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Hormonal rhythm and behavioural trends in insects

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Abstract. Circadian rhythmicity in the timing of secretion and release of many of the neurohormones appears to be a common phenomenon in insects. Involvement of hormonal components in the locomotor activity rhythm in cockroaches, crickets etc. has not yet been proved unequivocally eventhough some of the findings along these lines support this. Many of the physiological events in insects occur only once in each individual's life-time-gated events. Release of eclosion hormone in insects is determined both by a circadian clock and by the developmental competence of the insect. Periodic release of PTTH which influence the moulting process in larvae has been established to be gated. Induction of prodromal signs of pupation as a result of gated release of PTTH in some insects have been confirmed. Intrinsic neurosecretory cells of CC release a hormone (calling hormone) in a rhythmic fashion which affect the pheromone release and subsequent initiation of calling behaviour in some of the lepidopteran virgin females. Production of proctodone by the epithelial cells of hindgut also follows a rhythm bringing about diapause in some of the insects.

Keywords. Rhythm; endocrines; eclosion; hatching; moulting, bursicon; calling hormone; diapause.

1. Introduction

As in many other animals, insects are also known to possess internal clocks which can be synchronised and entrained by daily environmental periodicities. Since many of these clocks follow a periodicity of about 24 hr, these are designated as 'circadian clocks' and diverse types of behaviour in insects have been implicated to follow this cyclic pattern. The performance of behaviour is often directly coupled to the output of the clock with little or no influence from other stimuli. In many instances, the coupling between the driving circadian clock and the behaviour appears to be hormonal. Daily, seasonal and even annual phenomena are apparently linked to biological clock functions. Daily and seasonal responses are the results of the effects of photoperiodic stimulation on the clocks of the neural and endocrine cells that control the behavioural, physiological or developmental processes (Beck 1980).

Circadian rhythms have a major influence on insectan endocrine activity. Daily fluctuations of the titers of circulating hormones may reflect daily alterations in the activity of endocrine organs themselves or they may reflect daily alterations in specific or general metabolic pathways that serve to clear hormonal activity from the blood (Truman 1978). Monitoring the daily cycles of activity in the endocrine system of insects have attracted attention because of the probable importance of this system in the control of overt rhythms of physiology and behaviour.

2. Endocrine rhythms

2.1 *Rhythms in the brain neurosecretory cells*

The involvement of circadian rhythmicity in the determination of timing of secretion of insect neurohormones now appears to be a general phenomenon and a precise

knowledge of the nature of the clock that controls hormone release is a prerequisite for a thorough understanding of any developmental problems related to hormone action. This circadian clock of timing of release of insect neurohormones was demonstrated unequivocally for the eclosion hormone of silkmoths, a hormone which triggers their adult eclosion (Truman and Riddiford 1974; Truman 1971a, b).

The first report of a circadian cyclic pattern of activity in the neurosecretory cells was that by Klug (1958) in the brain of a beetle, *Carabus nemoralis*. Rensing (1964, 1966) used a sort of microspectrophotometer to compare the absorption of neurosecretory material in the region around the nucleus with that in the axon 'hump' of brain neurosecretory (NS) cells of *Drosophila melanogaster*. A maximum accumulation was noticed near the nucleus around 'dawn' and 'dusk' i.e. some 3 hr after their peak nuclear size. Larvae also showed similar but less marked changes.

Dutkowski *et al* (1971) made some ultra-structural investigations to demonstrate the circadian cycle of neurosecretory activity. Brain NS cells of *Acheta domesticus* recovered after sacrificing them 30 min after 'dawn' and 30 min after 'dusk' (i.e. at the time of minimum and maximum locomotor activity) showed marked differences between the two groups. The cells of the inactive animals contained extensive ER and secretory vesicles in the golgi region and in the axon only and nuclei with smooth membranes. On the other hand cells of the active ones housed only fragmented ER, apparently quiescent golgi, secretory vesicles in the perikariyon, but not in the axon and nuclei with undulating membranes. It was therefore concluded that the brain cells of the inactive animals were synthesising and releasing neurosecretion whereas the cells of the active animals had ceased (temporarily) to synthesise actively or to release the secretion and thus accumulated secretion in the perikaryon. The potential value of such EM studies is revealed from these investigations in elucidating the circadian secretory cycles.

