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Embryonic Development of Nervous System in the Alderfly, *Sialis mitsuhashii* Okamoto (Megaloptera, Sialidae)*

Nobuo SUZUKI

Satoru SHIMIZU

and

Hiroshi ANDO

Synopsis

The nervous system formation in the embryo of the alderfly, *Sialis mitsuhashii* Okamoto (Megaloptera, Sialidae), is described. The ventral nerve cord is formed mainly from neuroblasts occurred in three gnathal, three thoracic, and ten abdominal segments. The median cord seems to contribute the formation of the transverse commissure and dorsal cortical layer of the ganglion. The modified outer ganglion cells give rise to the perilemma. The embryogenesis of the brain occurs in usual manner as in most insects. The stomatogastric nervous system is derived from three evaginations along the medio-dorsal part of the stomodaeal roof. The anterior evagination provides the frontal ganglion, and the middle and posterior ones the hypocerebral ganglion and small ganglion-like swelling, which degenerates up to hatching, respectively.

Introduction

The Megaloptera has been considered as the most primitive order of holometabolous insects because of its fossil record, and the embryology of this order, which consists of two families, Sialidae and Corydalidae, is poorly known.

As far as the authors are aware, there are only six papers, concerning *Sialis* (Siali-

* Contributions from Sugadaira Montane Research Center, University of Tsukuba, No. 113.

dae): Strindberg (1915) — general embryology, Du Bois (1938) — experimental research with brief description of normal embryology, Suzuki *et al.* (1981) — early embryogenesis, Ando *et al.* (1985) — external morphology of embryogenesis; and *Protohermes* (Corydalidae), Miyakawa (1979) — external features of embryos through development, Miyakawa (1980) — embryogenesis of abdominal appendages.

As mentioned above, the embryology of the Megaloptera is known only briefly, espcially as for the organogenesis. Accordingly, this paper deals with the formation of the nervous system, and this study might furnish data useful in understanding the relationship between the Megaloptera and other lower holometabolans.

Materials and Methods

Pregnant females and eggs of *Sialis mitsuhashii* Okamoto were captured at the Goshiki Lake, Fukushima Prefecture of Japan in mid-June, 1978－1982. Fixation and sectioning of the eggs were done in same manner as described in the previous paper (Suzuki *et al.*, 1981).

