An analytical study of the neurosecretory cells of the brain in *Philosamia ricini* (Hutt.) (Lepidoptera: Saturniidae) during post-embryonic development

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Summary. A multiplicity of types of neurosecretory cell (NSC), and uniformity in the numbers occurring in medial, lateral, posterior and tritocerebral groups, have been observed in *P. ricini*. 3 types of cells A, B and C, with their subtypes (A-1, A-2, A-3; B-1, B-2; C-1 and C-2) have been reported. Only B-cells increase in numbers during post-embryonic development. Others (A and C) remain constant throughout development. Abnormal staining behaviour of A-cells has also been reported.

Although much work has been done on the number and distribution of neurosecretory cell-types in lepidopteran insects[2-12], very few attempts have been made to study them in detail during post embryonic development (i.e. from 1st instar to 5th instar larva, pupa and adult). Mitsuhashi[5] has reported considerable variations in the types and numbers of cells in Lepidoptera; Panov and Kind[6], Herman and Gilbert[4] and Hinks[8] have reported a multiplicity of cell types but a remarkable uniformity in the number of cells. Singh and Arif[11] reported variations in the number of cells in *Philosamia ricini*. The present observations on the numbers, types and groups of cells in *Philosamia ricini* differ considerably from those of Singh and Arif[11].

The central aim of the present study is to examine and confirm the occurrence of various types, numbers and groups of NSCs in the brain of *Philosamia ricini* during post-embryonic development.

Materials and methods. The eri-silkworms, *Philosamia ricini* were reared and maintained in the laboratory on the leaves of castor oil plants *(Ricinus communis)* as described earlier[13]. The brains of larvae of each instar, pupae and adults of the appropriate ages were dissected out in saline-water and fixed in Bouin's fluid, then processed using the paraldehyde fuchsin[14] technique on whole brains as well as on sections of 8–10 µm in thickness.

Results. Based on the staining properties as described earlier[12], 3 main types of cells viz., A, B and C have been observed in *P. ricini*. The A-cells have been further classified into A-1, A-2, A-3; B-cells into B-1, B-2, and C-cells into C-1 and C-2 subtypes.

Larva. 1st instar. The neurosecretory cells (NSCs) are clearly visible in the anterodorsal part of the protocerebrum in 1-day-old larvae (figure 1). In addition to medial groups, the NSCs in the lateral and posterior groups have also been observed. Only the A-cells are observed in the 1st instar larvae (table 1). The B- and C-cells are either inactive or absent. The medial cells move towards the mid-dorsal part of the protocerebrum in the later stages of larval life, but the position of other groups of cells remains the same.

2nd instar. The A-cells can be clearly divided into their subtypes in the 2nd instar larvae. In the medial group there are only 2 subtypes of A-cells (A-1 and A-2). Besides these, B-1 and C-1 cells are also observed. The lateral groups contain A-2 and B-2, and the posterior A-3 and B-2 cells. In the tritocerebral group only B-2 cells are present (table 1).

3rd instar. All the 4 groups of cells viz., medial, lateral, posterior and tritocerebral, have been observed to contain a moderate amount of neurosecretory material (NSM). The distribution of cells in these groups is similar to that seen in the second instar. The A-3 cells of the medial groups, described by Singh and Arif[11], are absent. Each medial group is composed of 4A-1 and 4A-2 cells and not 5A-1 cells as described by Singh and Arif[11]. The number of various sub-types of A-cells remains the same as in the 2nd instar but the number of B-cells increases (table 1). The C-1 cells have also been found but the C-2 are either inactive or absent.

4th instar. No alteration in the numbers and types of A-cells was observed in the 4th instar larvae (table 1) except an increment of the B-types of cells. In medial groups there are 2 subtypes of B-cells i.e. B-1 and B-2. The positions of cell groups remain the same as in the 3rd instar.

Just after moulting, the lateral A-2 cells show enhanced activity. The pathways are also clearly marked out due to presence of NSM under transport in them. In certain larvae, there are 2 lateral groups in each brain lobe, of which one group is composed of 4 A-2 cells close to the medial group and the other group is formed by the remaining 2 A-2 cells.