We have noticed a distinct circadian rhythmic pattern of secretory activity in the PINSC (pars intercerebralis neurosecretory cells) *A* cells of the late instar larvae (4th and 5th) of castor semilooper, *Achoea janata*. During the early hours of the day, the activity was minimum and around mid-day at peak level and with a gradual drop towards evening and night. Topical application of an anti-allatotropin, Precocene-II, completely upsets this rhythm of neurosecretory activity and the level of secretory activity as well (Mohanakumar and Muraleedharan 1985).

Autoradiographic techniques have been used as another tool in the elucidation of cyclical metabolic activities of endocrine cells. Cymborowski and Dutkowski (1969) used this technique in *Acheta domesticus* to relate neurosecretory cell function to the control of locomotor activity. They showed that there is a sharp diel rhythm in RNA synthesis (as indicated by ^3H incorporation) in the median NS cells of the brain and the NS cells of the suboesophageal ganglion of crickets.

Fowler and Goodnight (1966) succeeded in cluturing isolated brains from an opilionid, *Leiobunum longipes* for 80 days in L:D at the end of which there was a distinct residual rhythm of 5-hydroxy tryptamine accumulation. Rensing (1969) also studied the 24 hr rhythmic pattern of secretory activity in the salivary glands by measuring the size of nuclei of the gland cells of *Drosophila melanogaster* which were cultured.

2.2 *Rhythms in the NS cells of ventral nerve cord*

In the ventral nerve cord of almost all insects studied, four major categories of NS cells

are to be noticed. However, the pathways and release sites of these NS cells-products have not yet been completely elucidated (Raabe 1983). Naturally, very few attempts have been made to study the activity rhythms of these cell types as well. A reasonably clear diel change in the nuclear size of NS cells of the SOG (suboesophageal ganglion) was demonstrated in *Drosophila melanogaster* (Rensing 1964, 1966; Rensing *et al* 1965). In the cockroach *Periplaneta americana* cyclic changes in the SOG NS cells were noticed (Cymborowski and Flisinka–Bojanowska 1970). A similar rhythm was also noticed in the 'C' cells of SOG of the stick insect, *Clitumnus extradentatus*. Harker (1960a, b, c) traced the rhythm inducing factor of the SOG as two pairs of NS cells located on the lateral aspects of SOG, one pair on each side. During the active period 'A' type neurosecretory cells in the ventral ganglia of *Leucophaea maderae* contained greater quantities of NS granules (De Besse 1965). In *Acheta domesticus* a bimodal rhythm in the SOG NS cells was noticed with a maximum in the midphotophase and midscotophase (Cymborowski and Dutkowski 1969). Protein synthesis in the NS cells were also found to be rhythmic. In the light of all the above findings they suggest that in response to photoperiod, the brain begins RNA synthesis at the onset of light followed by protein synthesis and subsequent elaboration of neurohormone which is being translocated to the SOG where it stimulates RNA synthesis and neurosecretion.

2.3 *Rhythms in corpus allatum and prothoracic glands*

A daily rhythm of nuclear and nucleolar size in the cells of corpus allatum (CA) and prothoracic glands (PTG) was noticed in the larvae of *Drosophila melanogaster* (Rensing 1964, 1966; Rensing *et al* 1965). Ecdysteroid titers in the different tissues like the ovaries, fatbody and haemolymph of the cricket, *Gryllus bimaculatus* were monitored by Hoffmann *et al* (1982) and it was noticed that ecdysteroid was present in small quantities in all young organs which increased markedly during ovarian maturation and decreased again during the last days of adult maturity. Two peaks of haemolymph ecdysteroids during larval-pupal development, one at the transition from the feeding stage to post-feeding prepupa and the other in association with pupal cuticle formation were demonstrated in *Manduca sexta* by Bollenbacher *et al* (1975) and the same observations were later confirmed in *Calpodes ethius* by Dean *et al* (1980). Later Fujishita and Ishizaki (1982) have demonstrated that in the larvae of *Samia cynthia ricini* haemolymph ecdysteroid titer begins to rise at 1800 hr of the day preceding the gut purge under LD 12:12 to reach a maximal level 4·5 hr before the purge.