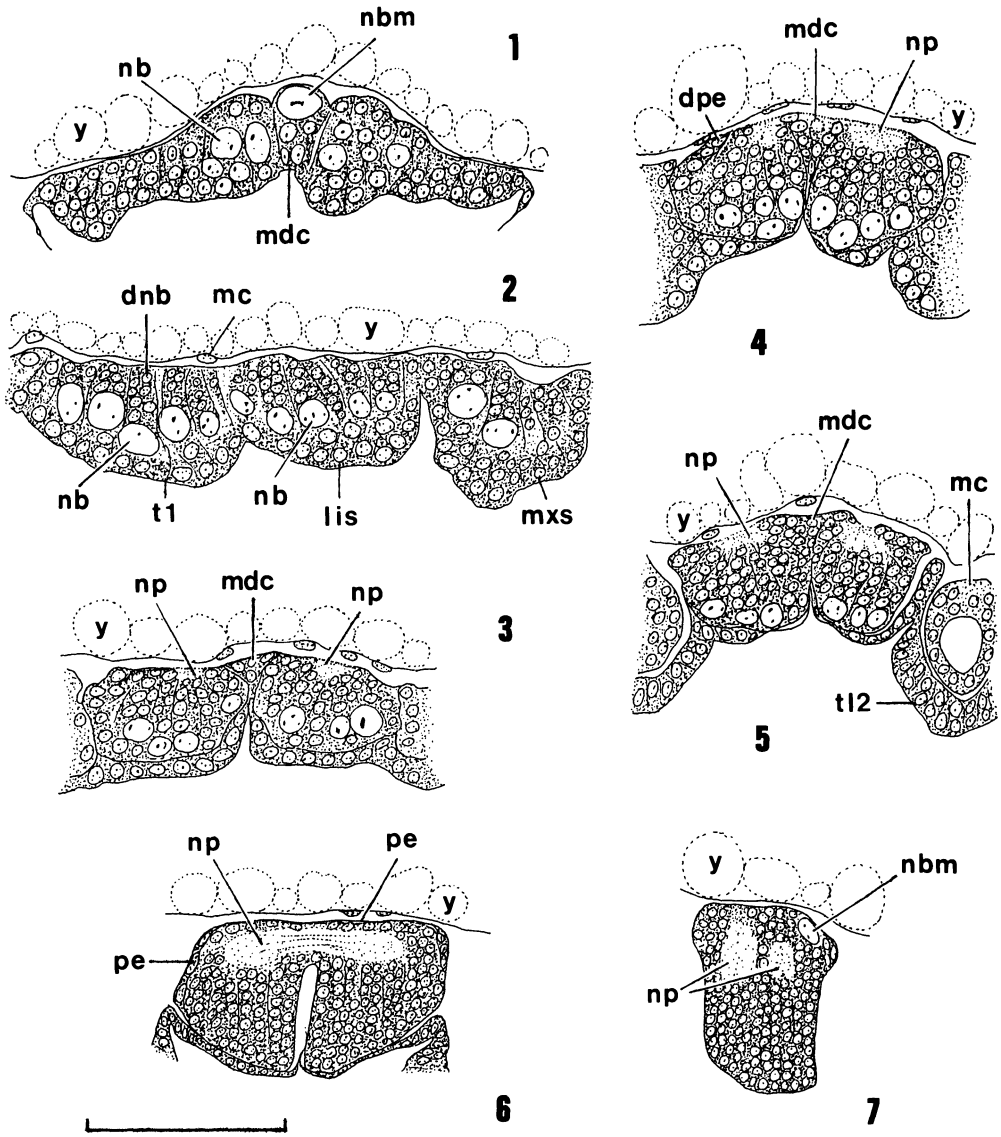
Observations

a) *Ventral nerve cord*

At 55 hours after oviposition, two to three neuroblasts differentiate in the each lateral plate of gnathal, thoracic, and first and second abdominal segments (in cross and longitudinal sections), though there is no neuroblast in the developing median cord yet.

At 60 hours after oviposition, mesodermal cells on the lateral plates become a thin single layer, and the number of neuroblasts contained in these plates increases to four to five in longitudinal section (two to three in cross section). The median cord of the inter- and intrasegmental regions in cross section is rather broad at this stage. The posterior region of the median cord has a cell, stained lightly with hematoxylin, and has a large nucleus in the medio-dorsal portion (Fig. 1), and this may correspond to a neuroblast of the lateral plate.

At about 70 hours after oviposition, the neuroblasts appear in the remaining segments, *i. e.*, to the tenth abdominal segment. The number of neuroblasts at lateral plate of each segment becomes to three to four in number in cross section and five to six in longitudinal section, however four to five in longitudinal section of the tenth abdominal segment. The neuroblasts repeat mitoses to produce their daughter cells above them, so that the columns of nerve cells are formed above these neuroblasts (Fig. 2). As development proceeds the daughter cells, at dorsal portion of the column, start to form the neuropile (Fig. 3). There is a thin mesodermal cell layer on the neuropile, however this layer never differentiates into the perilemma. As ganglia increase in size, the medio-ventral part of the intersegmental median cord becomes narrow in cross section (Fig. 3). On the other hand, the cord of the posterior part of each segment and intrasegmental region still possesses its original feature, and contains a neuroblast in its