5th instar. In the medial groups only 2 subtypes of A-cells viz. A-1 and A-2 (figure 2) are present, and not three as described by Singh and Arif[11]. The B-1, B-2 and C-1, C-2 cells in the medial (figure 10) and B-2 cells in the lateral (figure 6) groups are quite distinct. The number of different

Table 1. Showing the average number of medial, lateral, posterior, and tritocerebral neurosecretory cells occurring in each cerebral lobe in *P. ricini* during post embryonic development

Groups of cells	Larval instar					Pupa			Adult	
	I	II	III	IV	V	Prepupa	Mid-pupa	Late-pupa	Male	Female
Medial										
A-1	4	4	4	4	4	4	4	4	4	4
A-2	4	4	4	4	4	4	4	4	4	4
B-1	–	5–7	6–8	8–11	10–14	11–13	11–13	11–15	13–17	13–18
B-2	– – – – – –	Indistinct	– – – – –	6–10	10–15	11–15	14–18	14–20	–	–
C-1	–	2	2	2	2	2	2	2	2	2
C-2	–	–	–	2	2	2	2	2	2	2
Lateral										
A-2	4–5	6	6	6	6	6	6	6	7–8	7–8
B-2	–	3–5	4–5	5–7	6–9	6–9	6–8	6–9	7–10	7–9
Posterior										
A-3	2	2	2	2	2	2	2	2	2	2
B-2	–	3–5	5–6	5–6	6–8	6–8	8–11	11–13	13–18	13–19
Tritocerebral										
B-2	–	2–3	3–4	4–6	6–7	6–7	8–9	8–10	10–12	10–12

subtypes of A, B- and C-cells in the medial, lateral, posterior and tritocerebral groups are given in table 1. It was found that the lateral A-2 cells remain active in the early stages which is indicated by the presence of a moderate amount of NSM in the cell bodies and transportation of NSM via axons. Meanwhile, the A-1 cells are in the initiation of the active phase. The activity in A-1 cells increases further at the middle of the larval period (figure 8) and that can also be seen in the axons showing transport of NSM. Before pupation, cells become inactive and are packed with undischarged NSM.

An interesting observation that has been made in the present work, is the domonstration of different staining properties in A-types of cells as reported in *Nezara*[15]; where a portion, or the upper half portion of the cell(s) stained dark purple like the A-cells while the remaining lower half, stained orange-green or green, like the B-cells (arrows, figure 9). This shows that some biochemical change has occurred in the A-cells at a very specific stage. Such properties have been observed at the last stages of the 5th instar.

Pupa. The life span of the pupae was broadly divided into 3 stages i.e. prepupa, mid-pupa and late-pupa, and during these stages the following observations were made:

Prepupa. At this stage, i.e. from spinning to pupation, there is no change in the number of NSCs (table 1). The A, B and C-cells are present in their respective groups as found in larval stages (table 1). Most of the A-1 cells are provided with a small amount of NSM, while the A-2 cells are heavily loaded (figure 3). The lateral A-2 cells in the beginning are lightly stained indicating poor activity. At later stages, A-2 cells contain a moderate amount of NSM