Eventhough certain amount of work has already been done in investigating the activity rhythms of endocrines in some of the selected insect species, we are still unaware of the exact rhythmic activity patterns (whether it is daily modulation of hormone titers, gating of hormone secretion or photoperiodic control of hormone release) of most of these hormones especially juvenile hormone, ecdysone etc. in most of the insects. Even attempts made on neurohormones along these lines are mostly observations made on the basis of daily changes in the histological appearance of NS cell groups or endocrine organs. In certain cases rhythmic changes in the target tissues have provided evidence for rhythmic endocrine activity. Knowledge about the factors initiating release of JH, ecdysone, PTTH, JH esterase or proctodone and the time of their release are prerequisites for the proper interpretation of events controlling metamorphosis in insects. One of the methods which can be relied upon is by quantitatively

monitoring the titer of a particular hormone by using radioimmunological, mass-spectrometric and other techniques.

3. Hormones and circadian rhythms

Morphogenesis in insects are known to be controlled by different hormones. Hence it is not very surprising to see that the timing of larval-larval moulting, pupation, adult emergence etc. are also effected *via* hormones. The circadian control of gated—once in a lifetime—programmes and the more typical daily, repeated, ongoing behaviour of the type that is measurable in individual insects appears different. For gated rhythms, control is in the form of a 'single shot' hormonal release of each unique and largely fixed behaviour pattern. For ongoing behaviour continuous control must be exerted right round the clock.

3.1 *Locomotion*

Harker (1954) reported that rhythmic activity could be induced in arryhythmic *Periplaneta americana* by parabiosing them to rhythmic animals. Later Cymborowsky and Brady (1972) demonstrated in both crickets (*Acheta demesticus*) and cockroaches (*Periplaneta americana*) that headless animals take up the rhythm of the intact animal stuck on their backs, but significantly more rhythms are induced if the haemocoels are interconnected than if they are not. Thus some sort of influence affecting rhythmicity in locomotor activity apparently passed *via* the haemolymph from the intact donor to the headless recipient. Harker (1956) showed that the transplantation of SOG from the rhythmic donor induced rhythm in .a headless arrhythmic cockroach recipient. Subsequent experiments implied that if two pairs of lateral NS cells of SOG are destroyed, no rhythms were induced even when the ganglia were implanted. So it was inferred that these four lateral NS cells of SOG act as an autonomous hormonal clock (Harker 1960c, 1961, 1964). However subsequent work by many others failed to confirm the induction of activity rhythm in the cockroach itself by any sort of SOG transplant (Leuthold 1966; Roberts 1966; Brady 1967a). Observations made in the grasshopper, *Romalea microptera* and in the beetle, *Blaps mucronata* also failed to support the above finding by Harker (Fingerman *et al* 1958; Thomas and Finlayson 1970). The implicated NS cells could be successfully removed by microcautery without impairing the periodicity of activity from the cockroach and even the removal of a great bulk of cell bodies and neuropile from SOG ventral region—leaving little more than the thorough tracts—did not stop the rhythm (Brady 1967c; Nishiitsutsujii-Uwe and Pittendrigh 1968). So it becomes almost clear that SOG NS cells are in no way essential to the timing or control of cockroach locomotor activity.

Many of the experiments conducted by different workers to establish the role of CC-CA complex also demonstrated that CC (corpus cardiacum) may be involved in judging the amount of locomotor activity (Shepard and Keeley 1972; Michel 1972) but appear not to be involved with its periodicity (Roberts 1966; Brady 1967b; Weber and Gaude 1971; Brady 1971). Attempts were also made to demonstrate the effect of hormonal principles from the brain NS cells on the cockroach locomotor activity rhythms (Harker 1956; Brady 1967b; Nishiitsutsujii-Uwo *et al* 1967) and the results showed no influence

as such on the timing of activity rather than destroying the behavioural integration.

