Memoirs of the College of Science, University of Kyoto, Series B.
Vol. XX, No. 3, Article 3, 1953.

Studies on the Insect Metamorphosis. III. Activity of the Brain in the Post-Embryonic Development of Lepidopterans[1)]

By

Mamori ICHIKAWA, Kanji YASHIKA and Junko NISHIITSUTSUJI

Zoological Institute, University of Kyoto

(Received March 31, 1953)

In the previous works with *Bombyx*, *Philosamia* and *Luehdorfia*, we verified the fact that the brains of these worms, at least of the first two species, release a hormone which is indispensable to evoke the activity of the prothoracic gland so as to secrete the metamorphosis-promoting hormone. Experiments to be described in this paper were carried out with the intention of determining whether the brain in all larval stages has the faculty of secreting the hormone or its faculty is restricted to the mature stage before pupation. The second aim of this work is to ascertain whether or not the same faculty of the brain is maintained in the various stages of the pupal life. The following is the results of these experiments.

Results of Experimants

Materials used were Eri-silkworms, *Philosamia cynthia ricini*. The brains to be tested were removed from the larvae and pupae in various stages from the second day of the second instar to the eighth day after pupation: the first instar having been omitted because of the technical difficulty of isolating the sufficient number of the brains alone in this instar. Five brains were implanted into each brainless recipient worm which was in the mounting stage of the last instar; namely, about 48 hours before the critical period for pupation, and about 108 hours before pupating. Needless to say, the recipient worms were equipped with their own prothoracic glands, from which the pupation hormone is released, provided that there is sufficient brain hormone. Therefore, in the case when the implanted brains would have the faculty of secreting the brain hormone, it should be natural for the recipient worms to undergo their pupation earlier than the control animals which were previously deprived of their own brains without subsequent trans-

1) The work was aided by a grant for Scientific Research from the Ministry of Education

plantation of the brains that were removed from the other worms.

The results are arranged in two tables, since the temperature of cultivating was different between the experiments on larvae and those on pupae.

Table 1. Activity of larval brains removed in the various stages and instars.

Stage of donor	No. of Experiments	No. of death without pupation	No. of pupated specimens	Days required for inception of pupation at 21°C	Average days required for inception of pupation
2nd~3rd day of 2nd instar	13	2	11	**11**, 13, 13, 14, 14, 15, 15, 17, 18, 18, 21,	14
2nd sleeping st.	18	1	17	**11**,12,12,12,13,13,13, 13,13,13,14,15,16,19, 20,21,24.	15
4th sleeping st.	7	1	6	**12**, 13, 13, 14, 15, 18.	14
1st day of 5th instar	18	3	15	**10**,**11**,**11**,**11**,**12**,13,14, 14,14,16,18,23,23.23, 24.	16
3rd day of 5th instar	10	3	7	**9**, **11**, **11**, **12**, 14, 15, 19.	13
4th day (st. 3) of 5th instar	12	5	7	**10**, **12**, **12**, 13, 14, 15, 20.	14
5th day (st. 4) of 5th instar	16	2	14	**9**, **10**, **10**, **10**, **11**, **11**, **12**, **12**, **12**, 13, 14, 14, 20.	12
Mounting stage (st. 5)	16	3	13	**8**, **10**, **10**, **11**, **11**, **12**, **12**, 13, 13, 14, 15, 16, 16.	12
Spinning~early prepupal st. (st. 6~7)	12	6	6	**9**, **10**, **12**, 13, 15, 17.	13
Control	25	2	23	13,14,17,17,17,19,21, 23 24.31,35,42,42,45, 46,49,50,51,52,56,57, 63,66.	33

As shown in Table 1, the brains removed from the larvae in every stage of larval life are demonstrated to have the ability of secreting the brain hormone, when implanted into the larvae in the mounting stage of the last instar, although there exists the considerable variation in the time required for the initiation of pupating. The cause of this variation may be ascribed to some possibilities that, for instance, some of the implanted brains would fail to function in the abnormal surrounding tissues of the host or that the responsibility of the host's prothoracic glands to the stimulating brain hormone would vary to some extent from individual to individual or that the tissues of the host would vary in susceptibility to the pupation hormone from the prothoracic glands. At any rate it is fairly apparent that the brains of the larvae in stage 4 of the last instar are more effective than those removed

from the larvae in younger instars as well as in the other stages of the same instar. But it is uncertain whether or not there is an actual difference between younger and older larvae concerning the activity of their brains, for when larvae are younger, their brains are smaller, and when larvae become older, their brains become larger. Consequently, it would be assumed that accumulation of a sufficient amount of the brain hormone may require much more time when younger brains are planted than when the older brains are inserted. However, that the brains taken from the larvae of the first day of the last instar were the least effective is perhaps due to the temporary lessening of function in this stage.

