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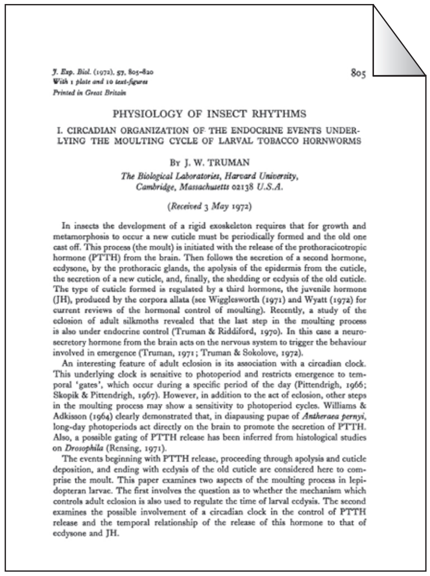
JEB Classics

CIRCADIAN WINDOW OF OPPORTUNITY: WHAT HAVE WE LEARNED FROM INSECTS?



J. Exp. Biol. (1972), 57, 805–820
With 1 plate and 10 text-figures
Printed in Great Britain

805

PHYSIOLOGY OF INSECT RHYTHMS

I. CIRCADIAN ORGANIZATION OF THE ENDOCRINE EVENTS UNDERLYING THE MOULTING CYCLE OF LARVAL TOBACCO HORNWORMS

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(*Received* 3 *May* 1972)

In insects the development of a rigid exoskeleton requires that for growth and metamorphosis to occur a new cuticle must be periodically formed and the old one cast off. This process (the moult) is initiated with the release of the prothoracicotropic hormone (PTTH) from the brain. Then follows the secretion of a second hormone, ecdysone, by the prothoracic glands, the apolysis of the epidermis from the cuticle, the secretion of a new cuticle, and, finally, the shedding or ecdysis of the old cuticle. The type of cuticle formed is regulated by a third hormone, the juvenile hormone (JH), produced by the corpora allata (see Wigglesworth (1971) and Wyatt (1972) for current reviews of the hormonal control of moulting). Recently, a study of the eclosion of adult silkmoths revealed that the last step in the moulting process is also under endocrine control (Truman & Riddiford, 1970). In this case a neurosecretory hormone from the brain acts on the nervous system to trigger the behaviour involved in emergence (Truman, 1971; Truman & Sokolove, 1972).

An interesting feature of adult eclosion is its association with a circadian clock. This underlying clock is sensitive to photoperiod and restricts emergence to temporal 'gates', which occur during a specific period of the day (Pittendrigh, 1966; Skopik & Pittendrigh, 1967). However, in addition to the act of eclosion, other steps in the moulting process may show a sensitivity to photoperiod cycles. Williams & Adkisson (1964) clearly demonstrated that, in diapausing pupae of *Antheraea pernyi*, long-day photoperiods act directly on the brain to promote the secretion of PTTH. Also, a possible gating of PTTH release has been inferred from histological studies on *Drosophila* (Rensing, 1971).

The events beginning with PTTH release, proceeding through apolysis and cuticle deposition, and ending with ecdysis of the old cuticle are considered here to comprise the moult. This paper examines two aspects of the moulting process in lepidopteran larvae. The first involves the question as to whether the mechanism which controls adult eclosion is also used to regulate the time of larval ecdysis. The second examines the possible involvement of a circadian clock in the control of PTTH release and the temporal relationship of the release of this hormone to that of ecdysone and JH.

Jadwiga Giebultowicz discusses James Truman's 1972 paper entitled: 'Physiology of Insect Rhythms: I. Circadian Organization of the Endocrine Events Underlying the Moulting Cycle of Larval Tobacco Hornworms'.

A copy of the paper can be obtained from http://jeb.biologists.org/cgi/content/abstract/57/3/805

Diurnal rhythms in plants and animals have been observed for centuries, but their endogenous and circadian nature were not acknowledged until the 1960s (Menaker, 1969). Research during that decade provided ample evidence for an internal 'clock' generating daily rhythms in behavior through biochemistry; however, the nature and anatomical location of this clock were a mystery. Insects helped to unveil this paradox, providing critical early insights into both the genetic and the physiological bases of the clock. The discovery of the first circadian clock gene *period* in *Drosophila melanogaster* was a milestone for chronogenetics (Konopka and Benzer, 1971). The overt behavior used to identify arrhythmic *period* mutants in flies was the rhythm of emergence of the adult from its pupal cuticle. This event, termed eclosion, is 'gated' by the circadian clock, which means that it can only occur in a restricted portion of a day/night cycle. This daily window of time is species specific; for example, *D. melanogaster* adults eclose in the early morning. Then, as now, *D. melanogaster* was an excellent organism to probe the genetic nature of the clock, but too tiny to explore the clock's physiological and anatomical nature with the resources available at the time. This quandary was approached by young James Truman working at Harvard with Lynn Riddiford. Using two species of silk moths which eclose at different times of day, they showed that eclosion gating is governed by the brain. By exchanging brains between the two species they 'transplanted' the timing of eclosion. These experiments provided compelling evidence that the moth brain contains a photoreceptor mechanism, a clock and a neuroendocrine output that are necessary and sufficient for the synchronization of eclosion behavior with the environmental photoperiod (Truman and Riddiford, 1970).

