Neural Organization of the Circadian System of the Cockroach *Leucophaea maderae*

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**ABSTRACT**

The cockroach *Leucophaea maderae* was the first animal in which lesion experiments localized an endogenous circadian clock to a particular brain area, the optic lobe. The neural organization of the circadian system, however, including entrainment pathways, coupling elements of the bilaterally distributed internal clock, and output pathways controlling circadian locomotor rhythms are only recently beginning to be elucidated. As in flies and other insect species, pigment-dispersing hormone (PDH)-immunoreactive neurons of the accessory medulla of the cockroach are crucial elements of the circadian system. Lesions and transplantation experiments showed that the endogenous circadian clock of the brain resides in neurons associated with the accessory medulla. The accessory medulla is organized into a nodular core receiving photic input, and into internodular and peripheral neuropil involved in efferent output and coupling input. Photic entrainment of the clock through compound eye photoreceptors appears to occur via parallel, indirect pathways through the medulla. Light-like phase shifts in circadian locomotor activity after injections of γ-aminobutyric acid (GABA) or Masallatropin into the vicinity of the accessory medulla suggest that both substances are involved in photic entrainment. Extraocular, cryptochrome-based photoreceptors appear to be present in the optic lobe, but their role in photic entrainment has not been elucidated.
been examined. Pigment-dispersing hormone-immunoreactive neurons provide efferent output from the accessory medulla to several brain areas and to the peripheral visual system. Pigment-dispersing hormone-immunoreactive neurons, and additional heterolateral neurons are, furthermore, involved in bilateral coupling of the two pacemakers. The neuronal organization, as well as the prominent involvement of GABA and neuropeptides, shows striking similarities to the organization of the suprachiasmatic nucleus, the circadian clock of the mammalian brain.

Key Words: Insect brain; Circadian clock; Pigment-dispersing hormone; Light entrainment; Accessory medulla; Cockroach; Leucophaea maderae.

INTRODUCTION

Internal circadian clocks are universal adaptations of living organisms to the 24h periodicity of the environment. Their molecular organization and localization, the analysis of clock-controlled effectors, and mechanisms for entrainment of these clocks have been the focus of intensive research in organisms of all main phyla. Work in insects has focused heavily on the fruit fly Drosophila melanogaster, owing largely to the multitude of available molecular genetic tools, and has greatly advanced our understanding of the molecular machinery underlying the circadian system (Allada et al., 2001; Stanewsky, 2002; Young and Kay, 2001). These studies also revealed: (i) in addition to a "master clock" in the brain for the control of behavioral output, additional clocks exist in the nervous system and in peripheral organs (Giebultowicz, 1999); (ii) parallel mechanisms and pathways operate for light entrainment of the clock (Helfrich-Förster et al., 2001); and (iii) distinct neural and molecular pathways are required for the control of specific outputs of the clock (Helfrich-Förster, 1998; McNeil et al., 1998; Zhang et al., 2000). Comparative studies, however, showed that other insects may considerably differ from D. melanogaster with respect to clock localization, entrainment mechanisms, and molecular basis of the clock (Helfrich-Förster et al., 1998). For a general understanding of the circadian system in insects, studies on a variety of insect species are, therefore, essential. Insect species with a larger brain size than that of D. melanogaster, such as the cricket Gryllus bimaculatus, the cockroach Leucophaea maderae, or the moth Antheraea polyphemus, furthermore, offer the advantage of a cellular and physiological approach to the circadian system.

Research on the circadian system of the cockroach L. maderae is favored by the prominent circadian rhythm of locomotor activity of this insect. Owing to its large size and robustness, L. maderae was the first animal in which lesion experiments have localized the circadian clock to a particular brain region (Nishiitsutsuji-Uwo and Pittendrigh, 1968b). More recently, the organization of the putative circadian timekeeping center in the brain, the accessory medulla, has been analyzed at the cellular level. The present paper reviews the neuronal organization of the circadian system of L. maderae with special emphasis on its internal organization, distribution of putative neurotransmitters and neuropeptides operating in the circadian system, light entrainment pathways, clock outputs, and bilateral coupling mechanisms.
LOCALIZING THE INTERNAL CLOCK THROUGH LESIONS AND TRANSPLANTATIONS