In the control animals the first pupating occurred 13 days after the decapitation. But a majority of specimens pupated after 17 days subsequent to the operation. However, as may be seen in the following Table 2, to use the decapitated worms as control would not be quite adequate. In addition to the decapitation, a wound through which the brains are to be inserted should be made in them at least. By this procedure the time required for the first appearance of pupating was prolonged 3 days more. If the control would be made in this manner, the effect of the transplanted brains would become more distinct than that presented in Table 1.

Table 2. Activity of pupal brains removed in various days after pupating as well as of larval brains in the last instar.

Stage of donor	No. of Experiments	No. of death without pupation	No. of pupated specimens	Days required for inception of pupation at 27°C	Average days required for inception of pupation
Mounting stage	34	16	18	8, 8, 9, 9,10,10,11, 11,12,12,12,13,13,15, 15,15,19,19.	12
Late prepupal st.	24	16	8	11, 12, 13, 14, 16, 17, 18, 19.	15
Just after pupating	21	10	11	10, 12, 12, 14, 15, 16, 16, 17, 17, 18, 19.	15
3rd day after pupating	27	7	20	12,13,14,15,15,17,17, 18,18,18,18,19,20,21, 23,24,25,27,28,29.	20
5th day after pupating	17	5	12	12, 13, 13, 14, 15, 16, 16, 18, 19, 19, 20, 21.	16
8th day after pupating	20	9	11	13, 14, 15, 20, 20, 21, 22, 22, 25, 26, 27.	21
Decapitated and wounded control	20	13	7	17, 20, 20, 23, 24, 25, 28.	22
Decapitated control	25	16	9	14, 15, 16, 17, 17, 20, 20, 22, 22.	18

Next the pupal brains were examined in the same way as described above, but in this case the decapitated and wounded specimens served as a control in addition to the merely decapitated worms. As the temperature at which the worms were reared was higher by about 6 degrees in this case than in the preceding one, some of the experiments on the larval brains were repeated in additton to those on the pupal brains. The results are described in Table 2 on the preceding page.

As is shown in the table, mortality is high in every series of the experiments presumably owing to the high temperature, but it is found that the brains in the mounting stage are the most effective in this case too, and that those in the late prepupal stage become a little less effective. The brains that were taken from the pupae just after pupating are shown to hold nearly the same degree of secretory function as those in the late prepupal stage. On the third day after pupating they seem to decrease the function. If this is the case, on the fifth day they would regain it upto about the same degree as in the late prepupal stage. Then the function seems to fall again with the elapse of time. However, whether the function of the brain in pupal life is cyclic or not requires further investigation.

Discussion

From the results of the present experiments, it is evident that the brains in every instar of larvae have the ability to release the brain hormone, although it is uncertain whether the minor change concerning the secretory faculty of the brain occurs within the each larval instar. It is also shown that the same secretory function is maintained in the pupal life.

In the case of such diapausing species as *Luehdorfia japonica* and *Platysamia cecropia*, brains apparently begin to decrease the activity of secreting the brain hormone immediately after pupating. Consequently the prothoracic gland cannot be stimulated so as to secrete a different hormone which is directly responsible for the metamorphosis. Thus, the pupae cannot metamorphose into butterflies or moths, till the brain hormone is produced or introduced someway into them. Williams (1947, '52) succeeded in interrupting this prolonged pupal diapause of *cecropia*-silkworm by transplanting the chilled brains removed from pupae of the same species and Ichikawa and Nishiitsutsuji (1951) could also induce the precocious metamorphosis of *Luehdorfia japonica* by introducing the active brains isolated from *Bombyx*-mature larvae. These facts demonstrate that the prolonged pupal diapause of these insects is solely conditioned by temporary blocking of the secretory function of their brains and that the prothoracic glands are always competent to release the hormone, if they are activated continuously by the brain hormone. Further it is evidenced that the skin and other tissues can react on the

hormone from the prothoracic glands whenever the latter is sufficiently supplied.