If the compound eyes are severed from the optic lobes, the activity in *Leucophaea maderae* remained normally rhythmic but became uncoupled from the environment. In effect the animals became blind and their rhythm freeran unentrained by light/dark cycle in which they were kept. On the other hand, if the optic tracts were cut between the optic lobes and the protocerebrum, the animals became arrhythmic even in LD (Nishiitsutsujii-Uwo and Pittendrigh 1968). Later this finding was confirmed by Roberts (1971) by removing the optic lobes along with the compound eyes which resulted in arrhythmicity. However, as Brady (1974) suggests a possibility still exists for the involvement of hormonal components in the cockroach locomotor activity rhythms since it has already been demonstrated in the NS cells in the optic lobes (Beattie 1971) and also their axons in the circumoesophageal connectives of locusts (Michel 1972).

Experimental work on crickets (*Acheta domesticus*) by Cymborowski (1970) demonstrated hyperactivity and superficial arrhythmicity due to pars intercerebralis ablation eventhough autopsy of operated animals showed equivocal stainable material in the pars intercerebralis region. So it appears premature to conclude that brain NS cells ablation in crickets necessarily disrupts its rhythm at least in LD. In some of the noctuid moths, ablation of median NS cells has a different effect; the normal night disappears, but is replaced by a burst of activity for an hour or two after dawn.

3.2 *Gated events*

There is a whole class of physiological events that occur once only in each individual's life time, but which are nevertheless timed by a circadian rhythm. This sort of phenomenon cannot be detected as a rhythm in an individual; it becomes apparent only in mixed-age populations. Here the individual completes the morphogenetic aspects of its development at random with respect to time of the day for its emergence. Thus although individuals become ready to emerge at all times, they only do so through a narrow span of time each day, when a so-called circadian gate is open.

3.2a *Eclosion*: The concept of gating events by a circadian clock grew out of the studies of the rhythm of adult eclosion in *Drosophila* by Pittendrigh and Skopik (1970). In these flies eclosion is restricted to a specific temporal gate, the time of which is determined by an interaction between the photoperiod and the fly's circadian clock. Experimental evidence for a triggering effect of hormone on adult eclosion in the moth *Manduca sexta* was given in detail (Truman 1970, 1971a, b; Reynolds 1977). When blood was removed from eclosing animals and injected into pharate moths prior to their normal gate, the recipients showed precocious eclosion. So also extracts from the brains or CC of pharate moths contained eclosion stimulating activity which was depleted during eclosion. This 'eclosion hormone' proved to have a number of actions on the pharate moth including the behaviour release involved in emergence and wing spreading, the triggering of the break down of the intersegmental muscles and plasticising of the wing cuticle. The time of appearance of 'eclosion hormone' was determined by bleeding pharate *Manduca sexta* at various times of the day and assaying each sample for hormonal activity (Reynolds *et al* 1979). The hormone appears in the blood only at a restricted time of day about 2·5 to 3 hr before the moth subsequently emerges. At the gate, eclosion hormone was released as a rapid pulse which is then

gradually cleared from the blood. The appearance of the circulating hormone is complemented by an 85 to 90% depletion of activity stored in the CC.

Truman (1978) concludes in the light of earlier findings, that the gating of the 'eclosion hormone' release assumes that the time of release is determined both by a circadian clock and by the developmental competence of the insect. Therefore eventhough the proper circadian time has arrived secretion will not occur during that gate if development has not been completed. But when the ability of *Manduca* to respond to the eclosion hormone was examined, it was found that receptivity appeared only about 4 hr before the hormone was actually secreted. Thus eventhough the proper circadian time is arrived at, hormone release will not occur if the animal is not in the proper developmental state.

