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External and internal inputs affecting plasticity of dendrites and axons of the fly's neurons

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Neurons and glial cells in the fly's visual system exhibit circadian rhythms through changes in shape and size. Moreover, the number of synaptic contacts between these cells changes during the day and night and in the case of one type of synapses, feedback synapses, is maintained under constant conditions indicating an endogenous origin of this rhythm. The structural changes described above, involving the oscillations in the number of synapses and the size of interneurons and glial cells, are examples of plasticity in the central nervous system driven by internal inputs from a circadian clock and by external stimuli such as light. They are also modulated by visual and other sensory stimuli and by motor activity.

Key words: circadian clock, interneurons, glia, visual system, *Drosophila melanogaster, Musca domestica*

INTRODUCTION

The visual system of flies provides an excellent model for the study of transduction and transmission of information in the nervous system, interactions between neurons and glial cells, and circadian rhythms, i.e. endogenous oscillations, with a period of about one day, in various physiological processes (Pyza 2001).

The fly's visual system contains cell types similar to those of the retina of vertebrates and a striking homology in functions of these cells types has indeed been observed (Meinertzhagen 1993). As in other insect species, the visual system of flies is composed of the retina and three optic neuropils: the lamina, the medulla and the lobula (Fig. 1A,B). In flies the third neuropil is called the lobula complex and is divided into the lobula and lobula plate. The retina consists of modular units called ommatidia, the number of which makes 3 000 in *Musca domestica* and about 800 in *Drosophila melanogaster* (Fig. 1A). Each of them can be compared functionally to the vertebrate retina photoreceptor. Each ommatidium comprises eight photoreceptor cells. The photoreceptors receive photic and visual information, and after transduction, transmit the information to the first order interneurons, termed L1 and L2 large monopolar cells (LMCs), located in the lamina where these signals are filtrated, enhanced and transmitted to the second order interneurons in the medulla (Hardie et al. 1989). Functionally L1 and L2 monopolar cells have a similar role as the bipolar cells of the vertebrate retina. The structure of the lamina, the first optic neuropil of the optic lobe, is also modular and composed of cylindrical units called cartridges (Strausfeld and Nässel 1980) (Fig. 1C). Each cartridge receives six terminals (R1–R6) (Fig. 2A,D) originating from neighboring ommatidia and fibers of two other photoreceptors (R7, R8) which bypass the first optic neuropil and synapse in the second optic neuropil, the medulla. The cartridge also comprises five monopolar cells (L1–L5), including LMC L1 and L2 (Fig. 2B,E), α processes of amacrine cells, β processes of T1 cells and processes of C1 and C2 neurons with cell bodies located outside of the lamina (Strausfeld and Nässel 1980, Fischbach and Dittrich 1989). Each cartridge is surrounded by three epithelial glial cells (Boschek 1971) (Fig. 2C,F). In the lamina R1–R6 form synaptic contacts, the so-called tetrad synapses, with four postsynaptic elements (Fig. 1D). Two of them are LMCs