Cockroaches show robust circadian rhythms in wheel running activity (Roberts, 1960) and in electroretinogram recordings (Wills et al., 1985). Both assays have been used to monitor circadian clock function after various lesions to the cockroach brain. Transections of the optic lobe at various levels showed that the circadian pacemaker controlling locomotor activity (Nishiitsutsuji-Uwo and Pittendrigh, 1968b; Roberts, 1974), neural activity in the ventral nerve cord (Colwell and Page, 1990), and electroretinogram rhythms (Wills et al., 1985) resides in the optic lobe, and is associated with the medulla/lobula but not with the lamina. A sustained electroretinogram rhythm could be recorded from the isolated optic lobe for several days (Colwell and Page, 1990), and transplantation of the optic lobe into an animal, whose own optic lobes had been removed, restored circadian rhythmicity of locomotor activity after several weeks (Page, 1982). These experiments showed that the optic lobe contains a self-sustained circadian clock controlling locomotor behavior and visual sensitivity.

Microlesions using tungsten microelectrodes further specified the site of the clock (Page, 1978; Sokolove, 1975) and showed that lesions in or near the lobula, particularly in the ventral cell body cortex between the medulla and lobula, produced a high percentage of arrhythmic cockroaches. Immunocytochemical studies revealed that this area contains the cell bodies of neurons with arborizations in the accessory medulla, a small neuropil at the anterior ventromedian edge of the medulla (Homberg et al., 1991; Petri et al., 1995). Distinct and partly overlapping subpopulations of these neurons are immunoreactive with antisera against serotonin, γ-aminobutyric acid (GABA), and a variety of neuropeptides, including Mas-allatotropin, Dip-allatostatin, FMRFamide, pigment-dispersing hormone (PDH), and leucokinin (Petri et al., 1995; 2002). These neurons send processes into the accessory medulla, and most of them have additional projections into other areas of the brain. These studies suggest that neuropeptides play a key role in the circadian system of the cockroach and draw attention to the accessory medulla as the possible synaptic integration center of the clock.

Immunostaining with an antiserum against the crustacean peptide PDH (Rao, 2001) is particularly selective for neurons of the accessory medulla, and throughout the brain of the cockroach, only three groups of neurons are immunoreactive. Peptides closely related to crustacean PDH, termed pigment-dispersing factors (PDFs), have been identified in several insect species, including the cockroach Periplaneta americana (Rao, 2001). Two groups of PDH-immunoreactive neurons with cell bodies at the posterior dorsal and posterior ventral edge of the lamina were termed PDFLa cells and, a third group of 16 neurons near the accessory medulla, PDFMe neurons [Homberg et al., 1991; Reischig and Stengl, 1996; Fig. 1(A)]. The PDFLa neurons have arborizations throughout the lamina and probably contribute processes to a fan of immunostained fibers along the distal face of the medulla [Fig. 1(A)]. Their possible involvement in the circadian system is unknown. The role of the accessory medulla and the contribution of PDFMe neurons to the circadian system was addressed in lesion and transplantation experiments (Reischig and Stengl, 2002; Stengl and Homberg, 1994). Bilateral transections of the optic stalk in the cockroach abolished circadian wheel-running activity. In many individuals, however, the circadian rhythm reappears after several weeks corresponding with mass regeneration of fibers from the optic lobe to the median protocerebrum (Page, 1983a).
Immunocytochemistry using PDH antiserum shows a strong correlation between ingrowth and reinnervation of original targets in the lateral and superior protocerebrum by fibers from PDFMe neurons in animals that regained circadian activity after lesions (Fig. 2; Stengl and Homberg, 1994). This suggests that PDF acts as an output signal of the clock to particular brain areas involved in motor control. Finally, ectopic transplantations of

