Pigment-dispersing factor signaling and circadian rhythms in insect locomotor activity

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Though expressed in relatively few neurons in insect nervous systems, pigment-dispersing factor (PDF) plays many roles in the control of behavior and physiology. PDF's role in circadian timekeeping is its best-understood function and the focus of this review. Here we recount the isolation and characterization of insect PDFs, review the evidence that PDF acts as a circadian clock output factor, and discuss emerging models of how PDF functions within circadian clock neuron network of Drosophila, the species in which this peptide's circadian roles are best understood.

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Introduction

Pigment-dispersing factors (PDFs) are highly conserved 18-amino acid, α-amidated neuropeptides [1–3]. Though expressed in relatively few neurons in insect nervous systems [4–12], PDF plays many roles in the control of behavior and physiology, including circadian rhythmicity [13••], geotaxis [14], sleep and arousal [15–18], copulation [19], flight [20], the modulation of visceral muscle contraction [21] and tracheal growth [22]. PDF's role in circadian timekeeping is its best-understood function and the focus of this review.

Isolation and identification of PDFs as clock output components

PDFs were first identified based on their similarity to crustacean pigment dispersing hormones (PDHs), which regulate the dispersion and migration of pigment in chromophores and photoreceptors, including daily rhythms of the latter [1,23]. Insect PDFs were first isolated based on their ability to induce pigment dispersion in crustaceans when applied exogenously and have very high sequence similarity to crustacean β-PDHs (Figure 1a) [23]. Insect brains contain small numbers of neurons, approximately six to 20, that are immunoreactive to antisera raised against crustacean PDH (PDHir neurons) [4–12]. Though the numbers and positions of PDHir neurons vary among the insect orders, most species studied contain anterior-ventrally located somata near the accessory medulla, an accessory visual neuropil [4–12]. These neurons typically extend neural processes within the accessory medulla, the optic lobes and the dorsal protocerebrum, as schematized for Drosophila melanogaster in Figure 1b [4–12]. Some species display additional PDHir somata and projections in other regions of the central nervous system, most notably in the tritocerebrum, suboesophageal ganglia, and the corpora cardiaca (e.g. [9,24]).

PDHir neurons with somata situated near the accessory medulla were implicated as circadian pacemakers in orthopteroid insects based on anatomical criteria, physiological observations, and ablation and transplant experiments [25]. The discovery that the PDHir neurons of the Drosophila melanogaster brain express the circadian clock gene period (per) [26] and that these neurons are missing in visual system mutants with weak or absent circadian rhythms further supported the hypothesis that PDHir neurons function as circadian pacemakers in insects [4,27].

The cloning of Pdf from Drosophila melanogaster [2] and subsequently from other insects (e.g. [3,28,29]), made it possible to address the role that this peptide plays in the control of circadian locomotor rhythms using molecular/genetic approaches. Flies bearing the loss-of-function Pdf^{01} mutation display a syndrome of circadian phenotypes. These mutants are characterized by the loss of the anticipatory morning peak of activity and an advanced evening peak of activity under light/dark (LD) conditions [13••]. Pdf^{01} mutants also display significantly higher levels of arrhythmicity under constant darkness and temperature (DD), indicating that the ability to produce endogenous circadian rhythms is compromised in the absence of PDF [13••]. Pdf^{01} mutants that do display rhythmic locomotion under DD have relatively weak rhythms with significantly shorter periods [13••]. The loss of PDF in the fly is also accompanied by an inability to delay the evening peak of activity during long days [30] and the absence of increased nighttime activity in response to nocturnal light [31].

RNA-interference mediated knockdown of Pdf in the cockroach Blattella germanica resulted in significant