Once it was clear that ecdysis into the adult is controlled by the circadian clock, Truman asked whether the same holds true for larval ecdyses. He reported his findings in his 1972 paper in *The Journal of Experimental Biology* on the circadian organization of the endocrine events underlying the larval moulting cycle (Truman, 1972). From today's perspective, several features make this paper a classic, such as the use of the tobacco hornworm, *Manduca sexta*, which has subsequently become a favorite model for insect physiology research. Further, Truman showed in this study that larval ecdyses in *Manduca* occur at a specific time of day and then investigated how this rhythm may be controlled. The highlight of this paper was the determination that release of a major insect developmental neurohormone is gated by the circadian clock.

The insect moulting cycle starts with the release of the prothoracicotropic hormone (PTTH), which stimulates the production of ecdysone by the prothoracic glands. Ecdysone initiates secretion of a new cuticle and digestion of the old one, followed by the ecdysis behavior. In the JEB classic Truman showed that in contrast to adult eclosion, the synchrony of larval ecdysis does not arise from circadian gating of the ecdysis behavior itself. Simple but elegant experiments involving blood-tight ligations separating the brain from the rest of the body suggested that synchronous larval ecdysis is the result of gated PTTH release. These data highlight the fact that circadian clocks can control separate neuroendocrine targets to synchronize larval or adult ecdysis in the same species. Circadian rhythm-gated PTTH release initiates a linear sequence of events that are eventually manifested as rhythmic larval ecdysis, which occurs 1–2 days after PTTH release. In contrast, the moulting cycle that produces an adult moth takes a couple of weeks as new internal organs and the radically different adult cuticle are made during metamorphosis. An endocrine signal synchronizes the onset of ecdysis after these protracted processes have been completed so that ecdysis occurs in a narrow window of time. Truman and his co-workers later showed that the resynchronization of the eclosion behavior involves rhythmic release of the neuropeptide termed the eclosion hormone. Several laboratories built on Truman's foundational studies of neurohormonal regulation of ecdysis, and many more neuropeptides have been identified that play a role in synchronizing behavioral and physiological steps in the insect ecdysis cycle (Zitnan et al., 2007).

The initial discovery by Truman that the release of PTTH is gated by the circadian clock has been confirmed even in distantly related insect species. The best understood example involves the kissing bug, *Rhodnius prolixus* (a favorite 'model organism' of one of the JEB's first Editors, Sir V. B. Wigglesworth). In *Rhodnius*, PTTH is released daily in a gated fashion, and this rhythm is controlled by paired photosensitive clocks in the brain. Rhythmic PTTH release generates rhythmic synthesis and release of ecdysone from cells, which also possess clock properties (Steel and Vafopoulou, 2006). Rhythmic changes in ecdysone levels were recently demonstrated in the testis of adult moth (Polanska et al., 2009) leaving one to wonder about the significance of the parallel occurrence of steroidogenesis in testicular tissues in both insects and vertebrates.

Another surprising rhythm was detected in the levels of the juvenile hormone (Zhao and Zera, 2004). This hormone promotes the juvenile status of larva, and also acts as a gonadotropin in adult females. Juvenile hormone exhibits a large-amplitude daily cycle in the hemolymph of the cricket (Zhao and Zera, 2004). Truman is no doubt pleased to read about these recent developments. It is likely that clock-controlled hormonal rhythms are widespread in insects; however, additional supporting evidence may be slow to come as sample collection requires the sacrifice of a night's sleep!

In 1972, I was an undergraduate studying Insect Physiology at the University of Warsaw in Poland and learning English by reading Truman's papers, because I was fascinated by his discoveries and wanted to understand the figure captions. I was lucky to join his lab several years later and to observe first-hand his recipe for scientific pursuit: a mix of curiosity with a drive to address big biological questions using those miniature marvels of nature, insects. Over the years, the fertile research environment created by Jim Truman has produced a large cohort of scientists who continue to use their mentor's recipe to cook up a smorgasbord of scientific discoveries.

The concept of circadian gating that Truman helped to develop in his JEB classic and other papers, provided a useful framework which helped to move chronobiology from the black box era to the functional genomics era. We now know that rhythmic expression of genes can provide a switch or rate-limiting step that restricts a given process to a specific window of time. For example, mitosis in many mammalian tissues is associated with a specific time of day, and it has been demonstrated that the circadian clock controls the expression of cell cycle-related genes that in turn modulate the expression of a key regulator of mitosis (Matsuo et al., 2003). Thus, the specific window of time during which cells are permitted to enter mitosis is defined by the expression of genes that are necessary to initiate this process. Reflecting on the development of chronobiology since 1972, one can appreciate the immense progress that the field has made in defining the molecular basis of the clock and its outputs. Nevertheless, many important questions remain and, along with Jim Truman, I strongly believe that insects, those tiny scientific workhorses, will continue to play a central role in addressing these questions.

10.1242/jeb.034199

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