benzer, S. (1971). Clock mutants of Drosophila melanogaster. *Proc Natl Acad Sci. U S A* **68**, 2112–2116.

Krishnan, B., Dryer, S.E., and Hardin, P.E. (1999). Circadian rhythms in olfactory responses of Drosophila melanogaster. *Nature* **400**, 375–378.

Krishnan, B., Levine, J.D., Lynch, M.K., Dowse, H.B., Funes, P., Hall, J.C., Hardin, P.E., and Dryer, S.E. (2001). A new role for cryptochrome in a Drosophila circadian oscillator. *Nature* **411**, 313–317.

Krishnan, P., Chatterjee, A., Tanoue, S., and Hardin, P.E. (2008). Spike amplitude of single-unit responses in antennal sensillae is controlled by the Drosophila circadian clock. *Curr. Biol.* **18**, 803–807.

Kuhlman, S.J., and McMahon, D.G. (2004). Rhythmic regulation of membrane potential and potassium current persists in SCN neurons in the absence of environmental input. *Eur. J. Neurosci.* **20**, 1113–1117.

Kula-Eversole, E., Nagoshi, E., Shang, Y., Rodriguez, J., Allada, R., and Rosbash, M. (2010). Surprising gene expression patterns within and between PDF-containing circadian neurons in Drosophila. *Proc. Natl. Acad. Sci. U S A* **107**, 13497–134502.

Kula, E., Levitan, E.S., Pyza, E., and Rosbash, M. (2006). PDF cycling in the dorsal protocerebrum of the Drosophila brain is not necessary for circadian clock function. *J. Biol. Rhythms* **21**, 104–117.

Lear, B.C., Lin, J.M., Keath, J.R., McGill, J.J., Raman, I.M., and Allada, R. (2005 a) The ion channel narrow abdomen is critical for neural output of the Drosophila circadian pacemaker. *Neuron* **48**, 965–976.

Lear, B.C., Merrill, C.E., Lin, J.M., Schroeder, A., Zhang, L., and Allada, R. (2005 b) A G protein-coupled receptor, groom-of-PDF, is required for PDF neuron action in circadian behavior. *Neuron* **48**, 221–227.

Lebestky, T., Chang, J.S., Dankert, H., Zelnik, L., Kim, Y.C., Han, K.A., Wolf, F.W., Perona, P., and Anderson, D.J. (2009). Two different forms of arousal in Drosophila are oppositely regulated by the dopamine D1 receptor ortholog DopR via distinct neural circuits. *Neuron* **64**, 522–536.

Levine, J.D., Funes, P., Dowse, H.B., and Hall, J.C. (2002). Resetting the circadian clock by social experience in Drosophila melanogaster. *Science* **298**, 2010–2012.

Lin, Y., Han, M., Shimada, B., Wang, L., Gibler, T.M., Amarakone, A., Awad, T.A., Stormo, G.D., Van Gelder, R.N., and Taghert, P.H. (2002). Influence of the period-dependent circadian clock on diurnal, circadian, and aperiodic gene expression in Drosophila melanogaster. *Proc. Natl. Acad. Sci. U S A* **99**, 9562–9567.

Lin, Y., Stormo, G.D., and Taghert, P.H. (2004). The neuropeptide pigment-dispersing factor coordinates pacemaker interactions in the Drosophila circadian system. *J. Neurosci.* **24**, 7951–7957.

Loh, D.H., Navarro, J., Hagopian, A., Wang, L.M., Deboer, T., and Colwell, C.S. (2010). Rapid changes in the light/dark cycle disrupt memory of conditioned fear in mice. *PLoS One* **5**(9); pii: e12546.

Lyons, L.C., Rawashdeh, O., Katzoff, A., Susswein, A.J., and Eskin, A. (2005). Circadian modulation of complex learning in diurnal and nocturnal Aplysia. *Proc. Natl. Acad. Sci. U S A* **102**, 12589–12594.

Lyons, L.C., and Roman, G. (2009). Circadian modulation of short-term memory in Drosophila. *Learn Mem.* **16**, 19–27.

Ma, W.P., Cao, J., Tian, M., Cui, M.H., Han, H.L., Yang, Y.X., and Xu, L. (2007). Exposure to chronic constant light impairs spatial memory and influences long-term depression in rats. *Neurosci. Res.* **59**, 224–230.