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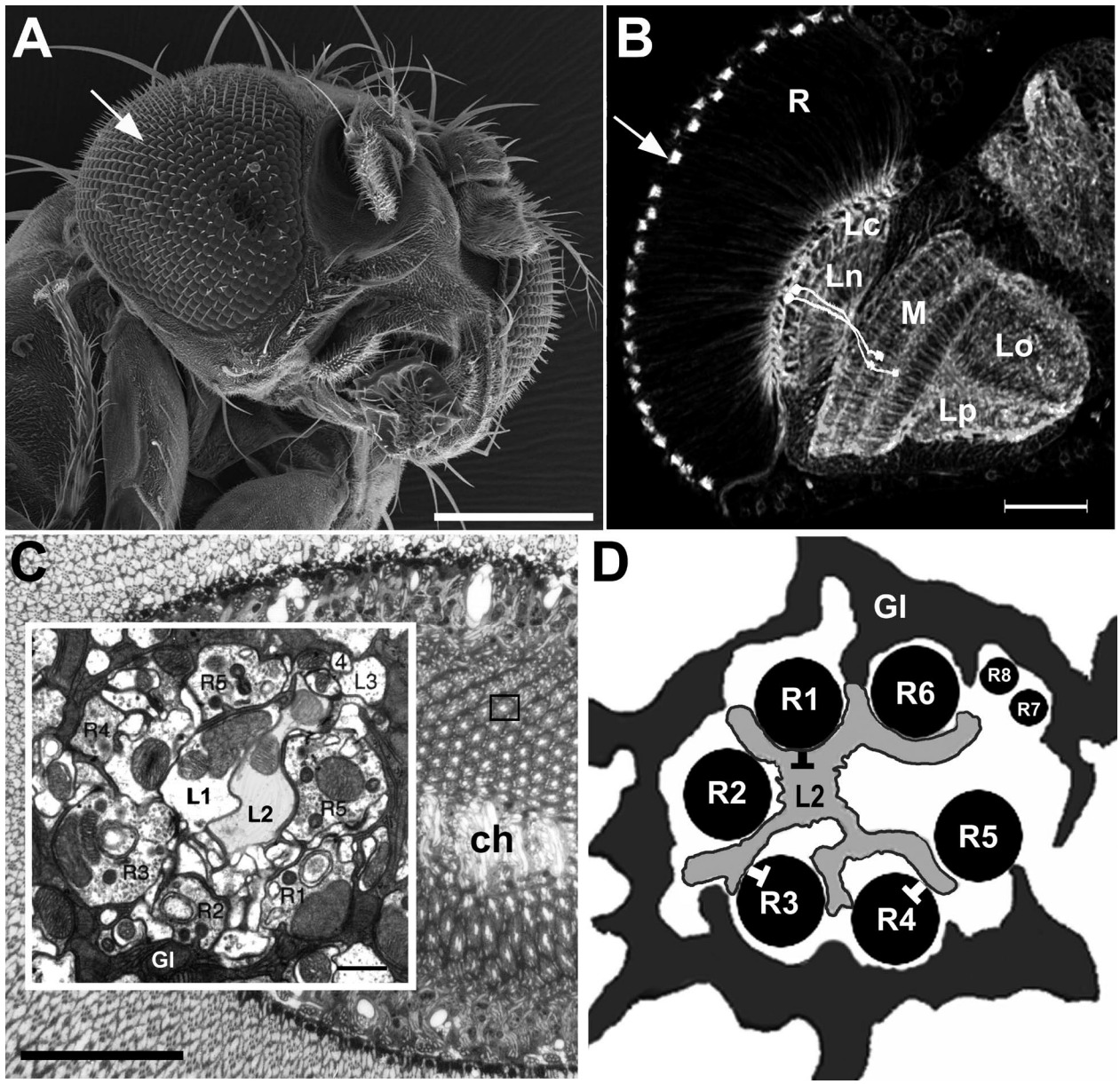

Fig. 1. The visual system of *Drosophila melanogaster*. (A) Scanning electron micrograph of the head and compound eye of *Drosophila* viewed from the right side. The fly's eye is composed of hexagonally arranged units/facets called ommatidia. Arrow- the surface of the eye. Scale bar is 200 μm. (B) The structure of the compound eye and optic lobe in horizontal section as revealed by immunostaining with an antibody against α-tubulin (Sigma). The ommatidial array of photoreceptors in the retina (R) innervates the first of a series of neuropils, the lamina (Ln), where the first order interneurons, L1 and L2 monopolar cells (marked in white) have their cell bodies and axons. L1 and L2 axons terminate in the second visual neuropil or medulla (M). (Lc) lamina cortex; (Ln) lamina neuropil; (M) medulla; (Lo) lobula; (Lp) lobula plate. (Arrow) The surface of the eye. Scale bar is 50 μm. (C) The structure of the lamina in cross-section. The lamina is composed of cylindrical modules called cartridges (the region enclosed in a small box), that comprise the same cell types and constitute synaptic units of this neuropil. (ch) Chiasma. Scale bar is 20 μm. (Insert) EM micrograph showing the magnification of a single cartridge with the profiles of L1 and L2 monopolar cells at its axis and the surrounding photoreceptors (R1–R6) and glial cells (Gl). (L3) Monopolar cell L3; (L4) monopolar cell L4. Scale bar is 1 μm. (D) Schematic representation of the lamina cartridge in cross section with the positions of photoreceptors axons (R1–R8), and the axon of L2 monopolar cell (L2), as well as presynaptic elements (T-bars) of synaptic contacts that are formed between these cell types; tetrad synapses (white T-bars) and feedback synapses (black T-bar). (Gl) Epithelial glia that surround each cartridge.

and two other elements include α processes of an amacrine cell, or a glial cell, or – in the distal lamina – L3 cell (Burkhardt and Braitenberg 1976, Meinertzhagen 1989, Meinertzhagen and Sorra 2001). In return the photoreceptor terminals receive feedback from the L2 and from the amacrine cells (Strausfeld and Campos-Ortega 1977, Meinertzhagen and Sorra 2001). The tetrad synapses use histamine as a neurotransmitter (Hardie 1987), while the L2 cells, sending feedback synapses back onto the photoreceptor terminals, are probably glutamatergic. While the lamina has been studied intensively, the medulla and the two neuropils of the lobula complex are more complicated and less studied (Strausfeld and Nässel 1980).

