DO THE BRAINS OF WAX MOTH LARVAE SECRETE AN ALLATOTROPIC HORMONE?

RUDOLPH L. PIPA
Division of Entomology and Parasitology, University of California, Berkeley, California 94720, U.S.A.

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Abstract—The hypothesis that the brains of young, last instar larvae of Galleria mellonella (L.) initiate supernumerary larval apolyses by secreting an 'allatotropic factor' was reexamined. It was confirmed that following bilateral allatectomy the larvae lose their ability to produce supernumerary instars (super-larvae) in response to implanted brains. The JH analog Altosid caused the allatectomized larvae to undergo extra apolyses irrespective of whether or not brains had been implanted. Although the percentage of superlarvae obtained following Altosid treatment was not increased by the implanted brains, the onset of extra apolyses was accelerated. This suggests that the brain can promote larval-larval apolyses without acting first on the corpora allata (CA). Presumably, it does so by producing prothoracotropic hormone.

The propensity to generate new larval structures was tested by injecting ecdysterone into larvae 48 and 65 hr after they had been allatectomized. Within 48 hr after both CA had been removed the precocious apolysis resulted in individuals with antennae that were partly larval and partly pupal, and by 65 hr the ability to reproduce larval parts had diminished further. Those that were hemi-allatectomized did not demonstrate this impairment. The results were consistent with the interpretation that allatectomy abolishes the capacity to produce superlarvae because the JH titer declines to a level insufficient to permit expression of the larval genetic program during the next moulting cycle. This is offered as an alternative to the hypothesis that allatectomy prevents implanted brains from producing superlarvae because the target organs of the 'allatotropic factor' have been removed.

An attempt was made to confirm the observation that brains from young, last instar larvae are more effective initiators of supernumerary apolyses than those from donors in the process of pupating. There was no evidence for a different endocrine function by the brain during the two stages.

INTRODUCTION

When last instar larvae of the greater wax moth, Galleria mellonella (L.), are deprived of free space by keeping them in food-filled containers, their pupation will be greatly delayed (WOOLEVER and PIPA, 1970). During a study of the endocrine mechanisms that might mediate this response (PIPA, 1971), it was found that the retardation was abolished if brains were implanted. Although the metamorphosis of the larvae, irrespective of their ages when space deprived, was effectively hastened by the implants, the immediate consequence of the operation was age dependent. Most larvae younger than 3 days beyond ecdysis underwent supernumerary apolyses; those that were older usually did not, and they pupated sooner.

This difference in the morphogenetic action of the brain implants was shown to be unrelated to the requirement of the larvae for free space. Instead, it seemed likely to be correlated with a changing titer of juvenile hormone (JH). The results obtained with the younger larvae would be anticipated if neurohormonal stimulation of ecdysone production occurred at a time when the JH concentration was high. In older larvae, with a supposedly diminished titer of JH, the prothoracotropic hormone would be expected to stimulate metamorphosis.

GRANGER and SEHNAL (1974) and SEHNAL and GRANGER (1975) were able to confirm that young, last instar Galleria larvae undergo extra apolyses (i.e. become superlarvae) in response to brain implants, and that their propensity to do so is lost as they approach metamorphosis. However, they discounted the candidate explanation mentioned above and proposed that the implants release a 'neurohumoral allatotropic factor'. According to their interpretation, the recipients of implanted brains become superlarvae when this blood-borne neurosecretory product stimulates the corpora allata (CA) to produce JH.

They based their hypothesis on the following observations: (1) implanting 3 to 6 pairs of corpora cardiaca—corpora allata (CC—CA) into young larvae that had these glands removed would produce superlarvae; (2) extirpating both CA would abolish the ability of implanted brains to induce additional larval apolyses; (3) brains from young, last instar larvae seemed to initiate supernumerary apolyses more effectively than did those from donors commencing to pupate. The increased potency during the first part of the instar corresponded to the period when the larval developmental program was most responsive to exogenous JH. This result would be anticipated, perhaps, if brains from young larvae produced more of the 'allatotropic factor'.
I have performed experiments designed to repeat or parallel those on which the hypothesis by Sehnal and Granger rests. The results of those experiments and my assessment of the validity of the conclusions that can be drawn from them will be presented.