Figs. 1—7. Embryonic development of ventral nerve cord in *Sialis mitsuhashii*.
1. Transverse section through posterior part of labium in a 60-hr embryo. 2. Parasagittal section through maxilla to first thorax in a 70-hr embryo. 3—6. Transverse sections through second thorax in a 72-hr embryo (Fig. 3), maxilla in a 90-hr embryo (Fig. 4), second thorax in a 4-day embryo (Fig. 5), and first thorax in a 5-day embryo (Fig. 6). 7. Sagittal section through first thorax in a 6-day embryo.
dnb, daughter cell of neuroblast; dpe, developing perilemma; lis, labial segment; mc, mesodermal cell; mdc, median cord; mxs, maxillary segment; nb, neuroblast; nbm, neuroblast of median cord; np, neuropile; pe, perilemma; tl, first thoracic segment; tl2 appendage of second thoracic segment; y, yolk. Scale: 50 μm.

medio-dorsal part.

At 80 hours after oviposition, the mesodermal layer almost disappears above the developing ganglia, and the ganglia increase in height as the neuroblasts repeat mitoses and produce nerve or ganglion cells. The neuropile at the dorsal part of each ganglion becomes apparent, though there are observed no intrasegmental connectives. The peripheral ganglion cells begin to be slightly flat and cover the developing ganglia. As development advances those flattened cells become membranous and seem to be a rudimental perilemma (Fig. 4). As a result of the development of ganglia, the ventral half of the intersegmental median cord of each segment becomes thin in cross section, though it connects to the epidermis. The dorsal half of the intersegmental median cord takes a feature of inverted triangle in cross section, and becomes to be light-stained, so that it becomes difficult to discriminate from the developing neuropile of the ganglia (Fig. 4).

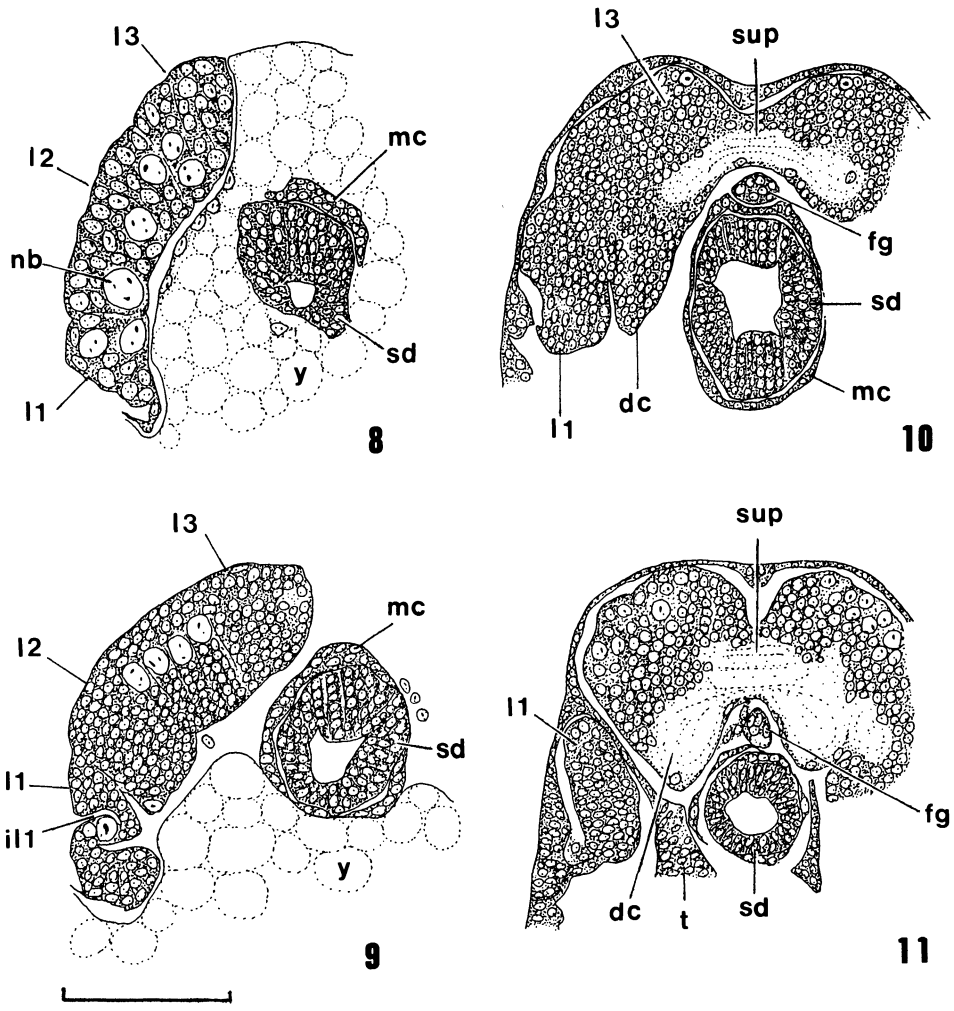
At 4 days after oviposition, the neuropile formation further undergoes, and the anterior and posterior commissures, though only one commissure in mandibular ganglion, and connectives of each ganglion are observed. Between both the commissures, the medio-dorsal region of the median cord seems to participate in the formation of the dorsal cortical layer of the ganglion (Fig. 5). There are a few mitotical figures of the neuroblasts in the ganglia now increased in height, and right and left ganglia begin to move towards the median line. Consequently, the median cord is almost detached from the epidermis throughout the intersegmental regions, on the other hand, they possess the connection with the epidermis at the intrasegmental regions. The ganglia of the three gnathal segments begin to gather.