The circadian system of the fly's brain

In insects the circadian system has been best described in *Drosophila melanogaster*. In *Drosophila* and in mammals it consists of cells expressing "clock genes" which show spontaneous cyclical expression (Stanewsky 2003, Helfrich-Förster 2004). In the brain

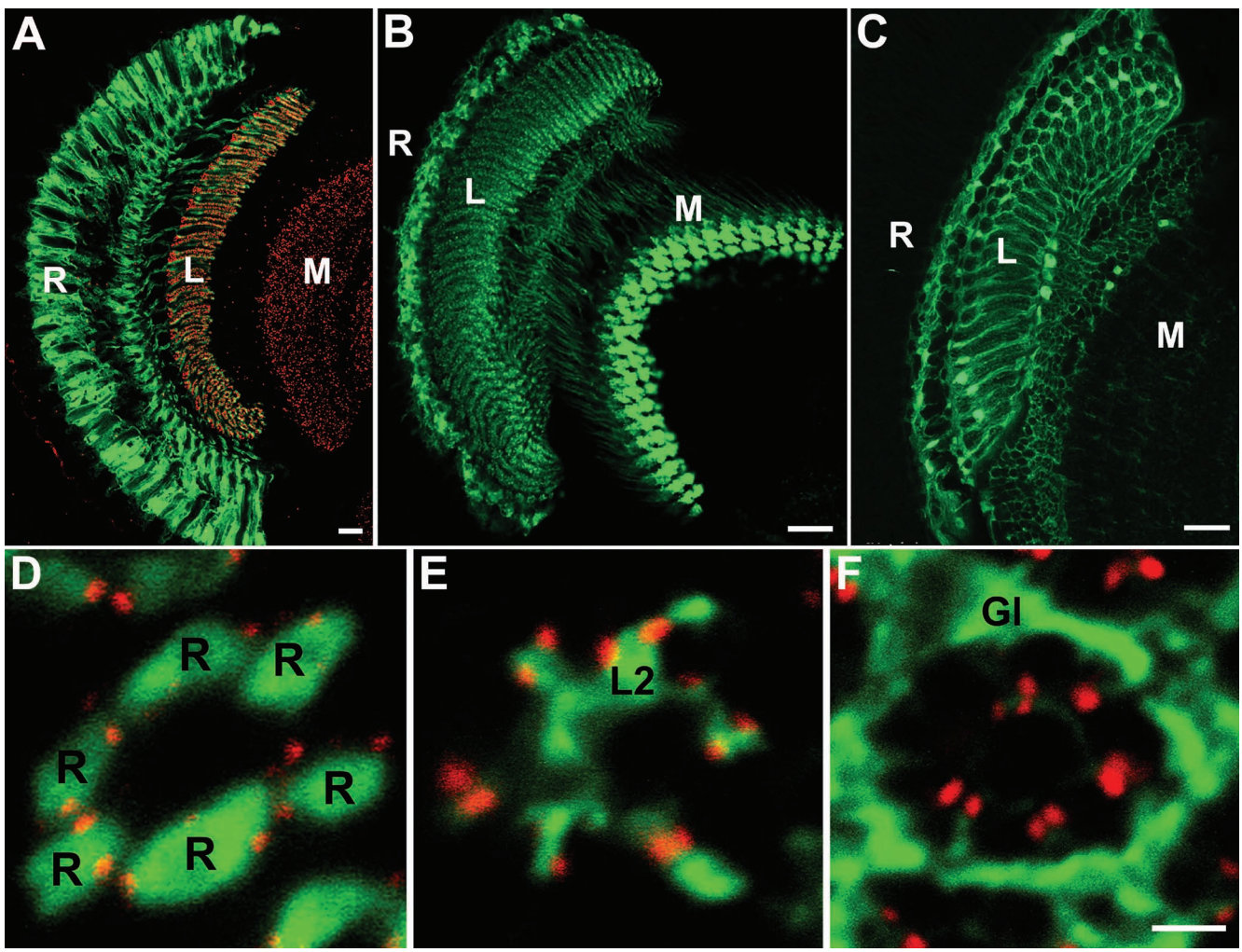

Fig. 2. Confocal images of GAL4/UAS transgenic lines of *Drosophila* showing targeted expression of green fluorescent protein (GFP) in particular cell types of the fly's visual system (green). (A); (B); (C) GFP expression in photoreceptors (GMR-GAL4/UAS-S65T-GFP), L2 monopolar cell (21D-GAL4/UAS-Act5.T-GFP), and epithelial glial cells (REPO-GAL4/UAS-S65T-GFP), respectively. (A) Frontal cryostat section of the optic lobe of GMR-GAL4/UAS-S65T-GFP transgenic *Drosophila* line. The optic lobe section was immunolabeled with Mab Nc82 (Hybridoma) which specifically label individual synapses by recognizing a presynaptic protein named Bruchpilot (red). (R) Retina; (L)lamina; (M) medulla. Scale bar is 20 μm. (D); (E); (F) Cross sections of cartridges of mentioned above transgenic lines immunostained with Mab Nc82. (R) Axons of photoreceptors; (L2) axon of L2 monopolar cell; (Gl) processes of epithelial glial cells. Scale bar is 1 μm.

of *Drosophila*, the PER protein, a product of the clock gene *period* (*per*), has been detected in about 150 neurons, divided into three lateral (LN) and three dorsal groups (DN), and also in a lateral-posterior group (Hall 2003, Shafer et al. 2006, Helfrich-Förster et al. 2007). Some lateral neurons, four to five large ventral lateral neurons (l-LNvs) and four small ventral lateral neurons (s-LNvs) also express a neuropeptide pigment-dispersing factor (PDF) while the fifth s-LNv and six dorsal lateral neurons are PDF-negative. Beside the circadian pacemaker cells, PER has been detected in the compound eye photoreceptors and in some glial cells (Siwicki et al. 1988, Ewer et al. 1992).