Marder, E., and Bucher, D. (2007). Understanding circuit dynamics using the stomatogastric nervous system of lobsters and crabs. *Annu. Rev. Physiol.* **69**, 291–316.

Marques, G., and Zhang, B. (2006). Retrograde signaling that regulates synaptic development and function at the Drosophila neuromuscular junction. *Int. Rev. Neurobiol.* **75**, 267–285.

Matsumoto, A., Matsumoto, N., Harui, Y., Sakamoto, M., and Tomioka, K. (1998). Light and temperature cooperate to regulate the circadian locomotor rhythm of wild type and period mutants of Drosophila melanogaster. *J. Insect. Physiol.* **44**, 587–596.

Matsumoto, G., and Tasaki, I. (1977). A study of conduction velocity in nonmyelinated nerve fibers. *Biophys. J.* **20**, 1–13.

McDonald, M.J., and Rosbash, M. (2001). Microarray analysis and organization of circadian gene expression in Drosophila. *Cell* **107**, 567–578.

Mehnert, K.I., Beramendi, A., Elghazali, F., Negro, P., Kyriacou, C.P., and Cantera, R. (2007). Circadian changes in Drosophila motor terminals. *Dev. Neurobiol.* **67**, 415–421.

Mehnert, K.I., and Cantera, R. (2008). A peripheral pacemaker drives the circadian rhythm of synaptic boutons in Drosophila independently of synaptic activity. *Cell. Tissue Res.* **334**, 103–109.

Mertens, I., Vandingenen, A., Johnson, E.C., Shafer, O.T., Li, W., Trigg, J.S., De Loof, A., Schoofs, L., and Taghert, P.H. (2005). PDF receptor signaling in Drosophila contributes to both circadian and geotactic behaviors. *Neuron* **48**, 213–219.

Michel, S., Geusz, M.E., Zaritsky, J.J., and Block, G.D. (1993). Circadian rhythm in membrane conductance expressed in isolated neurons. *Science* **259**, 239–241.

Michel, S., Manivannan, K., Zaritsky, J.J., and Block, G.D. (1999). A delayed rectifier current is modulated by the circadian pacemaker in Bulla. *J. Biol. Rhythms* **14**, 141–150.

Miskiewicz, K., Pyza, E., and Schurmann, F.W. (2004). Ultrastructural characteristics of circadian pacemaker neurones, immunoreactive to an antibody against a pigment-dispersing hormone in the fly's brain. *Neurosci. Lett.* **363**, 73–77.

Miyasako, Y., Umezaki, Y., and Tomioka, K. (2007). Separate sets of cerebral clock neurons are responsible for light and temperature entrainment of Drosophila circadian locomotor rhythms. *J. Biol. Rhythms* **22**, 115–126.

Moura, P.J., Gimenes-Junior, J.A., Valentinuzzi, V.S., and Xavier, G.F. (2009). Circadian phase and intertrial interval interfere with social recognition memory. *Physiol. Behav.* **96**, 51–56.

Myers, E.M., Yu, J., and Sehgal, A. (2003). Circadian control of eclosion: interaction between a central and peripheral clock in Drosophila melanogaster. *Curr. Biol.* **13**, 526–533.

Nagoshi, E., Sugino, K., Kula, E., Okazaki, E., Tachibana, T., Nelson, S., and Rosbash, M. (2010). Dissecting differential gene expression within the circadian neuronal circuit of Drosophila. *Nat. Neurosci.* **13**, 60–68.

Nitabach, M.N., and Taghert, P.H. (2008). Organization of the Drosophila circadian control circuit. *Curr. Biol.* **18**, R84–R93.

Oishi, K., Miyazaki, K., Kadota, K., Kikuno, R., Nagase, T., Atsumi, G., Ohkura, N., Azama, T., Mesaki, M., and Yukimasa, S et al., (2003). Genome-wide expression analysis of mouse liver reveals CLOCK-regulated circadian output genes. *J. Biol. Chem.* **278**, 41519–41527.

Panda, S., Antoch, M.P., Miller, B.H., Su, A.I., Schook, A.B., Straume, M., Schultz, P.G., Kay, S.A., Takahashi, J.S., and Hogenesch, J.B. (2002). Coordinated transcription of key pathways in the mouse by the circadian clock. *Cell* **109**, 307–320.