At 5 days after oviposition, the connectives are formed between each ganglion and covered with the perilemma, and it nearly covers the dorsal part of the each ganglion also (Fig. 6). From lateral sides of the gnathal and thoracic ganglia, a pair of neurofiber differentiates and extends into each appendage. The median cord finally loses the connection at the intersegmental regions, and is nearly detached at the intrasegmental regions. The gnathal ganglia further fuse, and become a rudimental suboesophageal ganglion.

At 6 days after oviposition, the embryo begins to revolve, and the perilemma completely covers the dorsal part of each ganglion. The eighth to tenth abdominal ganglia begin to gather and fuse. The cell mass of the intrasegmental median cord, which contains a neuroblast, exists at the medio-dorsal region of the posterior part of each ganglion (Fig. 7).

At 7 days after oviposition, the eighth to tenth abdominal ganglia become more compact, and situate just behind the seventh abdominal ganglion. The neuroblast of the median cord, located in the posterior margin of each ganglion, becomes undetectable from ganglion cells. As development advances, the epidermis begins to be thin, so that the ganglia are almost removed from the epidermis.

At about 9 days after oviposition, the ganglia are finally detached from the epidermis, and they approximately get the same features of those of the first instar larvae.



Figs. 8 — 11. Embryonic development of brain in *Sialis mitsuhashii*. Horizontal sections through head.
8. 55-hr embryo. 9. 70-hr embryo. 10. 4-day embryo. 11. 5-day embryo.
dc, deutocerebrum; fg, frontal ganglion; il1, invagination of lobus 1; l1 — 3, lobi 1 — 3 of protocerebrum; mc, mesodermal cell; nb, neuroblast; sd, stomodaeum; sup, supraoesophageal commissure; t, tentorium; y, yolk. Scale: 50 μm.

b) *Brain*

The ectodermal cells of protocephalon, antennal and intercalary segments are roughly uniform in size by 2 days. However, there arise several cells containing large nuclei in the ectoderm of those segments at 55 hours after oviposition. They are neuroblasts, and as development undergoes their nuclei become to be faintly stained with hematoxylin. The neuroblasts at the protocephalic lobes are distributed into three groups, which are corresponding to the future lobi 1−3 of the protocerebrum, on each lobe (Fig. 8). The neuroblasts of the antennal segment, which form the deutocerebrum later, are observed at the both sides of the developing stomodaeum, namely at the basal part of antennal anlagen, and those of the intercalary segment produce the future tritocerebrum, and are found just behind the neuroblasts of the antennal segment.