Some of the *per*-expressing neurons, specifically the s-LNvs, are regarded as crucial circadian pacemakers since *per* expression and their spontaneous activity are self-sustaining and necessary to maintain behavioral circadian rhythms (Veleri et al. 2003). The circadian clock in the brain of *Drosophila* and in other insects show striking similarities to the clock of the mammalian brain (Helfrich-Förster 2004). Beside clock cells in the brain, in many tissues of both insects and mammals, cyclical expression of *per* has been observed in cells called peripheral oscillators, but their oscillations dampen after several cycles in constant darkness (Giebultowicz 2001, Herzog and Tosini 2001, Balsalobre 2002).

Since *Drosophila* shows a bimodal pattern of locomotor activity with two morning and evening peaks in a 12 h day and 12 h night cycle (LD 12:12), different *per* expressing clock neurons contribute to maintain this bimodal pattern of activity (Grima et al. 2004, Stoleru et al. 2004).

At the molecular level, the circadian rhythm in both insects and mammals alike, is generated by two interconnected feedback loops (Stanewsky 2003). In

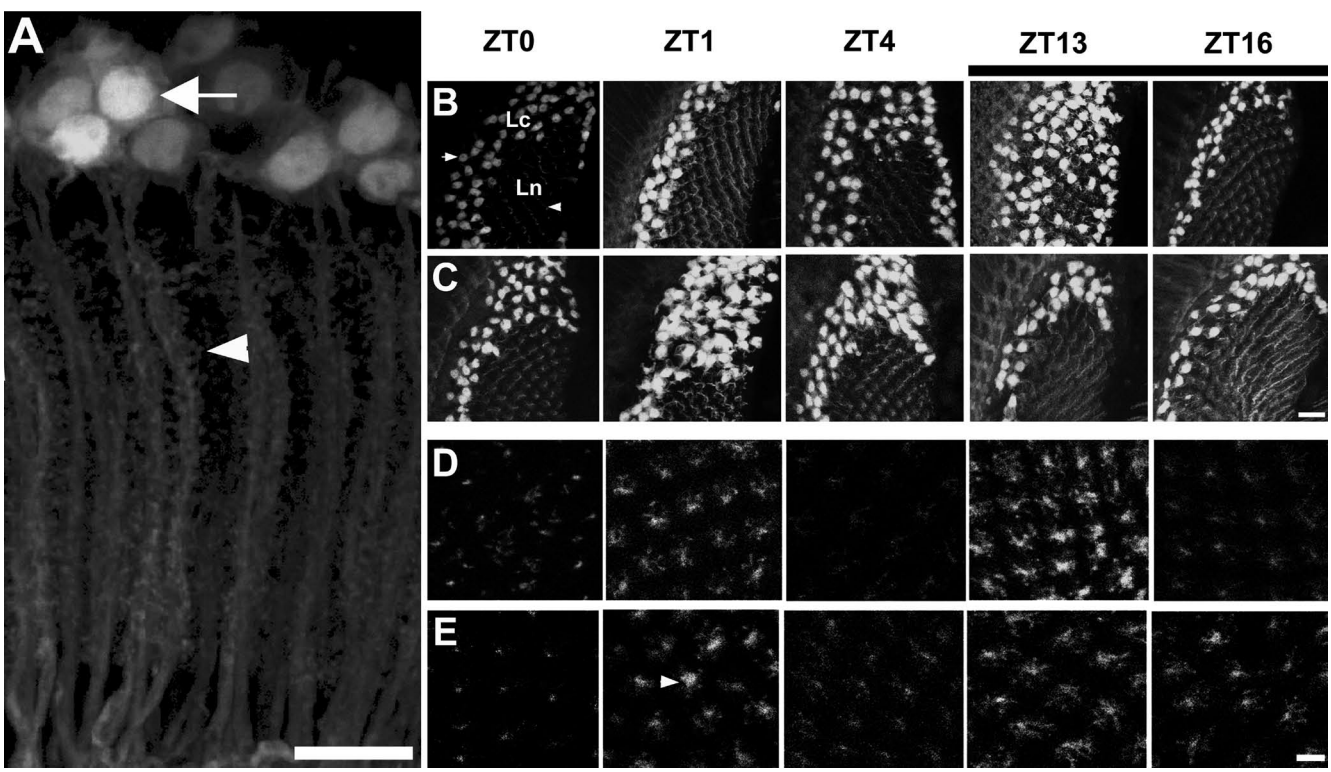

fig. 3. Daily changes in morphology of L2 monopolar cell in the lamina of 21D-GAL4/UAS-S65T-GFP transgenic line of *Drosophila*. (A) Confocal image showing the structure of L2 monopolar cells in the lamina. GFP expression labels nuclei of the somata (arrow) in the lamina cortex, as well as axons and dendrites (arrowhead) in the neuropil. Scale bar is 10 μm. (B); (C) Daily changes [ZT0-ZT16: (ZT0) the beginning of the day; (ZT 12) the beginning of the night] in the size of L2 cell nuclei in males (B) and females (C). The cross – sectional area of the nuclei appears to be largest 1 h after lights-on (ZT1, females) and 4 h after lights-on (ZT4, males) and smallest in the middle of the night (ZT16, females and males). Scale bar is 20 μm. (D); (E) Differences in morphology of the dendritic tree of L2 in males (D) and females (E). The cross-sectional area of L2 monopolar cell axons is larger at ZT1 and ZT13 than in other time points in both males and females. Scale bar is 5 μm.

Drosophila PER and TIM proteins enter the nucleus and inhibit the transcription of their own genes producing the negative feedback loop, while the positive one controls their rhythmic expression (Allada 2003).

CIRCADIAN RHYTHMS IN THE VISUAL SYSTEM

Circadian rhythms in structural changes and physiological processes have been detected in the retina of both vertebrates (Green and Besharse 2004) and invertebrates (Block et al. 1995, Chen et al. 1999, Barlow 2001). In the retina of vertebrates they include rhythms in changes of the electroretinogram (ERG) amplitude, in turnover of the photoreceptor outer segments, changes in concentration of neurotransmitters and many others. These rhythms are generated by the circadian pacemaker located in the suprachiasmatic nucleus in the hypothalamus as well as by circadian oscillators in the retina itself (Cahill and Besharse 1993).