3.2b *Hatching*: Since other developmental events are clearly gated by circadian clocks, it might have been expected that egg-hatch would also be so. The possibility have been examined thoroughly only in the pink bollworm, *Pectinophora gossipiella* (Minis and Pittendrigh 1968). In this particular species, the hatch rhythm is initiable until the 12th day of embryogenesis when the first cephalic pigmentation coincides with some essential link-up in the central nervous system. In *Aedes* mosquitoes, hatching occurs as a direct response to environmental amelioration, related only to the effects of temperature on embryogenesis and the presence of water after some sort of delayed developmental period (Gillett 1955) and unrelated to any rhythm (Corbet 1966). Pre-conditioned *Aedes taeniorhynchus* eggs hatch at any time of day within 15 min of emersion in de-oxygenated water (Nayar 1967).

3.2c *Larval moulting*: The release of PTTH to induce the periodic larval moults in *Manduca sexta* was established to be gated (Truman 1972). From an analysis of quantitative and qualitative differences in the responses of neck-ligatured *Manduca* larvae at various times of the day, it was found that the PTG needed contact with the brain for at least 1·5 hr before they were fully activated. Thus a minimal time interval was necessary for PTTH secretion by the brain. With the opening of the first gate, the larvae were apparently not competent to release PTTH but some gained this competence before the gate subsequently closed. The remainder attained competence during the succeeding day and were able to release PTTH as soon as the next gate opened. Thus the distribution in the first gate identified the closing of the gate and that in the second gate identified the time of the opening of the gate. Also the gates for the release of PTTH for the various instars occurred at essentially the same time of the day and the duration of gates tended to narrow as the animals grew.

Fujishita and Ishizaki (1981) demonstrated that in *Samia cynthia ricini* an endogenous circadian clock controls the timing of larval ecdysis and PTTH secretion preceding it. The clock upon reaching a specific phase point causes the brain to secrete PTTH provided that the brain has acquired the secretory competence. Full secretion of ecdysone occurred 6 hr after PTTH secretion and ecdysis ensued 34 hr thereafter to complete the ultimate sequence of ecdysis.

3.2d *Pupation*: The puparium formation in *Drosophila* is induced by the moulting hormone, ecdysterone. The process of metamorphosis starts with the puparium formation and can be regarded as a closed system. In *Drosophila lebabnonensis* puparium formation is a rhythmic process which can be characterised as a circadian

rhythm. The circadian oscillation regulates the timing of the ecdysterone mediated process of puparium formation. Jan Eeken (1978) opines that the influence of the circadian oscillation is not at the level of the ecdysterone concentration itself since no endogenous ecdysterone nor a changed ecdysterone degrading system is present at different phases of the circadian oscillation. So he suggests the interference system, coupled with circadian oscillation which determines whether or not the puparium formation can take place, seems to enforce its action at a level between transcription and translation.

The release of PTTH by the fifth instar larvae brings about the start of metamorphosis in *Manduca sexta*. Ligation experiments showed that the brain was required until an average time of 1600 hr in order to trigger the start of metamorphosis. However, a careful study of various ecdysone dependent epidermal changes in the larvae indicated that PTTH release probably began as early as 0100 hr on the preceding night. Thus the PTTH gate in the fifth instar appears to start at a time similar to that for other instars but the hormone release in this last case is greatly prolonged over about 15 hr (Truman 1978).

Considering the tropic action of brain on the PTG, one might expect that PTTH rhythm would result in the rhythmic secretion of ecdysone. Using the time of appearance of an ecdysone sensitive puff in *Drosophila*, Rensing (1966) postulated a rhythm of ecdysone release prior to pupariation.

In the last instar larva of *Samia cynthia ricini*, the initiation of development towards pupation as visualised by overt events such as gut purge and wandering occur with circadian rhythmicity (Ishizaki 1980; Fujishita and Ishizaki 1982), and the involvement of an innate circadian clock has been demonstrated. Fujishita *et al* (1982) have demonstrated that the timed surge of ecdysteroids is responsible for the gated occurrence of gut purge and that 18 hr before gut purge, larvae acquire the competence to undergo gut purge in a gated fashion provided that they are exposed to a sufficient surge of ecdysteroids. A gated release of PTTH was confirmed in the induction of prodromal signs of pupation in Manduca as well (Truman and Riddiford 1974).