The mitoses of neuroblasts occur, and at 70 hours after oviposition the neuroblasts frequently repeat teloblastical divisions. At the ectoderm of future lobus 1 of the protocerebrum, the teloblastical mitoses are not observed, but found the invagination of the ectoderm containing the neuroblasts (Fig. 9), so that the rudimental lobus 1 seems to be derived from the invaginated ectoderm at first. At this stage the protocerebral lobi 1−3 become easily recognizable, and piled daughter cells of the lobi 2 and 3 start to differentiate into the neuropiles at their dorsal regions. The neuropile of the brain is formed in the same way as that of the ventral nerve cord.

At 3 days after oviposition, developing deuto- and tritocerebra slightly shift forward, and the deutocerebrum locates behind the protocerebral lobus 3. The appearance of the neurilemma occurs, and its formation is similar to that of the ventral nerve cord. At 4 days, the distal region of the lobus 1 begins to be detached from the epidermis (Fig. 10). The supraoesophageal commissure makes its appearance between the lobi 3, and the neuropile is discernible in the commissure (Fig. 10). The neuropile between the lobi 2 and 3 is also visible, and the lobi 1 − 3 are removed from the epidermis. The deutocerebrum moves forward, and the tritocerebrum takes a position just behind the former. As development proceeds, the three cerebra connect with each other by each connective, and there occur the connectives between the tritocerebrum and mandibular ganglion also.

At 5 days after oviposition, the protocerebral lobi 1 and 2 shift forward and lobus 3 shifts medially. The distal end of the lobus 1 connects with the epidermis again, and this distal part and epidermis connecting with the lobus 1 later become the rudimental postretinal fiber and optic plate respectively. The commissure of the deutocerebrum, locating beneath the protocerebrum, becomes confluent with the protocerebral commissure, and it seems to form the supraoesophageal commissure (Fig. 11). There appears a tritocerebral commissure, *i. e.*, suboesophageal commissure under the stomodaeum. The tritocerebrum sends neurofibers to the frontal ganglion. The protocerebral lobi 1−3 shift mediad further, and the neuropilar region develops in them, though it is ill-developed in the lobus 1, at 6 days after oviposition. The lobus 1 is divided into distal and proximal parts, and the epidermis connected with the distal end is slightly thickened, so that the optic plate begins to differentiate. The deutocerebral nerves innervate the antennae.

At 7 days, the protocerebrum takes a position horizontal above the stomodaeum in the embryonic head capsule. The neuropiles further develop among the cerebra, and

also in the protocerebral lobus 1 as development advances. At 8 days after oviposition, the three cerebra consolidate into a single mass, *viz.*, brain. The brain assumes an ellipsoid shape and lies horizontally over the stomodaeum. In the cortical layer at each posterior margin of the protocerebrum, two large neurosecretory cells are constantly observed. No significant changes are found in the brain development up to the time of hatching.

