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ROLE OF THE CORPORA CARDIACA IN THE BEHAVIOR OF SATURNIID MOTHS. I. RELEASE OF SEX PHEROMONE

LYNN M. RIDDIFORD AND CARROLL M. WILLIAMS

The Biological Laboratories, Harvard University, Cambridge, Massachusetts 02138

Mating of Polyphemus moths under laboratory conditions requires the presence of a volatile emanation from oak leaves (Riddiford and Williams, 1967). The active material has been extracted from red oak leaves and shown to be *trans*-2-hexenal (Riddiford, 1967). Vapors of a dilute solution of this aldehyde were found to act upon the female antennae. The resulting nervous input to the brain provokes after a certain period of delay the "calling" behavior. The latter can be recognized by inspection in terms of the protrusion of the female genitalia thereby exposing the glands which emit the sex pheromone.

In the case of virgin Cercropia females, calling behavior is elicited, not by a chemical, but by photoperiod. Thus, under both long- and short-day conditions, calling begins 1.5 to 2 hours before dawn and often continues for as long as 0.5 hour after lights-on. During this same pre-dawn period, male Cecropia moths become hyperactive even in the absence of females.

The third silkmoth considered in the present study was *Antheraea pernyi*—a semi-domesticated species which, like the completely domesticated *Bombyx mori*, has for thousands of years been selected for ease of mating. Though virgin Pernyi females show no overt calling behavior under laboratory conditions, there is convincing evidence that the sex pheromone is continuously released to provoke mating at any time of day or night (Riddiford, 1970).

In the present study carried out on these three species we have sought to determine whether the corpora cardiaca or corpora allata are involved in the control of the release of sex pheromone.

Materials and Methods

1. *Experimental animals*

Pupae of *Antheraea polyphemus* and *Hyalophora cecropia* were purchased from dealers or reared outdoors on netted trees (Telfer, 1967). Cocoons of *Antheraea pernyi* were obtained from Japanese sources. The pupae were stored

at 5° C for at least 12 weeks; they were then returned to 25° C to provoke adult development.

2. Excision of corpora allata and/or corpora cardiaca

These organs were removed from pupae by the technique described by Williams (1959). To check the completeness of extirpation, the excised organs were placed in a black dish containing Ringer's solution and examined under a dissecting microscope. If the excised organs were not self-evident, the extirpation was considered incomplete and the animal was discarded. In certain individuals the glandular complexes were excised and three pairs of "loose" complexes reimplanted into the thoracic tergum. All individuals were placed at 25° C under controlled photoperiod (usually 17L:7D). Adult development was initiated after about 2 weeks and completed after an additional 3 weeks.

3. Behavioral assays

Female Polyphemus moths were caged in a darkened room and exposed to the vapors of 0.05% aqueous *trans*-2-hexenal solution. The experiment was usually begun in the early evening. At half-hour intervals for at least the first 4 hours, the moths were inspected under dim red light for calling behavior; they were again inspected the following morning.

In the experiments performed on Cecropia the female moths were reared and caged in two constant-temperature rooms, one programmed for a short day (12L:12D) and the other for a long day (17L:7D). Under dim red light the moths were inspected for calling behavior at hourly intervals throughout the scotophase.

In the experiments performed on *A. pernyi,* virgin females were caged with males and their mating behavior ascertained as described by Riddiford (1970).

Experimental Results

1. Delay in response of virgin female Polyphemus moths to vapors of trans-2-hexenal

As described under Methods, 46 normal females were caged in a darkened room in the presence of the vapors of *trans*-2-hexenal. Observations under dim red light at 0.5 hour intervals indicated that at least one hour was required for the initiation of calling behavior and that 74% of individuals were calling after a total of 4 hours.

More detailed observations were carried out on a series of 9 virgin females which were placed, 1 or 2 at a time, in a 2-liter glass chamber in a darkened room. The chamber was ventilated by a gentle stream of air containing the vapors of a 0.05% aqueous solution of *trans*-2-hexenal. Observations of the moths were made at 15-minute intervals under dim red light.