**Figure 1.** Arborizations of PDH-immunoreactive neurons in the brain of *L. maderae* (A) and anatomical organization of the accessory medulla (B). (A) Camera lucida reconstruction of PDH-immunoreactive neurons embedded in a computer-aided three-dimensional reconstruction of the cockroach brain (modified after Reischig and Stengl, 2002). PDFMe neurons have cell bodies near the accessory medulla (AMe). The neurons have dense ramifications in the AMe and send processes through the distalmost layer of the medulla (Me) to the lamina (La) and a second set of axonal fibers to various areas in the median protocerebrum. Immunoreactive fibers in the anterior and posterior optic commissures (AOC, POC) provide heterolateral connections between the two AMeas. Two additional groups of PDH-immunoreactive neurons with somata near the posterior dorsal (dPDFLa) and posterior ventral edge of the medulla contribute to immunostained arborizations in the lamina and first optic chiasma. (B) Computer-aided three-dimensional reconstruction corresponding to the boxed area in (A). Somata of the AMe-neurons are organized in six groups: median neurons (MNe), ventro-median neurons (VMNe), medial frontoventral neurons (MFVNe), ventral neurons (VNe), distal frontoventral neurons (DFVNe), and ventroposterior neurons (VPNe). The core of the AMe, composed of dense nodular neuropil (NN) and coarse internodular neuropil (IN) is surrounded by a shell of coarse neuropil (SN). Two of the six cell groups (VNe, VMNe) are shown separately in the right panel. Lo, lobula. Scale bars: 200 μm (A), 20 μm (B).
Accessory medullae and adjacent cell bodies into animals whose own optic lobes had been removed restored circadian activity (Reischig and Stengl, 2003). In these experiments, a small piece of tissue (about 200 μm diameter) including the accessory medulla and its neurons was transplanted from a donor cockroach to the optic or antennal lobe of a host cockroach whose accessory medullae or optic lobes had been removed. In nearly 30% of

Figure 2. Reappearance of circadian wheel-running activity after optic stalk severance correlates with mass regeneration of PDH-immunoreactive fibers from the AMe to the midbrain (modified after Stengl and Homberg, 1994). (A) Activity record of a cockroach kept in DD, which received transection of the right optic stalk and crushing of the left optic stalk at day 22 (arrowhead). After the operation, activity is reduced and the animal becomes arrhythmic. Five days after reappearance of rhythmic activity at day 40 the cockroach was sacrificed and the brain was examined for PDH immunostaining. (B) Pigment-dispersing hormone immunostaining in the brain of the cockroach in (A) at day 44. Mass regeneration of PDH-immunoreactive fibers has occurred predominantly from the right optic lobe to the median protocerebrum. Many fibers have reinvaded their original targets in the superior median, superior lateral, inferior lateral, and ventro-lateral protocerebrum (SMP, SLP, ILP, VLP) or have entered the posterior optic commissure (POC). Scale bar: 200 μm.
these animals, a circadian rhythm of locomotor activity reappeared and in all animals that regained rhythmicity, transplanted PDFMe neurons had regenerated fibers to their original targets in the protocerebrum (Reischig and Stengl, 2003). These data further confirm that the accessory medulla is the site of the circadian pacemaker controlling locomotor activity rhythms in the cockroach and suggest that neural connections by PDH-immunoreactive fibers from the accessory medulla to their original targets in the brain may be essential for clock control of locomotor activity.

INTERNAL ORGANIZATION AND CHEMOARCHITECTURE OF THE ACCESSORY MEDULLA

The accessory medulla has a pear-shaped form with a maximum longitudinal size of about 90 μm (Reischig and Stengl, 1996). It is composed of a core consisting of dense knots of neuropil termed noduli. The nodular core is embedded in coarse internodular neuropil and is surrounded by a shell of coarse neuropil, which is anteriorly continuous with the adjacent neuropil of the medulla [Fig. 1(B)]. Somata arranged in six groups in the cell cortex antero-ventrally to the accessory medulla contribute processes to the accessory-medulla neuropil [Fig. 1(B)]. Eight of the 16 PDFMe neurons have somata in the VNe group, four small somata are in the DFVNe group (see Fig. 1 for nomenclature), and the four remaining somata are in the cell cortex posterior to the medulla/lobula neuropil not shown in Fig. 1. The other cell groups contain accessory-medulla neurons immunostained with antisera against other neuropeptides and transmitter candidates, e.g., GABA-immunostained neurons in group MNe and Mas-allatotropin-immunostained neurons concentrated in the DFVNe and MFVNe cell groups (Petri et al., 1995; see Fig. 1 for nomenclature). The three compartments of the accessory medulla, noduli, internodular neuropil, and peripheral shell, are differentially supplied by immunocytochemically characterized populations of AMe neurons. Neurons immunostained with antisera against GABA, Mas-allatotropin, and leucokinin have processes concentrated in the nodular core of the accessory medulla (Petri et al., 1995; 2002). These neurons are either local interneurons of the accessory medulla (Mas-allatotropin) or connect the distalmost layer of the medulla (leucokinin) or a median layer of the medulla (GABA) to the nodular core of the accessory medulla. In contrast, neurons immunostained with antisera against serotonin, PDH, FMRFamide, Dip-allatostatin, and corazonin have processes concentrated in the internodular neuropil and peripheral shell of the accessory medulla (Petri et al., 1995; 2002). These neurons connect the accessory medulla through a fan of fibers with the distalmost layer of the medulla and with the lamina (PDH, FMRFamide, serotonin) and have axonal projections to several areas in the median protocerebrum and to the contralateral optic lobe (PDH, FMRFamide). The concentration of neuropeptides in the accessory medulla correlates with an abundance of dense core vesicles in fiber profiles in the accessory medulla revealed by ultrastructural examination. Based on differences in size and electron density, at least four different types of dense core vesicles have been distinguished, which are differentially distributed in the nodular core, in the internodular and shell neuropil. Granular vesicles are almost completely confined to the nodular core, while subpopulations of profiles with PDH-immunoreactive large dense-core vesicles are concentrated in the internodular neuropil, and
PDH-immunoreactive medium size dense-core vesicles are most common in the shell neuropil of the accessory medulla (Reischig and Stengl, 1996).