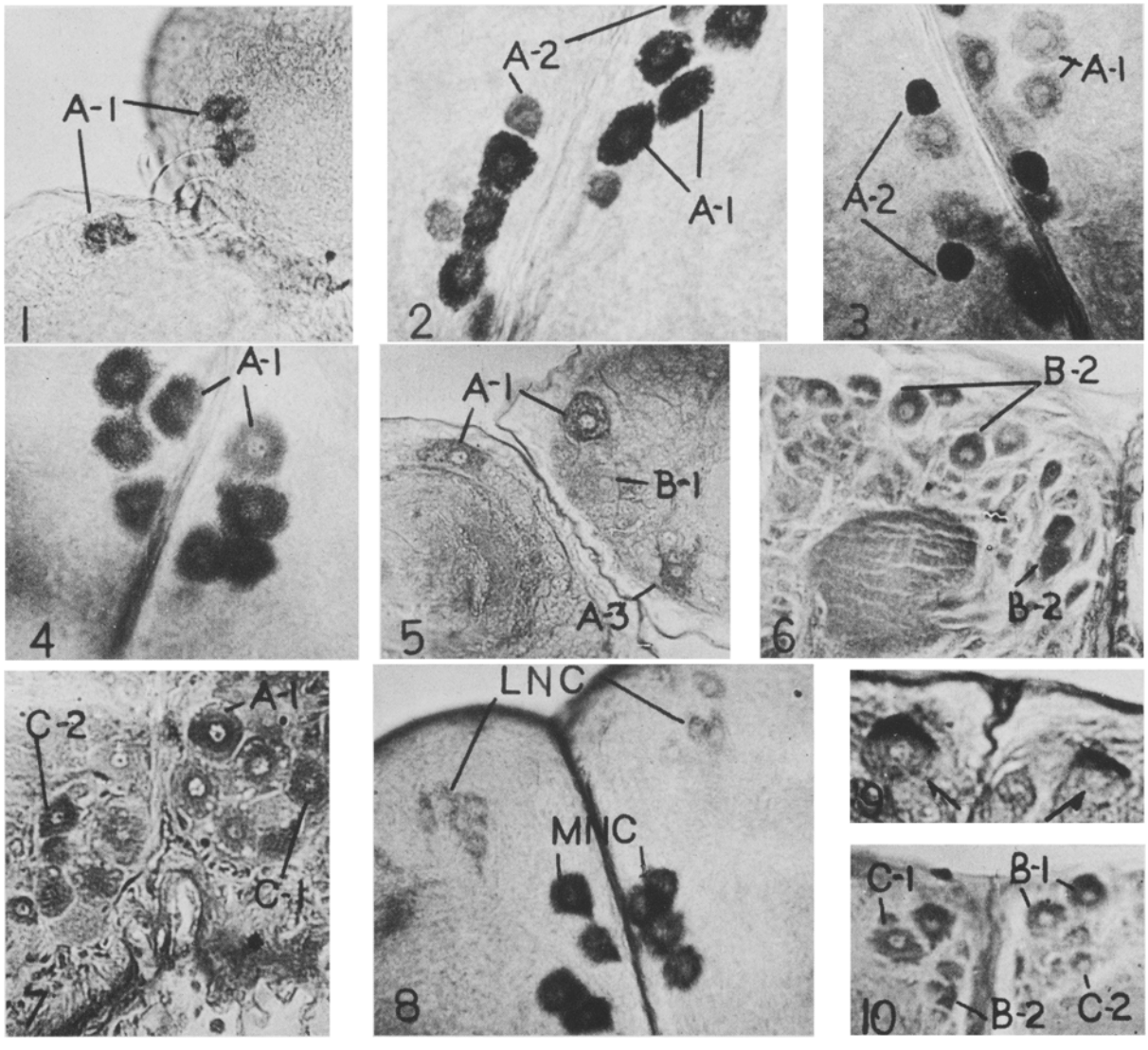

Fig. 1. Whole mount of brain of a 1st instar larva showing 4+4 A-1 cells in each medial group. ×250. Fig. 2. Dissected pars intercerebralis of the protocerebrum of a 5th instar (5-day-old) larva showing A-1 and A-2 cells. Out of 4 A-2, only 2 are visible in each lobe, the other two are seated deeply and thus are out of focus. ×366. Fig. 3. Dissected pars intercerebralis of the protocerebrum of a pre-pupa showing A-1 and A-2 cells. The A-1 cells contain a small amount of NSM, the A-2 cells are packed with NSM. ×300. Fig. 4. Dissected pars intercerebralis of the protocerebrum of a male moth showing active A-1 cells, the A-2 being inactive and are not clearly seen. ×300. Fig. 5. F.S. of brain of a 5th instar larva showing medial A-1, B-1 and posterior A-3 cells with a small amount of NSM. ×245. Fig. 6. F.S. of brain of a 5th instar larva showing medial and lateral B-2 cells. ×500. Fig. 7. T.S. of brain of a late-pupa, showing vacuolated A-1 cells. The C-1 as well as C-2 cells are also seen. ×266. Fig. 8. Whole mount of brain of a 5th instar larva showing medial and lateral neurosecretory cells (MNC, LNC). The medial cells contain a moderate amount of NSM, the lateral cells also indicate activity. ×285. Fig. 9. T.S. of brain of a mature larva showing A-1 cells whose upper part is purple and lower is orange-green (arrows) ×366. Fig. 10. T.S. of brain of the mature larva showing medial B-1, B-2 and C-1, C-2 cells. ×266.

Table 2. Showing the average size of NSCs types (in μm) in the brain of *P. ricini* during post embryonic development