In the retina of insects circadian rhythms have been detected in the ERG, the migration of screening pigment granules, and in changes of the rhabdomere structure in the photoreceptors (Sakura et al. 2003). In addition to circadian rhythms observed in the retina, other circadian rhythms have also been detected in the lamina. In the housefly daily rhythms have been found in the frequency and size of the presynaptic elements of tetrad and feedback synapses, but only in the case of feedback synapses the rhythm is circadian since it is maintained in conditions of constant darkness (DD) (Pyza and Meinertzhagen 1993). Structural changes of presynaptic elements, the photoreceptor synaptic ribbons, have also been reported in mammals. Balkema and coauthors (2001) have found that the synaptic ribbons vary in length at different times of the day/night cycle in mice and are largest 2 h after light onset when the visual sensitivity is the highest. Although the tetrad number in the fly's photoreceptor terminals does not oscillate in constant darkness, the photoreceptor exhibits circadian rhythms in vertical migration of screening pigment granules and in inter-receptor invaginations indicating the endogenous reorganization of organelles in these cells (Pyza and Meinertzhagen 1997a). In turn, the postsynaptic neurons of tetrad synapses, the lamina monopolar cells L1 and L2, show circadian structural plasticity changing the girth of their axons. These rhythms, first detected in the housefly *Musca domestica* (Pyza and Meinertzhagen 1995), have also been observed in *Drosophila melanogaster* and *Calliphora vicina* (Pyza and Meinertzhagen 1999, Pyza and Cymborowski 2001). In *M. domestica*, axons of L1 and L2 are larger during the day than during the night and these changes are maintained in DD. They also persist in continuous light (LL), in contrast to the behavioral circadian rhythms observed in most animals. In LL the rhythm in locomotor activity is abolished and animals become arrhythmic because the LNvs are molecularly arrhythmic (Murad et al. 2007) and PDF release is inhibited (Picot et al. 2007). The rhythms in changes of L1 and L2 axon sizes have different patterns in each species, however, and are correlated with species-specific patterns of locomotor activity. Since *M. domestica* and *C. vicina* are diurnal animals, the L1 and L2 cells are larger during the day than at night, while in *D. melanogaster* they become larger twice within a 24 h period, in the morning and in the evening, correlating with the morning and evening peaks of locomotor activity. The changes in sizes of monopolar cells are offset by changes in sizes of the epithelial glial cells surrounding cartridges in the lamina which swell during the night and shrink during the day (Pyza and Górska-Andrzejak 2004). In contrast to the L1 and L2 axons and the epithelial glia, the photoreceptor terminals in the lamina do not change their girth significantly at different times during a 24 h cycle.