MATERIALS AND METHODS

Descriptions of the techniques that were used to rear the larvae, to extirpate their CA, or to implant brains have been noted elsewhere (Pipa, 1963, 1971, 1976), so I shall not reiterate them. After surgery, and before administering the compounds described below, the larvae were kept at room temperature (22 to 25°C) without food for ca. 18 hr. Subsequently, a pair of larvae were placed into each wire gauze-topped, snap-cap vial (30 × 40 mm) containing ca. 5 ml of artificial diet. Except during brief daily inspections, all experimental insects were kept at 33 to 35°C in constant darkness.

The effects of an altered JH titer on the ability of young, last instar larvae to undergo supernumerary apolyses were explored in two ways: (1) By administering topically to each allatectomized larva 100 μg of the growth regulator Altosid (ZR 515), a compound that resembles JH both structurally and by its influence on morphogenesis (Henrick et al., 1973). This was done in a manner described previously (Pipa, 1976); (2) By injecting into each larva, at increasing intervals after allatectomy, 6 μg of ecdyserone. The hormone was dissolved in 10% ethanol at a concentration of 3 μg/ml. Forty-eight hours after the hormone was administered remnants of the larval cuticle were removed, and the morphology of the insect that resulted from the precocious apolysis was examined.

During the experiment designed to test the relative capabilities of brains to stimulate extra apolyses, these organs were removed from donors that were just commencing to pupate (i.e. were in eye stages 3 to 4 according to Piepho's (1938) classification), or from last instar larvae younger than 18 hr before ecdisis. Two brains were implanted into each recipient, a last instar larva that had completed ecdisis less than 18 hr previously. To assure that the results obtained were attributable specifically to the implanted brains, a control group consisting of larvae that had received two thoracic ganglia was included for each of the three replications of this experiment.

The period required for the last instar larvae to initiate extra apolyses or to pupate was measured from the time they were put into the incubator. In the case of Galleria, larval or pupal ecdisis normally occurs within 48 to 72 hr after apolysis, so the former event is a convenient index of when the moulting process begins. Where, due to injury, ecdisis did not follow apolysis after the usual delay, the presence of the darkening cuticle of the pharate pupa or the protrusion of the outer head capsule by the pharate superlarva was used to estimate when ecdisis should have occurred (Pipa, 1976).

RESULTS

Effects of allatectomy, brain implants and Altosid on the capacity to produce superlarvae

The responsiveness of allatectomized last instar larvae to Altosid (Table 1A) resembled that noted by Sehnal and Granger (1975) after they implanted 3 to 6 pairs of CC–CA: superlarvae or intermediate forms resulted. The intermediates were pharate individuals, mostly with pupal exoskeletons, but possessing the larval features described previously (Pipa, 1976). Larvae transforming to intermediates did so following a delay approximately 4 times that taken by individuals apolysing to superlarvae. None of the control larvae (Table 1B) that received 1 μl of acetone became superlarvae or intermediates, nor was their pupation retarded.

Also confirmed was the observation that by removing the CC–CA the capacity of brain implants to initiate supernumerary apolyses is abolished (Sehnal and Granger, 1975). Young larval recipients of brain implants consistently produce superlarvae (Pipa, 1971, 1976; Granger and Sehnal, 1974), but none of the 13 that had been allatectomized beforehand did so (Table 1D).

Although Altosid caused a majority of the allatectomized recipients of three implanted brains to undergo extra larval apolyses, the percentage of superlarvae obtained was not significantly greater than amongst the controls that had received three thoracic ganglia (for 1C vs 1E, t = 0.0845). Nevertheless, the brains were not without effect in this experiment, for they clearly accelerated onset of the larval–larval apolysis (P = < 0.01).

Diminution of JH titer in allatectomized larvae estimated by injecting ecdysterone

Young, last instar larvae that had had their CA removed lose their ability to undergo supernumerary larval apolyses in response to implanted brains. Is this because the hypothetical 'allatotropic factor' from the brain no longer stimulates the target organs, or could it be that without the CA the JH titer soon diminishes to a level insufficient to permit expression of the larval genetic program during the next moulting cycle? The latter possibility was explored indirectly, by injecting ecdysterone into larvae that had been partly or totally allatectomized.