The results summarized in Figure 1 once again show that at least an hour elapses before the first individual initiates calling. Fifty per cent of individuals initiated calling within the first 2.25 hours and 100% within the first 4 hours. From these observations we learn that a latent period intervenes between the

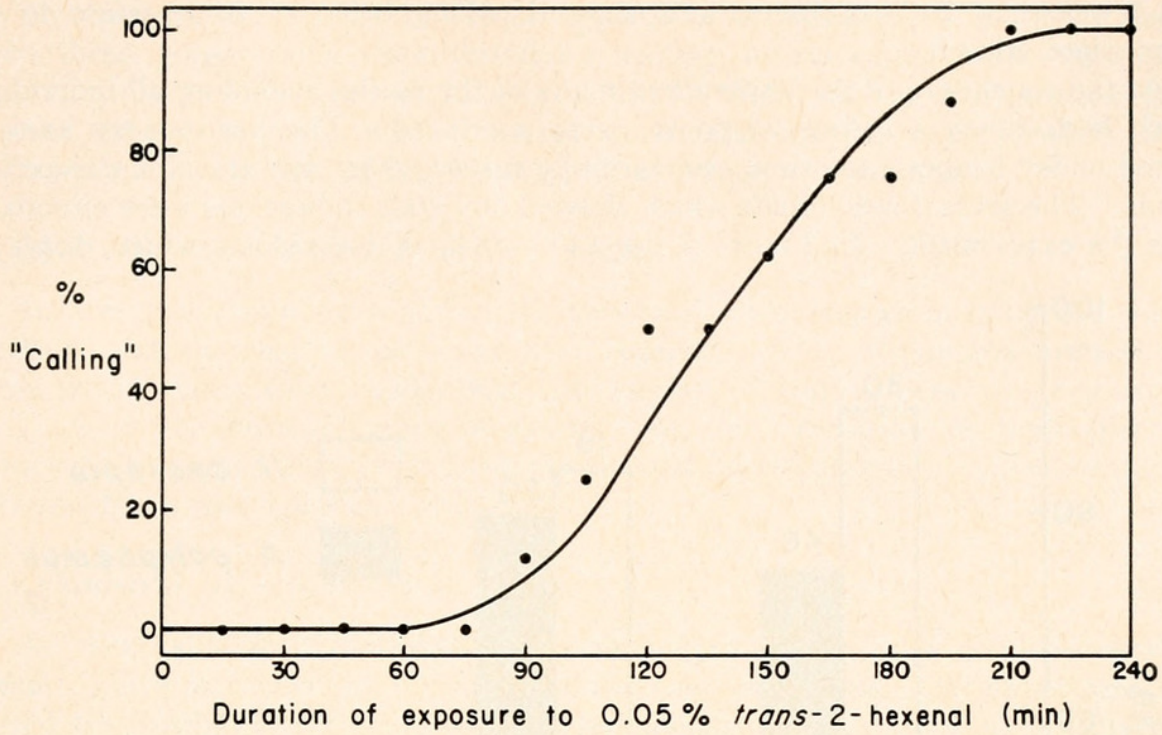

FIGURE 1. The time required for virgin Polyphemus females to begin releasing sex pheromone ("calling") after exposure to vapors of an aqueous solution of 0.05% *trans*-2-hexenal in the apparatus described under Materials and Methods. A total of 9 moths was used in these determinations.

presentation of the chemical stimulus and the initiation of the behavioral response. This delay was the first indication that a neuroendocrine relay mechanism might be involved.

2. *Effects of allatectomy*

The corpora allata were excised from 20 female Polyphemus pupae without any damage to the nearby copora cardiaca. The allatectomized pupae were then placed at 25° C and allowed to develop into adult moths. The latter's response to *trans*-2-hexenal was then determined as described under Methods. The results summarized in Figure 2 are the same as seen for unoperated Polyphemus moths. Moreover, the allatectomized females mated when placed with males and oviposited a normal number of eggs which hatched as normal first-instar larvae.

The experiment was repeated on 19 allatectomized Cecropia moths exposed to the short-day regimen of 12L:12D. Normal behavior was observed in terms of the presence of calling behavior during the final hour before lights-on. The results summarized in Figure 2 show that the absence of corpora allata in no way affected the response to photoperiod.

3. *Effects of removal of the complex of corpora cardiaca and corpora allata*

The experiment described in the preceding section was repeated on 29 Polyhemus and 38 Cecropia except that in this case the moths were derived from pupae lacking the entire complex of corpora allata and corpora cardiaca. The results summarized in Figure 2 show a great departure from normal behavior in

that fewer than 20% of individuals showed calling behavior in response to the appropriate stimuli.

At the conclusion of the experiment many of the moths, including all individuals which had shown a calling response, were sacrificed. The heads were excised, pinned under Ringer's solution, and carefully inspected for any trace of the excised glands. The several individuals which showed any such indications were eliminated from the experiment. In Figure 2 the 14 to 18% of individuals which displayed