Cell-groups	Larva					Pupa			Adult	
	1st instar	2nd instar	3rd instar	4th instar	5th instar	Prepupa	Mid-pupa	Late-pupa	Male	Female
Medial										
A-1 Cell body	15.7× 11.75	20.75× 15.25	24.00× 22.5	29.1× 20.2	30.5× 26.00	29.90× 25.25	28.75× 27.5	32.75× 30.75	38.25× 34.25	36.92× 33.45
Nucleus	6.17	6.25	9.25	9.25	11.25	10.50	7.5	9.5	10.00	10.00
A-2 Cell body	10.5× 9.75	11.5× 10.25	12.5× 11.65	14.5× 13.33	15.75× 14.87	16.80× 14.5	23.00× 21.0	22.75× 20.00	22.5× 18.5	23.75× 20.4
Nucleus	4.5	5.00	5.00	5.50	5.75	5.5	5.5	7.00	6.75	6.82
B-1 Cell body	-	13.25× 12.00	15.82× 14.15	18.75× 15.2	20.25× 18.17	22.25× 20.43	26.25× 22.75	27.5× 25.00	28.00× 24.27	26.65× 24.57
Nucleus	-	5.5	6.92	6.75	7.5	7.45	7.5	7.5	7.5	8.15
B-2 Cell body	-	-	-	11.07× 10.70	13.50× 12.75	15.80× 14.08	17.60× 16.00	20.75× 17.5	-	-
Nucleus	-	-	-	4.5	4.15	5.00	6.00	6.3	-	-
C-1 Cell body	-	16.25× 14.25	22.12× 17.12	22.5× 17.5	25.00× 18.75	28.15× 25.43	32.25× 28.75	31.80× 27.75	35.7× 31.25	34.37× 30.5
Nucleus	-	6.5	7.05	7.00	6.95	7.85	9.75	11.25	10.75	11.00
C-2 Cell body	-	-	-	12.5× 11.87	13.25× 13.5	14.12× 12.00	16.25× 15.00	17.82× 15.75	19.37× 15.62	20.82× 17.5
Nucleus	-	-	-	5.00	5.00	5.15	5.00	6.25	6.00	6.25
Lateral										
A-2 Cell body	10.0× 8.75	12.85× 10.35	13.35× 12.02	15.0× 14.3	16.8× 15.00	18.78× 15.65	23.75× 22.75	25.25× 23.75	23.12× 20.00	23.45× 19.78
Nucleus	4.25	4.75	5.75	5.75	7.25	6.90	6.25	5.78	5.75	5.95
B-2 Cell body	-	12.25× 11.25	13.5× 12.5	16.00× 15.00	18.92× 16.07	22.25× 18.98	24.5× 22.5	27.00× 23.75	27.8× 25.00	26.00× 23.25
Nucleus	-	5.00	5.4	6.65	6.5	6.15	6.25	7.78	8.25	7.85
Posterior										
A-3 Cell body	12.5× 11.25	12.5× 12.5	18.15× 17.25	19.0× 17.0	20.00× 19.75	21.25× 19.80	21.93× 20.23	25.18× 23.01	26.12× 25.00	25.80× 24.50
Nucleus	4.30	7.5	7.5	9.75	10.00	10.15	10.67	10.70	8.00	8.01
B-2 Cell body	-	12.07× 11.79	15.00× 13.32	18.75× 13.25	20.75× 18.97	21.75× 19.28	24.00× 22.00	25.00× 22.5	27.5× 23.12	27.75× 24.00
Nucleus	-	5.25	6.00	6.25	7.75	7.80	9.5	11.5	10.5	10.9
Tritocerebral										
B-2 Cell body	-	15.00× 12.5	15.83× 14.25	19.00× 13.25	21.4× 17.5	22.00× 18.15	21.5× 18.5	25.00× 22.5	25.00× 22.5	25.82× 24.00
Nucleus	-	7.5	6.25	6.25	8.00	7.85	7.75	11.5	9.25	10.00

Longer and shorter diameter of each type of cell and nucleus is taken and average is recorded.

in cell bodies as well as in axons, showing enhanced activity.

Mid-pupa. In 9- to 11-day-old pupae, due to changes in the size and shape of the brain (from larva to pupa), re-orientation of all groups takes place. The tritocerebral group comes toward the posterior side and lies horizontally and the cells of the posterior group are scattered in the posterior part of the brain. The lateral cells are scattered on the antero-lateral part of the protocerebrum. In certain individuals, in each brain lobe 2 lateral groups of cells are observed as in the 4th instar larvae. The number of A-1 and A-2 cells in the medial and A-3 cells in the posterior groups remains the same. The B- and C-cells of all the groups stained more deeply, and contained a large amount of their specific secretions.

Late-pupa. At this stage i.e. 2–3 days before imaginal ecdysis, all the 3 types of NSCs are present (table 1). No decrease in the number of cells is observed as described by Singh and Arif[11]. The number and size of medial B-cells increases further (tables 1 and 2). Posterior cells are also observed clearly which have been reported to be absent by Singh and Arif[11]. The A-1 cells contain a large number of vacuoles towards the periphery, with a fair amount of NSM (figure 7), indicating secretory activity. Such vacuoles are not observed in C-1 and C-2 cells.