Parisky, K.M., Agosto, J., Pulver, S.R., Shang, Y., Kuklin, E., Hodge, J.J., Kang, K., Liu, X., Garrity, P. A., and Rosbash, M et al., (2008). PDF cells are a GABA-responsive wake-promoting component of the Drosophila sleep circuit. *Neuron* **60**, 672–682.

Park, D., and Griffith, L.C. (2006). Electrophysiological and anatomical characterization of PDF-positive clock neurons in the intact adult Drosophila brain. *J. Neurophysiol.* **95**, 3955–3960.

Park, J.H., and Hall, J.C. (1998). Isolation and chronobiological analysis of a neuropeptide pigment-dispersing factor gene in Drosophila melanogaster. *J. Biol. Rhythms* **13**, 219–228.

Park, J.H., Helfrich-Forster, C., Lee, G., Liu, L., Rosbash, M., and Hall, J.C. (2000). Differential regulation of circadian pacemaker output by separate clock genes in Drosophila. *Proc. Natl. Acad. Sci. U S A* **97**, 3608–3613.

Peng, Y., Stoleru, D., Levine, J.D., Hall, J.C., and Rosbash, M. (2003). Drosophila free-running rhythms require intercellular communication. *PLoS. Biol.* **1**, E13.

Pennartz, C.M., de Jeu, M.T., Bos, N.P., Schaap, J., and Geurtsen, A.M. (2002). Diurnal modulation of pacemaker potentials and calcium current in the mammalian circadian clock. *Nature* **416**, 286–290.

Perez-Cruz, C., Simon, M., Flugge, G., Fuchs, E., and Czeh, B. (2009). Diurnal rhythm and stress regulate dendritic architecture and spine density of pyramidal neurons in the rat infralimbic cortex. *Behav. Brain Res.* **205**, 406–413.

Peschel, N., and Helfrich-Forster, C. (2011). Setting the clock—by nature: circadian rhythm in the fruitfly Drosophila melanogaster. *FEBS Lett* **585**, 1435–1442.

Picot, M., Cusumano, P., Klarsfeld, A., Ueda, R., and Rouyer, F. (2007). Light activates output from evening neurons and inhibits output from morning neurons in the Drosophila circadian clock. *PLoS Biol.* **5**, e315.

Pittendrigh, C.S.a.D.S. (1976). A functional analysis of circadian pacemakers in nocturnal rodents: V. Pacemaker structure: A clock for all seasons. *J. Comp. Physiol. [A]* **106**, 333–355.

Pyza, E., and Gorska-Andrzejak, J. (2004). Involvement of glial cells in rhythmic size changes in neurons of the housefly's visual system. *J. Neurobiol.* **59**, 205–215.

Pyza, E., and Gorska-Andrzejak, J. (2008). External and internal inputs affecting plasticity of dendrites and axons of the fly's neurons. *Acta Neurobiol. Exp. (Wars)* **68**, 322–333.

Pyza, E., and Meinertzhagen, I.A. (1996). Neurotransmitters regulate rhythmic size changes amongst cells in the fly's optic lobe. *J. Comp. Physiol. A* **178**, 33–45.

Pyza, E., and Meinertzhagen, I.A. (1999). Daily rhythmic changes of cell size and shape in the first optic neuropil in Drosophila melanogaster. *J. Neurobiol.* **40**, 77–88.

Reijmers, L.G., Leus, I.E., Burbach, J.P., Spruijt, B.M., and van Ree, J.M. (2001). Social memory in the rat: circadian variation and effect of circadian rhythm disruption. *Physiol. Behav.* **72**, 305–309.

Renn, S.C., Park, J.H., Rosbash, M., Hall, J.C., and Taghert, P.H. (1999). A pdf neuropeptide gene mutation and ablation of PDF neurons each cause severe abnormalities of behavioral circadian rhythms in Drosophila. *Cell* **99**, 791–802.

Rhee, J., Buchan, T., Zukerberg, L., Lilien, J., and Balsamo, J. (2007). Cables links Robo-bound Abl kinase to N-cadherin-bound beta-catenin to mediate Slit-induced modulation of adhesion and transcription. *Nat. Cell Biol.* **9**, 883–892.

Rhee, J., Mahfooz, N.S., Arregui, C., Lilien, J., Balsamo, J., and VanBerkum, M.F. (2002). Activation of the repulsive receptor roundabout inhibits N-cadherin-mediated cell adhesion. *Nat. Cell Biol.* **4**, 798–805.