In *D. melanogaster*, in addition to the L1 and L2 axon sizes, the structure of the entire L2 cell has been analyzed using transgenic lines expressing green fluorescent protein (GFP) driven by a promotor specific for the L2 cell gene (Fig. 2B,E). This techniques enabled the visualization of not only axons but also somata, nuclei and dendrites of the L2 (Fig. 3A). By comparing their structures in the transgenic flies, diurnal rhythms were found in changes of the size of nuclei (Fig. 3B,C), axons (Fig. 3D,E) and dendrites, but not in the L2 somata (Górska-Andrzejak et al. 2005). The L2 nuclei and perimeter of dendritic trees are larger during the day than at night and the oscillations in length of dendrites also persist under DD. In the case of L2 axons, their cross-sectional area measured at the proximal lamina at electron microscope level (Pyza and Meinertzhagen 1999) and analyzed in confocal microscope at different lamina depths (Górska-Andrzejak et al. 2005), is larger at the beginning of both day and night than in other time points (Fig. 3D,E).

Cyclical dynamic changes in structure of neurons are not unique for flies, however. Daily changes in the

structure of neurons have also been found in the retina of vertebrates. In fish retina the morphology of bipolar cell terminals shows adaptation-dependent changes, being irregular in light but smooth in dark-adapted animals (Behrens and Wagner 1996). In rats, on the other hand, an irregular structure was observed in dark-adapted bipolar cells while in light-adapted cells the shape of axon terminals was round and smooth (Behrens et al. 1998).

Effects of light on the circadian rhythms in the lamina

Measurements of the cross sectional area of L1 and L2 axons in different light conditions have shown that their axon sizes depend on light conditions during rearing. In *M. domestica* L1 and L2 are largest in LL, middle size in LD12:12 and smallest in DD (Pyza and Meinertzhagen 1995). Direct light exposure affects also the tetrad synapse number. When an one hour light pulse is applied at any time during the subjective day and subjective night in DD, the number of tetrad presynaptic element profiles increases (Pyza and Meinertzhagen 1993). Moreover, even a short light pulse after dark rearing induces a pronounced increase in the number of tetrad profiles (Rybak and Meinertzhagen 1997). The effect of a light pulse has not been observed in the case of the feedback presynaptic profiles, the number of which shows robust circadian oscillations. These results indicate that the daily synaptic plasticity of tetrad synapses depends on direct light exposure during the day while feedback synapse number is controlled by a circadian clock and does not depend on light conditions.

Effects of locomotor activity and sensory stimulation on the circadian rhythms in the lamina

The stimulation of the compound eyes of immobilized houseflies using a conventional grating has shown that visual stimulation alone has almost no effect on sizes of L1 and L2 axons, while stimulation of free flying flies to continuous motor activity for one hour during the day increases cell sizes by 50% (L1) and 40% (L2) in males (Kula and Pyza 2007). Similar results have been obtained in females, but the increase of L1 and L2 girths of axons was not as pronounced in females as in males, probably because of sexual dimorphism in the visual system and behavior (Hornstein et al. 2000). This suggests that not only visual stimuli but also other inputs, such as feedback from the motor system, affect the activity of the visual system. The increase of the size of L1 and L2 axons is significant if stimulation is carried out during the fly's period of high locomotor activity in LD, which is species-dependent and for *M. domestica* takes place during the day, but has no effect if applied in DD or LL. This means that locomotor activity and stimulation of all sensory systems result in the enlargement of L1 and L2 axons, correlated with clock-controlled phase of high motor activity and activity of the visual system in the 24 h day/night cycle.

Mechanisms of circadian plasticity of L1 and L2 monopolar cells

The swelling and the shrinking of the L1 and L2 cells continue after 12 h of dehydration applied at the beginning of day or at the beginning of night, so these processes are not the result of simple osmotic shifts (Pyza and Meinertzhagen 1995). These changes of cells' axon structures can be mimicked by injections of neurotransmitters identified in the lamina (Pyza and Meinertzhagen 1996). The effects of these injections, however, are not always the same for both cells. They also depend on the time of treatment. Serotonin (5-HT) injected during the day significantly increases the L1 cells, while injections during the night increase mostly the L2 cells. Injections of a specific toxin for serotoninergic neurons, 5,7-dihydroxytyptamine, shrink both cell types though significantly only the L2 cells. This indicates that changes of the concentration of 5-HT released in the lamina may modulate, in a different way, the sizes of both cells. In turn, histamine, when injected next to the lamina, significantly affects only the size of L1, by increasing it, while glutamate and GABA shrink the axons of both cell types. Applications of GABA into the medulla also affect the feedback synapses, decreasing their number (Pyza and Meinertzhagen 1998). The effect of glutamate, on size of neurons and glial cells, has also been reported in the guinea pig retina (Uckermann et al. 2004).

