Circadian growth layers in the cuticle of behaviourally arrhythmic cockroaches (*Blaberus fuscus*, Ins., Blattoidea)

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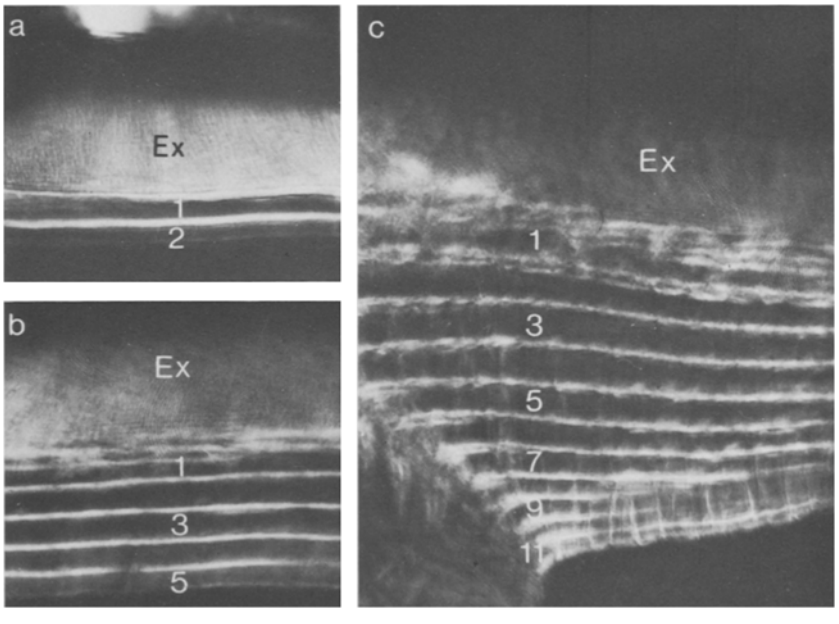
Summary. Circadian growth layers are still formed in the endocuticle of freshly emerged cockroaches, whose optic lobes had been excised during the last nymphal stage. The circadian locomotion rhythm disappears immediately after this operation. From these results we conclude that there are at least 2 self-sustained circadian oscillators in cockroaches.

In Orthoptera and Blattoidea, the circadian locomotion rhythm is controlled by an endogenous oscillator, which is closely connected with the optic lobes. After bilobectomy, the running activity is arrhythmic[2]. It is a question of general interest whether physiological rhythms are also abolished by the inactivation of the 'optic lobe clock'. The answer could contribute to the solution of the problem of how many self-sustained oscillators interact in 1 organism. In molluscs[3,4] and mammals[5], 2 independent circadian pacemakers have already been found. Comparable results in insects are lacking. This work is concerned with the effects of the removal of the optic lobes on the circadian growth of the endocuticle.

Methods. Pieces of the metathoracic tibiae of freshly emerged adults, whose optic lobes had been excised during the last nymphal stage, were used for the analysis of the endocuticular layers. After the operation, the animals were kept in constant darkness (DD) or constant dim light (LL, 1 Lux) at 25 or 30 °C. The leg pieces were frozen-sectioned and examined under polarizing microscope.

As in locusts[6], the rhythm continues in tibial pieces which were implanted into the abdominal haemocoel of adult hosts, even if the hosts had finished their own cuticular growth some months before.

Discussion. For the 1st time, these experiments demonstrate the persistence of a physiological circadian rhythm in insects behaviourally arrhythmic after bilobectomy. Obviously in cockroaches there are at least 2 circadian oscillators which are able to run independently. Probably other physiological rhythms are also independent of the 'optic lobe clock' which otherwise is not restricted to the control of the locomotion rhythm[7]. Neville suggests[6] that the epidermal cells are endogenously rhythmic themselves. The results presented here agree with this hypothesis: 1. the nervous and hormonal centres of the head do not play any role in the control of the cuticle rhythm; 2. the rhythm continues in mature hosts. Generally, the experiments confirm the largely accepted hypothesis[8,9] that multicellular organisms are controlled by a hierarchically organized system of multiple oscillators.



Circadian deposition of endocuticular layers in bilobectomized cockroaches. a, LL, 25 °C, 2 days; b, LL, 30 °C, 5 days; c, LL, 30 °C, 11 days after imaginal ecdysis. Ex = exocuticle; 1, 2, 3 ... the endocuticular isotropic lamellae in the sequence of their deposition. In *b* the 1st and in *c* the 1st 2 anisotropic lamellae are split by sectioning. × 650.

Results. After imaginal ecdysis, circadian endocuticular layers are still formed in bilobectomized cockroaches. Under constant conditions, one light (anisotropic) and one dark (isotropic) lamella is deposited during each circadian period lasting about 24 h (figure, a–c).

No differences were found between bilobectimized and control animals, or between operated animals kept at different temperatures (25 or 30 °C) or different light regimes (DD or LL). Up to 7 days, the circadian rhythm of deposition is also found in cockroaches decapitated 4–20 h after imaginal ecdysis.

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