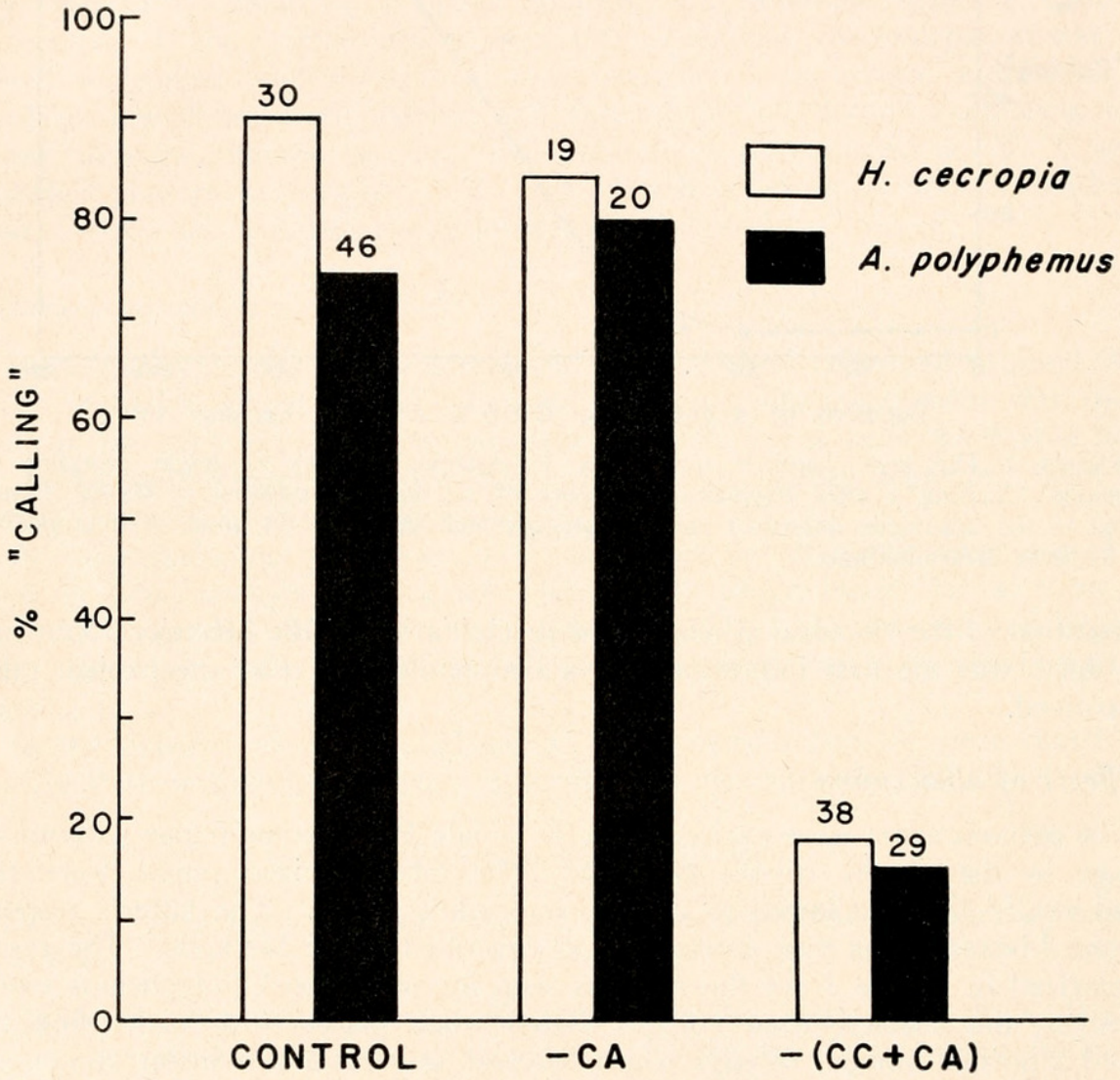

FIGURE 2. The effect of allatectomy and allatectomy-cardiactomy on the "calling" response of Polyphemus and Cecropia females to vapors of 0.05% *trans*-2-hexenal and to photoperiod respectively. The numbers in parentheses above the bars indicate the number of females tested.

the calling response showed no trace of corpora cardiaca. However, the dissection is a difficult one so there remains the possibility that the extirpation may have been incomplete in these individuals.

4. *Re-implantation of the corpora allata-corpora cardiaca complexes*

The glandular complexes were excised from 11 female Polyphemus pupae and 2 female Cecropia pupae. Into the thoracic tergum of each individual were imme-

diately reimplanted 3 pairs of "loose" glandular complexes. The moths derived from these preparations were tested for calling in response to the appropriate stimuli. The results were as follows: only 2 (18%) of the Polyphemus moths showed calling behavior when exposed to *trans*-2-hexenal vapors; none of the Cecropia moths showed calling behavior in response to photoperiod.

5. *Effects of denervating the corpora cardiaca*

In 5 female Polyphemus pupae the two pairs of nerves connecting the corpora cardiaca with the rear of the brain were severed. When the moths derived from these preparations were challenged with vapors of *trans*-2-hexenal, they showed no trace of the normal calling response. At the conclusion of the experiment autopsies performed on all 5 individuals showed no regeneration of the connections between brain and corpora cardiaca.