c) *Stomatogastric nervous system*

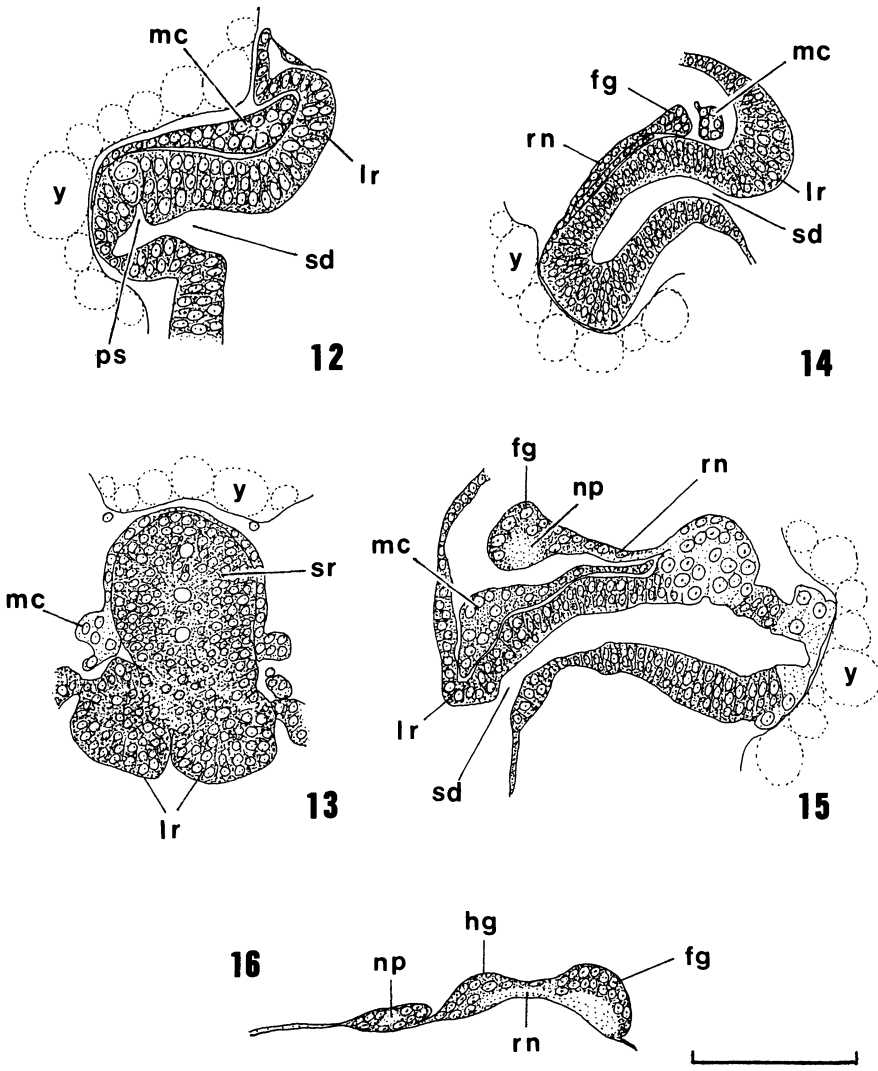
At 60 hours after oviposition, there arise three pits along the median line of the dorsal wall of the developing stomodaeum. These pits later become three evaginations, by which the stomatogastric nervous system is produced. The ectoderm which lies on the pits has a few large cells (Fig. 12). At 70 hours, there occur three small evaginations along the median line of the stomodaeal roof (Fig. 13) and they are covered with mesodermal cells. At 3 days after oviposition, the evaginations change into three cell masses and begin to extend forward. At 5 days after oviposition, the anterior cell mass from the anterior evagination produces the anlage of frontal ganglion (Fig. 14). Simultaneously the anterior and middle cell masses are detached from the stomodaeal roof. As for the posterior mass, it has still a connection with the roof.

At 6 days after oviposition, the rudimental frontal ganglion migrates forward and locates in front of the supraoesophageal commissure. There appear the nervefibers between the frontal ganglion and tritocerebrum, and are recognizable the neuropilar regions in these ganglia. The middle cell mass becomes corded and forms a recurrent nerve running from the frontal ganglion to the osesophagus (Fig. 15), and then the cord gets a small thickening, which is a future hypocerebral ganglion and corresponds to the oesophageal ganglion in *Sialis lutaria* (Strindberg, 1915), at its middle portion. The posterior mass exists on the stomodaeal roof near its blind end, and consists of light-staining cells. The anterior part of the posterior mass extends forward and connects with the developing recurrent nerve. At 7 days after oviposition, the posterior cell mass finally begins to be detached from the stomodaeal roof. There appears the neuropile within the middle swelling of the cord. At 8 days, the frontal ganglion further migrates forward, and the posterior cell mass produces a ganglion-like swelling. The neuropile differentiates within the swelling, which situates just behind the anlage of hypocerebral ganglion (Fig. 16). This swelling becomes ill-developed up to the time of hatching.

Discussion

Median cord

As for the fate of the median cord, Baden (1936) concluded that it does not participate in the ganglion formation in orthopteran *Melanoplus*. According to general agreement about role of the cord, however, it shows a contribution to the ganglion formation in many insects.