3.2e *Bursicon release*: Another hormone whose appearance is gated is the tanning hormone, bursicon. In *Manduca sexta* this hormone is produced in the abdominal nerve cord and released from the periviceral organs. Bursicon release occurs during wing inflation by newly emerged moths and it triggers the tanning of the freshly expanded wing (Truman 1973). Under normal conditions wing inflation behaviour is well under way by 15 min after eclosion and thus bursicon secretion was estimated to occur about 3 hr after the eclosion hormone peak. The time course of bursicon appearance was followed in individual cannulated *Manduca* (Reynolds *et al* 1979) by means of an isolated wing assay that responded to bursicon by tanning the wing veins. Within one to two minutes after eclosion, no hormonal activity was detected and 2 min later substantial increase in bursicon level was noticed in the blood and a peak titer was reached within 5 to 10 min. Thus the secretion of bursicon also occurs as a large pulse. The secretion of bursicon is dependent on the prior release of eclosion hormone (Truman 1973). When moths were induced to emerge early by eclosion hormone injection, they also showed early bursicon secretion. In fact the release of bursicon by neurones in the abdominal ganglia of *Manduca* appears to be a part of the complex neural programme that is triggered by the eclosion hormone. The gated appearance of

bursicon in newly emerged moths is a consequence of the prior gating of eclosion hormone release.

3.2f *Calling hormone*: This hormone in moths also show a daily pattern of secretion. The hormone differs from those discussed above in that its release is not gated but presumably occurs on a daily basis as long as the female is unmated. In virgin females of silkmoths, the behaviour involved in pheromone release—calling behaviour—shows a rhythmic occurrence (Riddiford and Williams 1971) and is triggered by a hormone released from the intrinsic NS cells of the CC. Blood from calling females when injected into non-calling individuals readily induced the characteristic behaviour. At other times of the day, this activity was reduced or absent. Consequently, the rhythmic display of calling behaviour is apparently triggered by the rhythmic secretion of this hormone from the CC.

Truman (1978) is of the opinion that in the moth, the cerebral lobe area contains the gating centre for at least one hormone. Further, the rhythm of hormone secretion could be a direct or indirect result of an interaction with a circadian clock. The rhythm of ecdysone and bursicon release are secondary gated rhythms since they result only from the tropic actions of PTTH and eclosion hormone respectively. By contrast, the latter two hormones and the calling hormone appear to be primary gated rhythms, the rhythm is enforced through direct association of the circadian clock(s) and the endocrine courses. Most likely the rhythmic centres which control the release of the other two hormones discussed above also reside in the same region of the brain. Whether these centres are distinct from one another is unknown at this time. Also, there is no evidence to indicate whether the respective endocrine cells contain the entire rhythmic system.

3.3 *Diapause*

The epithelial cells of part of the hindgut (ileum) of mature larvae of the European corn borer were implicated in the production of a hormone (proctodone) involved in the physiology of diapause (Beck and Alexander 1964). Part of the evidence offered in support of the postulated endocrine function of the ileal cells was the appearance of secretory granules within the cytoplasm. The secretory rhythm of these granules had an 8 hr periodicity with phase setting being effected by photoperiod. The cells released their secretory products shortly after the beginning of the scotophase, after which cytoplasmic granules would again accumulate and again disappear at 8 hr intervals. Although the postulated hormonal function of these cells remains questionable, the rhythmic secretory cycle is striking.

4. Conclusion

As is evident from the foregoing discussion, research work already done on the endocrine rhythms in insects have been mainly restricted to only a few species of insects. A thorough understanding about the rhythmic activity patterns of the entire array of hormones in insects and the controlling centres for the release of these hormones are immensely useful. Once we know the exact activity patterns of all these developmental, metabolic and other hormones, one can artificially manipulate the hormone titer at the wrong time in the insecton life resulting in its maldevelopment promising a novel approach in pest management.

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