PARALLEL PATHWAYS FOR LIGHT ENTRAINMENT

Light entrainment of the circadian clock of *L. maderae* occurs through photoreceptors in or near the compound eye. Bilateral transections of the optic nerves connecting the compound eyes to the optic lobes result in free-running circadian locomotor activity, while removal of the ocelli had no effect on light entrainment (Nishiitsutsuji-Uwo and Pittendrigh, 1968a; Roberts, 1965; 1974). These data indicate that ocellar photoreceptors are not essential for photic entrainment and provide no evidence for the involvement of other nonretinal photoreceptors. A series of bilateral lesion experiments, moreover, demonstrated that light entrainment of each of the two bilaterally distributed circadian pacemakers occurs via photoreceptors in the ipsi- and contralateral compound eye (Page, 1978; Page et al., 1977).

Recent studies from our laboratories showed that neural connections from photoreceptors to the accessory medulla involve intercalated interneurons and may be organized in several parallel pathways. Histamine immunostaining used to label all compound-eye photoreceptor cells showed that photoreceptors terminate in the lamina and in distal layers of the medulla, but not in the accessory medulla (Loesel and Homberg, 1999). Intracellular recordings showed that only certain cell types of the accessory medulla respond to photic stimuli (Loesel and Homberg, 2001). Excitatory responses to light were found in neurons with tangential dendritic processes in median layers of the medulla, projections to the nodular core of the accessory medulla, and fan-shaped, apparently axonal, processes to the lamina [Fig. 3(A); Loesel and Homberg, 2001]. These neurons are, therefore, likely candidates for photic input elements to the accessory medulla, but whether they receive direct input from compound eye photoreceptors remains to be demonstrated. In the same study light-sensitive commissural neurons were identified, which interconnect median layers of the medulla and nodular core of the accessory medulla of both hemispheres. These neurons are candidates for providing light entrainment from the contralateral eye [Fig. 3(C)]. Another line of evidence supporting the role of the nodular core of the accessory medulla as the recipient for light entrainment signals comes from phase shifting experiments. Injections of GABA or Mas-allatotropin into the medulla result in phase-dependent phase shifts of circadian locomotor activity (Petri et al., 2002). The phase response curves have striking resemblance to phase response curves obtained for 6h light pulses [Fig. 4(A)], and suggest that both substances are involved in light entrainment. At least one GABA-immunoreactive neuron is a member of the light-responsive tangential inputs from the medulla, many others connect the medulla via the distal tract, a fiber bundle along the distal face of the medulla (Reischig and Stengl, 1996), to the nodular core of the accessory medulla. Mas-allatotropin-immunostained local neurons of the nodular core likewise appear to be involved in the photic input pathway.

In addition to light signalling pathways from compound-eye photoreceptors via medulla interneurons to the accessory medulla, two additional pathways might contribute to light sensitivity in the accessory medulla. A class of apparently centrifugal neurons with ramifications in the median protocerebrum and axonal projections to the accessory medulla is strongly inhibited by light (Loesel and Homberg, 2001). One of the recorded
neurons was an ocellar interneuron. A prominent role of this pathway in photic entrainment is unlikely, given the evidence from lesion studies cited above, but a modulatory role in adjusting light sensitivity, as shown for an ocellar contribution in a cricket (Rence et al., 1988), or a role in entrainment under particular lighting schedules as shown for the geniculo-hypothalamic pathway in rats (Edelstein and Amir, 1999), appears possible.