In the case of non-diapausing insects such as commercial silkworms and Eri-silkworms, larvae deprived of their own brains in the mounting stage can proceed on development, though slowly, and undergo the pupation by themselves. However, it is noticed here that this fact cannot refute the necessity of the brain hormone in the process of pupation. It is plausible in this case to consider that the prothoracic glands have already been stimulated to some extent to produce their hormone by the hormone which had come from the brain before its removal. The reason for this is the evidence that the brainless siklworms in which the most active brains are planted can pupate several days before the control silkworms. Here we can arrive at the same conclusion as stated in the previous paper that the hormone from the brain apparently governs the onset and maintenance of function of the prothoracic gland.

Histological examination of our insects, *Philosamia*, *Bombyx* and *Luehdorfia*, revealed the existence of large neurosecretory cells in the pars intercerebralis of the brain as in other insects such as *Rhodnius* (Hanström, 1938; Wigglesworth, 1940), *Leucophaea* (Scharrer, 1941~'52), *Eacles* (Day, 1940), *Ephestia* (Rehm, 1950) and *Calliphora* (Thomsen, 1948~'52). They have been demonstrated in larvae of all instars and in pupae. It is sure that they produce the brain hormone discussed above. The morphology of these neurosecretory cells, however, will be described in a separate paper.

Summary

1. From the endocrinological view point, activity of the brains removed from larvae and pupae of *Philosamia cynthia ricini* was examined by means of transplanting them into the larvae previously deprived of their own brains before the critical period for pupation in the last instar.

2. The brain is found to be already functional from the second day of the second instar. It becomes the most effective in the mounting stage of the last instar, so far as the present experiments are concerned. The pupal brain is also evidenced to be functional.

Literature

Day, M. F., 1940. Neurosecretory cells in the ganglia of Lepidoptera. Nature, **145**.

Ichikawa, M. and J. Nishiitsutsuji, 1951. Studies on the insect metamorphosis. I. Rôle of brain in the imaginal differentiation of lepidopterans. Annot. Zool. Japon., **24**.

_____. 1952. Studies on the insect metamorphosis. II. Determination of the critical period for pupation in the Eri-silkworm, *Philosamia cynthia ricini*. Ibid., **25**.

Rehm, M., 1950. Sekretionsperioden neurosekretorischer Zellen im Gehirn von *Ephestia kühniella*. Zeitsch. f. Naturforsch., **5** b.

Scharrer, B., 1941. Neurosecretion. II. Neurosecretory cells in the central nervous system of cockroaches. J. Comp. Neur., **74**.

———, 1951. The storage of neurosecretory material in the corpus cardiacum. Anat. Rec., **111**.

———, 1952. Ueber neuroendokrine Vorgänge bei Insekten. Pflügers Arch., **255**.

———, 1952. Neurosecretion. XI. The effects of nerve section on the intercerebralis-cardiacum-allatum system of the insect *Leucophaea maderae*. Biol. Bull., **102**.

Thomsen, E., 1948. Effect of removal of neurosecretory cells in the brain of adult *Calliphora erythrocephalla* Meig. Nature. **161**.

———, 1952 Functional significance of the neurosecretory brain cells and the corpus cardiacum in the female blow-fly, *Calliphora erythrocephalla* Meig. J. Exp. Biol, **29**.

Wigglesworth, V. B., 1940. The determination of characters at metamorphosis in *Rhodnius prolixus* (Hemiptera). J. Exp. Biol., **17**.

Williams, C. M., 1947. Physiology of insect diapause. II. Interaction between the pupal brain and prothoracic glands in metamorphosis of the giant silkworm, *Platysamia cecropia*. Biol. Bull., **93**.

———, 1952. Physiology of insect diapause. IV. The brain and prothoracic glands as an endocrine system in the *cecropia*-silkworm. Ibid., **103**.