The data should be interpreted cautiously. The possibility that there may be a 'developmental momentum' makes it inadvisable to assume that the capacity of the cells to reproduce a larval structure reflects the concentration of JH circulating in the blood at the time ecdysterone is injected. Nevertheless, I considered it acceptable to suppose that if certain of the cells had lost their former synthetic capability, and a mosaic of larval and pupal structures resulted, then the titer of JH must have diminished.

Within 48 hr after bilateral allatectomy the JH titer apparently had declined (Table 2). Though the result-
Table 1. Effects of altosid and brain implants on supernumerary apolyses by last instar *Galleria* larvae that had been allatectomized when less than 24 hr beyond ecdysis

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of replications</th>
<th>Fraction surviving to next apolysis</th>
<th>Per cent (± S.D.) apolysing to superlarvae (L), intermediates (M), or pupae (P)</th>
<th>Hours (range) and T_{50}% (± S.D.) required for apolysis to superlarvae (L), intermediates (M), or pupae (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Altosid</td>
<td>3</td>
<td>24/34</td>
<td>L 58 ± 24.5 42 ± 24.5</td>
<td>L 120-384 (185 ± 31.2) M 456-912 (720 ± 148.8)</td>
</tr>
<tr>
<td>B. Diluent (control)</td>
<td>3</td>
<td>19/31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. Brain implants + altosid</td>
<td>3</td>
<td>20/31</td>
<td>L 65 ± 9.9 35 ± 9.9</td>
<td>L 48-144 (72 ± 24.0) M 168-528 (312 ± 117.6)</td>
</tr>
<tr>
<td>D. Brain implants + diluent (control)</td>
<td>3</td>
<td>13/25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. Thoracic ganglion implants + altosid (control)</td>
<td>3</td>
<td>15/31</td>
<td>L 53 ± 22.5 47 ± 22.5</td>
<td>L 96-216 (151 ± 31.2) M 408-936 (638 ± 213.6)</td>
</tr>
</tbody>
</table>

* Average hours required for half the population to undergo apolysis.
Table 2. Effects of partial or total allatectomy on expression of larval features induced by injecting ecdysterone

<table>
<thead>
<tr>
<th>Nature of allatectomy</th>
<th>Hr beyond ecdysis to last instar</th>
<th>Hr ecdysterone injected after allatectomy</th>
<th>Fraction forming superlarvae</th>
<th>Per cent forming superlarvae</th>
<th>Per cent forming intermediates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilateral (test)</td>
<td>&lt; 24</td>
<td>48</td>
<td>$\frac{4}{12}$</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Unilateral (control)</td>
<td>&lt; 24</td>
<td>65</td>
<td>$\frac{4}{12}$</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

ing individuals closely resembled the superlarvae produced by the hemi-allatectomized controls, they differed in one important respect: instead of typical antennae, they had formed partly evaginated pupal antennal cases bearing larval sensilla apically. Such "mixed antennae" are often produced after Altosid or the JH mimic 1-4(Ethylphenoxoxy)6, 7-epoxy-3,7-dimethyl-2-octene (PALLOS et al., 1971; KAMIMURA et al., 1972) have been applied topically to late, last instar *Galleria* larvae (PIPA, unpublished).

When ecdysterone was injected 65 hr after allatectomy the difference between the test and control insects was more conspicuous. Again, those that had been hemi-allatectomized produced superlarvae. Those that had been allatectomized bilaterally, however, not only formed 'mixed antennae', but also possessed mandibles that were reduced, and thoracic tarsi that were incompletely formed. Abdominal prolegs and crochets were either vestigial or absent.

*Do brains from young, last instar larvae lose their capacity to initiate supernumerary apolyses as pupation approaches?*

If the brains of young, last instar larvae secrete a hormone that activates the corpora allata, it might be anticipated that their capacity to do so would diminish near pupation, when an increased titer of JH could have untoward effects. The results of my attempt to repeat the experiment by GRANGER and SEHNAL (1974) which suggested that this happens are presented in Table 3. Though brains from young, last instar larvae or pharate pupae clearly were more effective initiators of supernumerary apolyses than were the thoracic ganglia, they did not differ from each other significantly ($t = 1.45$). There was no evidence that the brains of older larvae produced less of the hypothetical 'allatotropic hormone'.