Figure 3. Camera lucida reconstructions of single intracellularly recorded and stained neurons of the accessory medulla (AMe) (modified after Loesel and Homberg, 2001). (A) Candidate neuron involved in photic entrainment of the clock. The neuron has its cell body in cell group MNe (see Fig. 1). It has tangential arborizations in a median layer of the medulla (Me), innervates the nodular core of the AMe and projects via a fan of fibers to the lamina (La) and to small accessory neuropils of the lamina (arrowhead). The neuron was strongly excited by light during daytime. (B) Candidate output neuron of the AMe with cell body in cell group VNe. The neuron has sparse ramifications in the shell and internodular neuropil of the AMe and sends axonal processes to the La and to the median protocerebrum near the pedunculus (P) of the mushroom body. The neuron was unresponsive to light stimuli at daytime. (C) Candidate neuron involved in light entrainment via the contralateral eye. Its cell body lies in group VMNe (see Fig. 1). The neuron has bilateral arborizations in the Me and AMe. The neuron was strongly excited by light and showed pronounced polarization sensitivity. al, Ca, a-lobe, and calyces of the mushroom body; Lo, lobula. Scale bars: 100 µm in (A) and (B); 200 µm in (C).
Finally, ultrastructural studies and immunoreactivity for the blue light photopigment cryptochrome provided evidence for the presence of two extraocular photoreceptor organs in the optic lobe of the cockroach (Fleissner et al., 2001). The lamina organ is an elongated structure which runs parallel to the lamina in front of the first optic chiasm, and the second organ, termed lobula organ, lies near the accessory medulla in front of the second optic chiasm. So far, however, a possible involvement of these organs in photic entrainment of the clock has not been investigated at any level.

**OUTPUT PATHWAYS**

Reappearance of circadian wheel-running activity after bilateral optic stalk severance or transplantations of the accessory medulla correlates with regenerated PDH-immunoreactive fibers from PDFMe neurons to the median protocerebrum (Fig. 2; Reischig and Stengl, 2003; Stengl and Homberg, 1994). Therefore, PDFMe neurons might provide the connection between the pacemaker and motor control areas in the protocerebrum. While in animals with optic stalk severance, equal reinnervation of all original targets in the protocerebrum was observed (Stengl and Homberg, 1994), regeneration in animals with transplanted accessory medulla occurred predominantly to the superior median and lateral protocerebrum, suggesting that these areas might be more important for clock functions associated with circadian behavior.
control of locomotion than others (Reischig and Stengl, 2003). PDFMe neurons also have
fan-like processes through distalmost layer 1 of the medulla toward the lamina [Fig.
1(A)], suggesting that they are also involved in circadian control of visual sensitivity at
the level of the lamina. In single-cell recordings, neurons of the accessory medulla that
were unresponsive to light stimuli during the day were regarded as candidate output
neurons of the clock, since light does not cause phase shifts during the day. Like PDFMe
neurons, these neurons had ramifications confined to the internodular and peripheral shell
neuropil of the accessory medulla. In all cases, their axonal fibers had two targets, fan-like
distal projections to the lamina or first optic chiasm, and proximal projections to certain
areas in the median protocerebrum [Loesel and Homberg, 2001; Fig. 3(B)].