6. *Experiment on female Pernyi moths*

As mentioned in the introduction, *Antheraea pernyi* is a semi-domesticated species which has been highly selected for ease of mating. In experiments reported by Barth (1965) the allatectomized female Pernyi moths were fully effective in attracting and mating with males. The question therefore arises as to whether they can do so if the corpora cardiaca are also extirpated. To answer this question we excised the complex of corpora allata and corpora cardiaca from 16 female Pernyi pupae. The moths derived from these individuals were tested for the release of sex pheromone by caging them with normal males. Fifteen of the 16 females mated within 15 minutes, thereby documenting the continuous release of sex pheromone peculiar to the virgin females of this species.

Discussion

1. *The role of the corpora cardiaca in the reproductive behavior of virgin female silkmoths*

In contrast to the continous and apparently spontaneous release of sex pheromone by virgin females of semi-domesticated Pernyi silkmoths, the undomesticated Cecropia and Polyphemus silkmoths possess a neuro-endocrine mechanism for the control of pheromone release. Our experiments strongly argue that the corpora cardiaca, but not the corpora allata, are necessary for the calling behavior which accompanies the release of sex pheromone by the virgin female moths. That being so, the excision of the corpora cardiaca blocks the normal release of pheromone in response to environmental signals. This loss is not repaired by the reimplantation of as many as three pairs of "loose" glandular complexes—a finding which suggests that the nervous connections between the brain and corpora cardiaca are necessary for the behavioral response. When these nervous connections were selectively severed, calling behavior was blocked despite the continued presence of the denervated corpora cardiaca.

Females lacking corpora cardiaca fail to mate because males are not attracted to them. However, when caged with males near a cage of normal calling females, they not only mate, but then go on to lay fertile eggs. The absence of corpora

cardiaca therefore interferes with the attraction of males but not with the ability to mate and reproduce.

2. Dual role of the corpora cardiaca

The corpora cardiaca serve as neurohaemal organs in which the products of the brain's neurosecretory cells are released into the blood. In addition, the corpora cardiaca contain intrinsic neurosecretory cells and therefore qualify as genuine endocrine organs. When the corpora cardiaca are excised, their intrinsic cells are permanently lost, whereas the neurohaemal portion promptly regenerates from the cut ends of *nervi corpora cardiaci* I and II (Stumm-Zollinger, 1957). So, for our present purposes it appears that the intrinsic cells of the corpora cardiaca are the source of the hormone which triggers the calling behavior of virgin females.

3. The minimal circuitry

In virgin Cecropia and Polyphemus moths the brain processes in-coming signals conveying specific environmental cues relating to the onset and timing of reproductive behavior. After this central integration, signals flow from the brain to the corpora cardiaca via the two pairs of nerves which interconnect them. On the basis of present knowledge we cannot say whether these signals are nerve impulses or neurosecretory agents. The signals in question converge on the intrinsic cells of the corpora cardiaca to provoke the release from these cells of a certain hormone. In the case of cockroaches, Milburn and Roeder (1962) have extracted from the corpora cardiaca a substance which causes rhythmic discharge in the phallic nerve when applied to the abdominal ganglia. Evidently, in the virgin moths an analogous factor is secreted by the intrinsic cells of the corpora cardiaca to promote the motor acts which comprise the calling behavior.

A similar type of mechanism involved in oviposition behavior will be examined in the further detail in the following communication (Truman and Riddiford, 1971).

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Summary

1. In virgin female silkmoths the protrusion of the genitalia or "calling" behavior signals sex pheromone release. The wild Polyphemus and Cecropia silkmoths "call" in response to specific environmental cues which are chemical and photoperiodic respectively. The semi-domesticated Pernyi silkmoth is exceptional in that it shows no overt "calling" behavior and pheromone is apparently released continuously.

2. By appropriate experiments it was possible to show that the corpora cardiaca but not the corpora allata are prerequisite for the "calling" behavior. Thus, when the corpora allata are removed from female pupae, the behavior of the resulting

moths is normal. By contrast, removal of the corpora allata-corpora cardiaca complex greatly reduces the number which "call."

3. In order to perform their function in the "calling" behavior the corpora cardiaca must have intact connections with the brain. Reimplantation of three pairs of corpora allata-corpora cardiaca complexes into animals lacking their own complexes fails to restore the ability to "call." "Calling" is also blocked when the nervous connections between the corpora cardiaca and the brain are severed.

4. Evidently, in response to the environmental signals the brain stimulates the release of a hormone from the intrinsic cells of the corpora cardiaca. This hormone then acts on the abdominal nervous system to provoke the protrusion of the female genitalia and the accompanying release of pheromone.

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