Rieger, D., Wulbeck, C., Rouyer, F., and Helfrich-Forster, C. (2009). Period gene expression in four neurons is sufficient for rhythmic activity of Drosophila melanogaster under dim light conditions. *J. Biol. Rhythms* **24**, 271–282.

Ruiz, S., Ferreiro, M.J., Casanova, G., Olivera, A., and Cantera, R. (2010). Synaptic vesicles in motor synapses change size and distribution during the day. *Synapse* **64**, 14–19.

Schneider, N.L., and Stengl, M. (2006). Gap junctions between accessory medulla neurons appear to synchronize circadian clock cells of the cockroach Leucophaea maderae. *J. Neurophysiol.* **95**, 1996–2002.

Sehadova, H., Glaser, F.T., Gentile, C., Simoni, A., Giesecke, A., Albert, J.T., and Stanewsky, R. (2009). Temperature entrainment of Drosophila's circadian clock involves the gene nocte and signaling from peripheral sensory tissues to the brain. *Neuron* **64**, 251–266.

Shafer, O.T., Helfrich-Forster, C., Renn, S.C., and Taghert, P.H. (2006). Reevaluation of Drosophila melanogaster's neuronal circadian pacemakers reveals new neuronal classes. *J. Comp. Neurol.* **498**, 180–193.

Shafer, O.T., Kim, D.J., Dunbar-Yaffe, R., Nikolaev, V.O., Lohse, M.J., and Taghert, P.H. (2008). Widespread receptivity to neuropeptide PDF throughout the neuronal circadian clock network of Drosophila revealed by real-time cyclic AMP imaging. *Neuron* **58**, 223–237.

Shafer, O.T., Rosbash, M., and Truman, J.W. (2002). Sequential nuclear accumulation of the clock proteins period and timeless in the pacemaker neurons of Drosophila melanogaster. *J. Neurosci.* **22**, 5946–5954.

Shang, Y., Griffith, L.C., and Rosbash, M. (2008). Light-arousal and circadian photoreception circuits intersect at the large PDF cells of the Drosophila brain. *Proc. Natl. Acad. Sci. U S A* **105**, 19587–19594.

Sheeba, V., Gu, H., Sharma, V.K., O'Dowd, D.K., and Holmes, T.C. (2008). Circadian- and light-dependent regulation of resting membrane potential and spontaneous action potential firing of Drosophila circadian pacemaker neurons. *J. Neurophysiol.* **99**, 976–988.

Shemesh, Y., Eban-Rothschild, A., Cohen, M., and Bloch, G. (2010). Molecular dynamics and social regulation of context-dependent plasticity in the circadian clockwork of the honey bee. *J. Neurosci.* **30**, 12517–12525.

Stanewsky, R. (2002). Clock mechanisms in Drosophila. *Cell Tissue Res.* **309**, 11–26.

Stanewsky, R., Kaneko, M., Emery, P., Beretta, B., Wager-Smith, K., Kay, S.A., Rosbash, M., and Hall, J.C. (1998). The cryb mutation identifies cryptochrome as a circadian photoreceptor in Drosophila. *Cell* **95**, 681–692.

Stoleru, D., Nawathean, P., Fernandez, M.L., Menet, J.S., Ceriani, M.F., and Rosbash, M. (2007). The Drosophila circadian network is a seasonal timer. *Cell* **129**, 207–219.

Stoleru, D., Peng, Y., Agosto, J., and Rosbash, M. (2004). Coupled oscillators control morning and evening locomotor behaviour of Drosophila. *Nature* **431**, 862–868.

Stoleru, D., Peng, Y., Nawathean, P., and Rosbash, M. (2005). A resetting signal between Drosophila pacemakers synchronizes morning and evening activity. *Nature* **438**, 238–242.

Tanoue, S., Krishnan, P., Chatterjee, A., and Hardin, P.E. (2008). G protein-coupled receptor kinase 2 is required for rhythmic olfactory responses in Drosophila. *Curr. Biol.* **18**, 787–794.

Tanoue, S., Krishnan, P., Krishnan, B., Dryer, S.E., and Hardin, P.E. (2004). Circadian clocks in antennal neurons are necessary and sufficient for olfaction rhythms in Drosophila. *Curr. Biol.* **14**, 638–649.