Figure 1
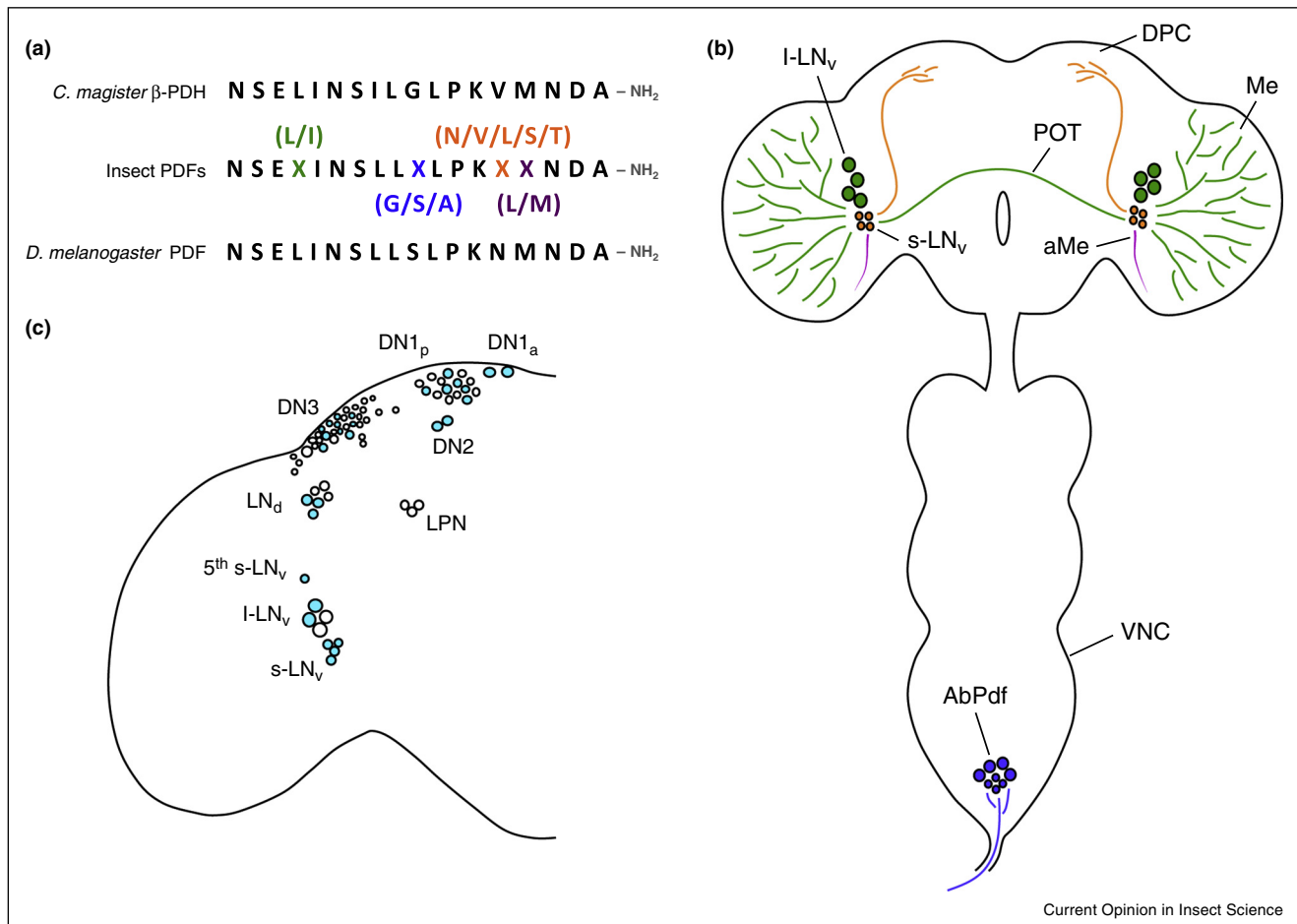
(a) The highly conserved sequence of insect PDFs. Insect PDFs have highly similar sequences to crustacean β-PDH hormones. Crustacean nervous systems also contain α-PDH, to which insect PDFs display no homology. Top: the sequence of β-PDH from *Cancer magister*. Middle: a generalized amino acid sequence for insect PDFs. Variable amino acids are indicated by a colored X with variants shown above or below the variable amino acid. Bottom: the sequence of PDF from *Drosophila melanogaster*. **(b)** A schematic of PDF neuron anatomy in the *Drosophila* central nervous system. The three classes of PDF neurons, the large ventral lateral neurons (l-LN$_v$s), the small ventral lateral neurons (s-LN$_v$s) and the abdominal PDF neurons (AbPdf) are indicated. The l-LN$_v$s innervate the medullae (Me) of the optic lobes and project across the posterior optic tract (POT). The s-LN$_v$s project to the dorsal protocerebrum (DPC). Both the l-LN$_v$s and s-LN$_v$s project to the accessory medulla (aMe), shown in magenta. The AbPdf neurons reside in the abdominal ganglia of the ventral nerve cord (VNC), project to the viscera and are a likely source of circulating PDF. Note, PDF is also expressed within neurons of the tritocerebrum of adult *Drosophila* (not shown), though they undergo programmed cell death in the days following adult eclosion [13••]. **(c)** A cell body map of clock neurons in the *Drosophila* brain. The various neuron classes are indicated. PdfR expression is indicated in cyan. PdfR expression in the lateral posterior neurons (LPNs) was not determined.

increases in arrhythmicity in both LD and DD conditions, but had no obvious effects on the period of locomotor rhythms [29]. In contrast, *Pdf* knockdown in the cricket *Gryllus bimaculatus* resulted in a shortening of the free-running period but did not result in increases in arrhythmicity under DD conditions [32]. *Pdf* knockdown also reduced levels of nighttime activity in the cricket, and caused a more rapid resynchronization to shifted LD cycles [32]. Injection of PDH or PDF into the brains of free-running cockroaches and crickets produces dose-dependent phase changes in both insects [33,34]. Thus, PDF is required for normal circadian locomotor rhythms in several insects and likely acts to adjust the period or

phase of circadian rhythms. However its specific roles may differ among species.

Mechanisms of PDF function in the clock neuron network of *Drosophila*

The circadian functions of PDF are best understood for *D. melanogaster*, whose clock neuron network can be manipulated with a precision unavailable in other species. This network consists of approximately 150 neurons, which support daily rhythms in clock gene expression and can be divided into nine distinct anatomical classes (Figure 1c) [35–37]. Two classes of clock neurons express PDF, which are readily divisible by anatomical criteria.