After injection, the swelling of axons of both cells induces PDF, regarded as a neurotransmitter in the circadian systems of insects. It is possible, however, that other neuropeptides in the fly's optic lobe are also involved in regulating circadian plasticity of neurons

and glial cells. Neural processes containing neuropeptide dense core vesicles, other than PDF, have been detected in the fly's medulla (Miskiewicz et al. 2008). A possible candidate is a neuropeptide similar to the molluscan FMRFamide. Injections of FMRFamide and related neuropeptides into the housefly's medulla showed that FMRFamide shrinks the L1 and L2 axons, thus opposing the effect of PDF injections (Pyza and Meinertzhagen 2003).

The results obtained after injections of the lamina neurotransmitters show that neurotransmitters released from different sets of neurons, having various functions in the visual system and conveying different types of information, either internal or external, affect the sizes of the L1 and L2 interneurons controlled by the fly's circadian system (Meinertzhagen and Pyza 1999).

The physiological significance of circadian morphological changes in L1 and L2 monopolar cells is unknown since electrophysiological intracellular recordings from these cells have not been carried out at different times of the day. It is known, however, that the L2 cells through the feedback synapses control the speed and amplitude of photoreceptor responses (Zheng et al. 2006) and their number in *M. domestica*, a diurnal species, increases at the beginning of night (Pyza and Meinertzhagen 1993). On the other hand, the L2 dendrites, postsynaptic cells of the tetrad synapses, are longest at the beginning of the day (Weber and Pyza 2006) when the number of the tetrad presynaptic elements is the highest (Górska-Andrzejak and Pyza, unpublished results). So, L2 cells not only control photoreceptor responses but also undergo an endogenous remodeling of the girth of axons and length of dendrites, needed to form more active synaptic contacts for faster transmission of information from the eye photoreceptors during the day.

The molecular mechanisms of circadian plasticity of neurons and synapses are still unknown but they involve remodeling of the microtubule and actin microfilament organization. When flies are treated with colchicine disrupting the microtubules, shrinkage of L1 and L2 during the night is observed (Pyza 2001). In turn cytochalasine D, disrupting the microfilaments increases the number of tetrad synaptic contacts and the sizes of L1 and L2 cells after night but not after day injections. As a result the daily pattern of the number of tetrads and of the structure of L1 and L2 interneurons is reversed (Pyza 2002). Thus both types of plasticity, synaptic and neuronal, require a functional cytoskeleton.

Differences in the cell structures after day and night treatments show that the swelling and shrinking of L1 and L2 seem to have different mechanisms. The swelling of both cells during the day depends on the synthesis of proteins at the beginning of the day (Kula and Pyza 2007). Injections of an inhibitor of protein synthesis, cycloheximide, prevent the L1 and L2 axons from swelling when carried out at the beginning of the day but have no effect on cell shrinkage during the night. In turn, changes of ion concentrations have no effect on cell sizes during the day but prevent their shrinkage during the night. When the vacuolar-type H+-ATPase (V-ATPase), the subunits of which have been localized in the fly's photoreceptors, is blocked by injecting of a specific blocker, bafilomycin, the axons of L1 and L2 increase but only if bafilomycin is applied during the night (Pyza et al. 2004). As a result, the rhythm in L1 and L2 sizes is abolished. The rhythm can also be disturbed by applications of chemicals which close gap junction channels connecting glial cells in the lamina, indicating an involvement of glial cells in regulating the rhythms in L1 and L2 interneurons (Pyza and Górska-Andrzejak 2004). This has been confirmed by blocking glial cell metabolism using fluorocitrate and iodoacetate, which affected the L1 and L2 axon sizes (Pyza and Górska-Andrzejak 2004).

Genetic control of structural circadian rhythms in the lamina

The clock that controls circadian rhythms in the visual system is located in the brain since the severance of the optic lobe from the rest of the brain abolishes the rhythmic swelling and shrinking of L1 and L2 cells in the housefly (Bałys and Pyza 2001). Using *D. melanogaster* clock mutants, it has been shown that these rhythms are controlled by the clock genes *per* and *tim* since in the per^{01} and tim^{01} null mutants they were not observed neither in LD 12:12 nor in DD. The lack of rhythm in the per^{01} mutant in LD conditions has not been observed in locomotor activity that is synchronized directly by alternating day and night even when a molecular clock does not function (Wheeler et al. 1993). It has been shown that transgenic flies carrying a 7.2 kb piece of DNA from the per gene can rescue the arrhythmicity in behavior but the rhythm has a period longer than in wild-type flies. Two rescue lines

studied, 7.2:2 and 7.2:9, have normal patterns of locomotor activity with two peaks but different PER expression in the brain (Frisch et al. 1994). In the 7.2:2 line the PER expression is only in the LNs and the day/night differences in L1 and L2 axons are reversed compared with wild-type flies (Pyza and Hardin, unpublished results). When the second line 7.2:9 was used, in which PER was not only detected in the LNs but also in the eye photoreceptors, the pattern of daily L1 and L2 changes in size was similar to the pattern of wild-type flies but differences in sizes at different time points were not significant. This confirms that the rhythm in remodeling of L1 and L2 axons and dendrites may depend on glial cells, particularly those expressing PER.