Adult. Male. The A-cells in the newly moulted individuals are stained faintly with fine granules showing initiation of the activity. The number of A-1 and A-2 cells in each medial group remains constant as in the larvae and pupae (table 1). The A-2 cells of lateral group are either arranged in a linear fashion or in clusters. The B-2 cells are haphazardly arranged among the A-2 cells. The posterior A-3 and B-2 cells are also present. Singh and Arif[11] reported that the tritocerebral cells (called posterior cells in the present work and by all other workers) are absent. The C-1 and C-2 cells are present in the medial group as in the larvae and pupae (table 1). Before or at the time of mating the A-1 cells become active, as indicated by a moderate amount of NSM, with vacuolated cytoplasm and transportation of NSM via axons. Before the death of the moths, the NSCs are heavily loaded with NSM, which indicates inactivity.

Female. The number and types of cells in the females are almost the same as found in the males (table 1). Singh and Arif[11] remarked that the number of medial A-1 cells vary in the 2 sexes i.e. 6 A-1 in female and 4 A-1 in male in each brain lobe. No such variation in the number of cells has been observed in the present work (table 1). The C-1 and C-2 cells are present in the medial group only. Cells of the newly-emerged females contain finely granulated cytoplasm, stained faintly and thus showing initiation of activity. At the time of egg laying and mating the cells are provided with a fair amount of NSM. At the same time the NSM is also observed in axons. Before death the cells become inactive as in the males.

Discussion. Numerous workers[4,6,8,12] have reported a multiplicity of cell-types and uniformity in their numbers but a few[5,11] have found a considerable variation in types as well as in numbers of cells in lepidopterans studied by them. In the present work, diversity of cell types and constancy in their numbers have been clearly observed at all the developmental stages. Three principal types of cells viz., A, B and C have been observed in *Philosamia,* instead of four as described by Singh and Arif[11]. The B-cells of Singh and Arif[11] seem to be ordinary neurons. The tritocerebral(?) group of Singh and Arif[11] is in fact the posterior group of other workers. Mitsuhashi[5] has also given a very ambiguous account of the cell types.

A remarkable uniformity in the numbers of A-cells (and their subtypes) has been reported by Herman and Gilbert[4], Panov and Kind[6], Awasthi and Singh[12], Raina and Bell[10] and also Hinks[8] in 23 species of Lepidoptera; Mitsuhashi[5] has given a very indistinct account of NSCs. Singh and Arif[11] observed variations in the numbers of cells in *Philosamia ricini* and reported that there were 5 A-1 cells in each medial group from 2nd instar larva to pupa, 6 A-1 cells in the female and 4 A-1 cells in the male; the number of A-2 was constant in larva, pupa and adult. However, we have observed in *P. ricini* a uniformity in the number of A-cells (subtypes) during the entire development (table 1) i.e. from 1st instar to 5th instar larva, pupa and adult of both sexes and thus disagree with the observations made by Singh and Arif[11] on *P. ricini.*

Mitsuhashi[5], McLeod and Beck[3], Herman and Gilbert[4], Sen and Gangrade[16], Raina and Bell[10] and Singh and Arif[11] have observed 3 paired groups; Hinks[8] found only 2 groups in 23 species of adult lepidopterans; Awasthi and Singh[12] reported 6 paired groups in *Amsacta.* In *P. ricini* we have observed 4 paired groups viz., medial, lateral, posterior and tritocerebral. The posterior group has been observed in all the Lepidopterans examined so far[2–5,9–13,16] and is one of the characteristic features of this order.

Further, the unequal or double but distinct staining patterns of cells as observed in the present work, and also reported earlier[15], indicates that there must be some biochemical changes occurring within the cells, because part of them (e.g. upper half) behave like A-cells and the remainder (i.e. lower half) like B-cells (as far as staining is concerned). What is actually going on inside the cell body at the molecular level, and the biochemical processes involved, is unknown. Due to the paucity of information on this type of behaviour of the cells, a correct interpretation will be possible only after ultrastructural and biochemical studies.

The number of A- and C-cells in the medial group remains constant throughout development; an increase in the number of B (B-1 and B-2) cells was observed as also reported by Singh and Arif[11]. However, the present observations do not support the view that there is a gradual decrease of cells from the beginning of spinning until emergence[11]. A brief mention may be made here of the secretory activity. The present work has revealed that all cell-types show a distinct cyclic activity within an instar as well as in each stage (larva, pupa, adult) of the post embryonic development, the longer and shorter diameters of cell bodies and nuclei are given in table 2.

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