Tully, T., and Quinn, W.G. (1985). Classical conditioning and retention in normal and mutant Drosophila melanogaster. *J. Comp. Physiol. A* **157**, 263–277.

Ueda, H.R., Matsumoto, A., Kawamura, M., Iino, M., Tanimura, T., and Hashimoto, S. (2002). Genome-wide transcriptional orchestration of circadian rhythms in Drosophila. *J. Biol. Chem.* **277**, 14048–14052.

Valentinuzzi, V.S., Menna-Barreto, L., and Xavier, G.F. (2004). Effect of circadian phase on performance of rats in the Morris water maze task. *J. Biol. Rhythms* **19**, 312–324.

Valentinuzzi, V.S., Scarbrough, K., Takahashi, J.S., and Turek, F.W. (1997). Effects of aging on the circadian rhythm of wheel-running activity in C57BL/6 mice. *Am. J. Physiol.* **273**, R1957–R1964.

Veleri, S., Brandes, C., Helfrich-Forster, C., Hall, J.C., and Stanewsky, R. (2003). A self-sustaining, light-entrainable circadian oscillator in the Drosophila brain. *Curr. Biol.* **13**, 1758–1767.

Vertes, R.P. (2006). Interactions among the medial prefrontal cortex, hippocampus and midline thalamus in emotional and cognitive processing in the rat. *Neuroscience* **142**, 1–20.

Vosko, A.M., Schroeder, A., Loh, D.H., and Colwell, C.S. (2007). Vasoactive intestinal peptide and the mammalian circadian system. *Gen. Comp. Endocrinol.* **152**, 165–175.

Wall, P.M., Blanchard, R.J., Markham, C., Yang, M., and Blanchard, D.C. (2004). Infralimbic D1 receptor agonist effects on spontaneous novelty exploration and anxiety-like defensive responding in CD-1 mice. *Behav. Brain Res.* **152**, 67–79.

Wang, L.M., Dragich, J.M., Kudo, T., Odom, I.H., Welsh, D.K., O'Dell, T.J., and Colwell, C.S. (2009). Expression of the circadian clock gene Period2 in the hippocampus: possible implications for synaptic plasticity and learned behaviour. *ASN Neuro* **1**(3); pii: e00012.

Weber, P., Kula-Eversole, E., and Pyza, E. (2009). Circadian control of dendrite morphology in the visual system of Drosophila melanogaster. *PLoS One* **4**, e4290.

Welsh, D.K., Takahashi, J.S., and Kay, S.A. (2010). Suprachiasmatic nucleus: cell autonomy and network properties. *Annu. Rev. Physiol.* **72**, 551–577.

Wulbeck, C., Grieshaber, E., and Helfrich-Forster, C. (2009). Blocking endocytosis in Drosophila's circadian pacemaker neurons interferes with the endogenous clock in a PDF-dependent way. *Chronobiol. Int.* **26**, 1307–1322.

Yan, J., Wang, H., Liu, Y., and Shao, C. (2008). Analysis of gene regulatory networks in the mammalian circadian rhythm. *PLoS Comput. Biol.* **4**, e1000193.

Yang, Z., and Sehgal, A. (2001). Role of molecular oscillations in generating behavioral rhythms in Drosophila. *Neuron* **29**, 453–467.

Yasuyama, K., and Meinertzhagen, I.A. (2010). Synaptic connections of PDF-immunoreactive lateral neurons projecting to the dorsal protocerebrum of Drosophila melanogaster. *J. Comp. Neurol.* **518**, 292–304.

Yoshii, T., Heshiki, Y., Ibuki-Ishibashi, T., Matsumoto, A., Tanimura, T., and Tomioka, K. (2005). Temperature cycles drive Drosophila circadian oscillation in constant light that otherwise induces behavioural arrhythmicity. *Eur. J. Neurosci.* **22**, 1176–1184.

Yoshii, T., Vanin, S., Costa, R., and Helfrich-Forster, C. (2009). Synergic entrainment of Drosophila's circadian clock by light and temperature. *J. Biol. Rhythms* **24**, 452–464.

Zhou, X., Yuan, C., and Guo, A. (2005). Drosophila olfactory response rhythms require clock genes but not pigment dispersing factor or lateral neurons. *J. Biol. Rhythms* **20**, 237–244.