Transmission of circadian information from the brain circadian clock to the lamina L1 and L2 interneurons

The effects of injecting PDF into the medulla of the optic lobe showed that circadian information may be conveyed to the lamina L1 and L2 monopolar cells by this neuropeptide (Pyza and Meinertzhagen 1996). PDF belongs to a family of peptides called pigment-dispersing hormones (PDHs) and were first identified in crustaceans (Rao and Riehm 1988, 1993). In these animals PDH not only disperses pigment granules in epithelial chromatophores but is also involved in the regulation of circadian rhythms (Rao 1985). It has been suggested that PDF may serve as a transmitter in circadian systems of several insect species (Petri and Stengl 1997, Saifullah and Tomioka 2003) and as a synchronizer of clock cells within the circadian system of *D. melanogaster* (Lin et al. 2004). Its expression in the LNvs of *D. melanogaster* has been proposed to drive the morning peak of locomotor activity in this species (Picot et al. 2007). The s-LNvs terminate in the dorsal protocerebrum and their terminals show circadian differences in size (Park et al. 2000) and complexity of arborization (Fernández et al. 2008), with larger fibers and more complex arborization during the day. These cyclical changes probably deliver circadian information downstream from the clock to the brain centers involved in control of locomotor activity. The lack of PDF cycling in the dorsal protocerebrum, however, does not produce arrhythmic flies (Kula et al. 2006). In turn the large LNvs possess dense fiber arborizations in the medulla and seem to propagate circadian information to the visual system, at least in the housefly. In this species PDF neurons have a similar localization in the anterior medulla, called the accessory medulla, but have a more complex arborization in the medulla and the lamina than in *D. melanogaster* (Meinertzhagen and Pyza 1996, Pyza and Meinertzhagen 1997b). In *M. domestica* the large and small PDF neurons appear early in postembryonic development, survive metamorphosis (Pyza et al. 2003) and show circadian structural changes (Pyza and Meinertzhagen 1997b). It has been shown that the varicosities (sites of neuropeptide release) of PDF fibers are larger during the night than during the day in LD 12:12. This pattern is reversed in DD, however, since PDF varicosities are larger during the subjective day than the subjective night, but the rhythm is abolished in LL and the varicosities are larger than in LD and DD. This suggests that light may stop the accumulation and release of PDF and mask circadian regulation of these processes. In addition cyclical changes of varicosity sizes indicate a paracrine release of PDF from clock neurons and its effect on L1 and L2 sizes. A recent study of PDF and other neuropeptide varicosities in the housefly's medulla at the electron microscope level confirmed that PDF cyclically accumulates in dense core vesicles during the day and is also cyclically released during the night in a paracrine fashion (Miskiewicz et al. 2004, 2008). When released it may directly or indirectly regulate circadian rhythms in neurons and glial cells in the visual system. Cells, which may intermediate between PDF releasing clock neurons (possibly the large LNvs) and neurons in the visual system seem to be PER-containing medulla glial cells. In the medulla PDF terminals neighbour these glial cell (Helfrich-Förster 1995) which in turn show cyclical expression of the b subunit of the Na^+/K^+-ATPase (Pyza et al. 2003). PDF may synchronize the *per* expressing glial cells and indirectly regulate the activity of neurons in the visual system.

CONCLUSIONS

Neurons, glial cells and synaptic contacts between cells in the fly's visual system show cyclical remodeling of structure controlled by the circadian system. The circadian system is composed of clock genes expressing neurons and glial cells, called pacemaker or clock cells, located in the brain. In *M. domestica* the circadian system and the molecular mechanisms of the

clock are not the same like in the fruit fly (Codd et al. 2007), however, the small and large PDF neurons seem to correspond to the LNvs of *D. melanogaster*. In the housefly the medulla PDF neurons appear early in development (Pyza et al. 2003), show circadian structural changes (Pyza and Meinertzhagen 1997b) and cyclically accumulate and release PDF (Miskiewicz et al. 2008). The large LNvs of *D. melanogaster* develop later (Helfrich-Förster 1997) and their cyclic expression of per dumps immediately when flies are transferred to DD (Grima et al. 2004, Lin et al. 2004). However, the similar structural rhythms in the visual system observed in *D. melanogaster* and the similar PDF arborization in the medulla indicate that also in *D. melanogaster* the PDF containing large LNvs may drive circadian rhythms in the fruit fly visual system. It has been reported that the large PDF neurons of *Drosophila* are regulated by the clock but they are also critical for the clock function (Park and Griffith 2006).

The structural circadian plasticity in the visual system is correlated with the circadian rhythm of rest and activity and is affected by sensory stimuli and a feedback from the motor centers. The circadian inputs enable the visual system cells to predict changes in light intensity during the day and night and reorganize their structure, synaptic contacts and metabolism. The effective adaptation to different light conditions includes not only changes in physiological processes in neurons and glial cells but also in their structure.

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