COUPLING OF THE BILATERALLY SYMMETRIC CLOCKS

The two pacemakers in the right and left optic lobe of the cockroach are coupled
through neural pathways (Page, 1978; 1983b; Page et al., 1977). Lesions of one of the two
pacemakers invariably leads to an increase in the free-running period of the rhythm,
indicating that coupling of both pacemakers accelerates the system, possibly through
mutual inhibition (Page, 1983b). Several lines of evidence suggest that PDF may be
involved in the coupling pathway. Dextran tracing studies and double-labeling of
commissural neurons and PDH immunostaining showed that three of the 16 PDFMe
neurons are commissural neurons that interconnect the two accessory medullae (Petri,
1998; Reischig and Stengl, 2002). The neurons project via the anterior and posterior optic
commissures, innervate the internodular and shell neuropil of the accessory medulla, and
send a fan of fibers along the distal surface of the medulla into the lamina [Fig. 5(B)].
Neurons of this morphological type were intracellularly stained [Fig. 5(A)] and were found
to be unresponsive to light stimuli during the day (Loesel and Homberg, 2001). Injections
of PDF into the medulla caused phase shifts in circadian wheel-running activity which
provides additional support for a role of PDF as a coupling signal in the circadian system
(Petri and Stengl, 1997). The resulting phase-response curve is monophasic with phase
delays during the late subjective day and, therefore, differs considerably from the phase-
response curve obtained with light pulses (Fig. 4). Pigment-dispersing factor is, therefore,
not part of the light entrainment pathway but participates in non-photic input to the clock.
Taken together with the immunocytochemical data, PDH-immunoreactive commissural
neurons are therefore, strong candidates for the bilateral coupling pathway of the two optic
lobe pacemakers. Evidence supporting a dual role of PDH-immunostained neurons as
output and coupling pathways is provided by ultrastructural studies, which revealed input
as well as output synapses from PDH-immunoreactive neurons in the accessory medulla
(Reischig and Stengl, 2003). Computer simulations, finally, demonstrated that in addition
to all-delay-type interactions as found with PDF injections, all-advance-type interactions
between the two pacemakers are required to explain all experimental findings on mutual
pacemaker coupling in *L. maderae* (Petri and Stengl, 2001). It is, therefore, likely, that
additional non-PDH-immunostained commissural neurons participate in bilateral coupling.
Candidates are commissural FMRFamide-immunostained connections between the two
accessory medullae, but the effects of FMRFamide-related peptides on locomotor activity
have not been tested so far.
COMPARISON BETWEEN FLIES, COCKROACH, AND MAMMALS

The circadian system of *L. maderae*, particularly with respect to the involvement of PDF-containing neurons shows striking similarity to the well-studied circadian system of the fruitfly *D. melanogaster*. In *D. melanogaster*, the so-called “lateral neurons” (LNs) near the medulla are circadian pacemaker cells (reviewed by Helfrich-Förster et al., 1998; Kaneko, 1998; Stanewsky, 2002). The neurons express the clock genes *period*, *timeless*, and *doubletime* and the presence of these neurons as well as their cyclical expression of *period* is essential for behavioral circadian rhythmicity (Helfrich-Förster, 1998; Kaneko et al., 1997). A subset of the LNs contain PDF and mutations of the *pdf*-gene or ectopic expression of *pdf* leads to severe deficits in circadian rhythms...
As in the cockroach, distinct subpopulations of pdf-expressing neurons with different branching patterns can be distinguished in the fly, consistent with a double role of the peptide as an output and bilateral coupling signal of the clock. Differences between D. melanogaster and L. maderae appear to exist with respect to light entrainment pathways; in the fly the pdf-expressing pacemaker cells possess their own extraocular photopigment, cryptochrome, which is apparently not present in the PDFMe neurons of the cockroach (Emery et al., 2000; Fleissner et al., 2001). Furthermore, a direct synaptic input from an extraocular photoreceptor organ, termed the Hofbauer-Buchner eyelet in the fly (Helfrich-Förster et al., 2001), has also not been found in the cockroach. While in D. melanogaster strong evidence suggests that the pdf-expressing cells are pacemaker cells, the identity of clock cells in the circadian system of L. maderae has not been determined. Current experiments aimed at identifying the clock molecules PER and TIM in the cockroach brain might help to solve this question.

While a distinct accessory medulla is hardly visible in D. melanogaster owing to fusion with the adjacent medulla, the anatomical and neurochemical organization of the accessory medulla of the cockroach bears considerable similarities to the organization of the mammalian circadian pacemaker, the suprachiasmatic nucleus (reviewed by Moore et al., 2002; Reghunandanan et al., 1993; van Esseveldt et al., 2000). Both areas are densely packed with neuropeptides and have a clear internal substructure into a core receiving retinal input, and a shell (internodular and shell neuropil in the cockroach) receiving nonphotic input. In mammals outputs to effector systems exit the core as well as the shell, while in the cockroach, outputs appear to exit primarily from the internodular and peripheral neuropil. These similarities may be accidental, but more likely represent similarly optimized solutions for the neural organizations of brain circadian pacemakers.

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