**DISCUSSION**

The observation by SEHNAL and GRANGER (1975) that allatectomy abolishes the capacity of brain implants to stimulate supernumerary apolyses by young, last instar *Galleria* larvae was confirmed; in the absence of the CC–CA all of the survivors pupated. Irrespective of whether or not brains were implanted, the JH analog Altosid led to the production of superlarvae by these allatectomized individuals. Extra apolyses also can be elicited from allatectomized larvae by implanting CC–CA (SEHNAL and GRANGER, 1975), or by injecting purified extracts of JH (SEHNAL and MEYER, 1968).

These results serve to illustrate the central rôle played by the juvenile hormones as regulators of gene

Table 3. Relative effectiveness of brains from donors in different developmental stages at stimulating supernumerary apolyses by young, last instar larvae

<table>
<thead>
<tr>
<th>Nature of implants</th>
<th>Stage of donors</th>
<th>Fraction surviving to apolysis</th>
<th>Per cent (± S.D.) forming superlarvae</th>
<th>Per cent (± S.D.) forming pupae</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 brains</td>
<td>Larvae &lt; 18 hr beyond ecdysis</td>
<td>$\frac{4}{4}$</td>
<td>65 ± 12.7</td>
<td>35 ± 12.7</td>
</tr>
<tr>
<td>2 brains</td>
<td>Pharate pupae</td>
<td>$\frac{4}{4}$</td>
<td>81 ± 14.1</td>
<td>19 ± 14.1</td>
</tr>
<tr>
<td>2 thoracic ganglia</td>
<td>Larvae &lt; 18 hr beyond ecdysis</td>
<td>$\frac{4}{4}$</td>
<td>10 ± 13.4</td>
<td>90 ± 13.4</td>
</tr>
</tbody>
</table>
expression, but have doubtful bearing on how the endoclines interact to initiate the larval moulting program. Because there is evidence that the juvenile hormones promote ecdysone production by certain lepidopteran pupae, probably by acting on their prothoracic glands (Gilbert and Schneiderman, 1959; Oberlander and Schneiderman, 1966; Siew and Gilbert, 1971), the possibility exists that supernumerary apolyses by wax moth larvae can be induced similarly. Nevertheless, the physiological parameters of the interaction are too poorly known to conclude that by exciting CA activity and raising the JH titer ecdysterone production is enhanced and superfalvae result.

The inability of brain implants to stimulate supernumerary apolyses by allatectomized larvae could denote a loss of the capacity to generate larval structures, not disruption of a brain hormone-CA interaction. Within 48 hr after total allatectomy the JH titer evidently had diminished, for exogenous ecdysterone caused the larvae to produce antennae possessing a mixture of larval and pupal characteristics. This decline in the ability to generate larval features was more widespread by 65 hr after the operation.

In this context, it is appropriate to note that most larvae must feed more than 48 hr after they have received brain implants if they are to produce supernumerary instars (Pipa, unpublished). Because this critical feeding period is probably longer after allatectomy, sufficient time would have elapsed for the JH titer to reach a level such that pupae, not larvae, would result.

The production of supernumerary instars by young, allatectomized larvae that had received Altosid was not increased significantly by implanting brains. Nevertheless, the implants did accelerate greatly the onset of the moulting program. The results indicate that the brain, presumably by producing a diffusible factor, can promote larval-larval apolysis without acting first on the CA. It remains to be demonstrated whether this factor is equivalent to the prothoracotropic hormone, though that is quite likely.

Had the brains from donors in the process of pupating been less effective initiators of supernumerary apolyses than those from young, last instar larvae, evidence for a changed endocrine function by the brain during development might have been at hand. This was suggested by Granger and Sehnal (1974), who reported that only 35% of 17 recipients of brains from 'prepupae' (eye pigment stage 3 to 4) became superfalvae, while 71% of 34 receiving brains from 0 to 12 hr last instar larvae did so. Their data are incomplete, however, for they did not indicate the number of times the experiment was replicated, nor did they present the variability.

If it could be demonstrated by assay that the JH titer within the larval haemolymph rises after brains have been implanted, the allatotropic hormone hypothesis would be substantiated. For an insect as small as Galleria the technical requirements of this procedure may be severely limiting, but it is difficult to foresee how incontrovertible evidence that would resolve the question can emerge from approaches that are less direct.

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REFERENCES