Figs. 12—16. Embryonic development of stomatogastric nervous system in *Sialis mitsuhashii*.

12. Slightly oblique sagittal section through head of a 60-hr embryo. 13. Transverse section through head of a 70-hr embryo. 14. Slightly oblique sagittal section through head of a 5-day embryo. Sagittal section through head of a 6-day embryo. 16. Sagittal section through stomatogastric nervous system of a 8-day embryo.

fg, frontal ganglion; hg, hypocerebral ganglion; lr, labrum; mc, mesodermal cell; np, neuropile; ps, pit of stomodaeal roof; rn, recurrent nerve; sd, stomodaeum; sr, stomodaeal roof; y, yolk. Scale: 50 μm.

For example, the median cord in part forms the transverse commissure and perilemma of ganglion in lepidopteran *Chilo* (Okada, 1960) and *Endoclita* (Tanaka *et al.*, 1985). The cord produces glial elements in hemipterans (Springer and Rutschky, 1969) and coleopteran *Lytta* (Rempel *et al.*, 1977). On the other hand, it participates in both formations of the glial element and dorsal cortical layer of the ganglion in trichopteran *Stenopsyche* (Miyakawa, 1974) and lepidopteran *Neomicropteryx* (Kobayashi and Ando, 1983).

In *Sialis*, the median cord principally contributes to the formation of the transverse commissure and the dorsal cortical layer of the ganglion, though there is a little possibility that the glial element partially develops from the cord. Consequently the role of the median cord in *Sialis* is thought to be similar to that of *Endoclita* (Tanaka *et al.*, 1985).

Brain

Miyakawa (1974) and Kobayashi and Ando (1983) reported that the lobus 1 of the protocerebrum is made from daughter cells of the neuroblasts. In *Sialis*, the lobus 1 occurs from the invagination of the ectoderm containing neuroblasts at embryonic head lobe. The neuroblasts, however, seem not to divide teloblastically and may correspond to the neuroblast-like cells which appear in the course of development of the stomatogastric nervous system in coleopteran *Tenebrio* (Ullmann, 1967), so that they may not be true neuroblasts in the strict sense. The fact that the lobus 1 occurs from the ectodermal invagination agree with the view of Ullmann (1967) and Rempel *et al.* (1977).

Although the deutocerebral commissure is absent in some insects (Eastham, 1930; Tanaka *et al.*, 1985; *etc.*), in *Sialis* the proto- and probably deutocerebral commissures form the supraoesophageal commissure as in *Tenebrio* (Ullmann, 1967) and *Stenopsyche* (Miyakawa, 1974), and the suboesophageal one occurs in usual manner observed in many insects.

Perilemma

The perilemma consists of a cellular uni-layer, the perineurium, and its secretion product represents the neurilemma (Rempel *et al.*, 1977). Though there are several opinions in regard to origin of the perilemma in ventral ganglion, they agree in point of the fact that it derives from the ectoderm, except for Baden's view (1936).

Okada (1960) concluded that the perilemma originates from the median cord, and more generally it is thought that it occurs from modified outer ganglion cells (Nelson, 1915; Miyakawa, 1974; Rempel *et al.*, 1977; Kobayashi and Ando, 1983; and in *Sialis*). Besides, according to Ullmann (1967) and Tanaka *et al.* (1985) the perilemma has a dual origin, namely formed from the modified ganglion cells and median cord. There may be no phylogenetic significance as for the origin of the perilemma as same as the fate of the median cord, judging from the facts mentioned above. However the ultras-

tructural investigations should be demanded promptly for those embryogenesis in detail.

Stomatogastric nervous system

The stomatogastric nervous system is derived from cell masses originated from the stomodaeal ectoderm along its dorso-median line. The number of the cell mass is one in *Chilo* (Okada, 1960), two in *Epiophlebia* (Ando, 1962) and *Pieris* (Eastham, 1930), and three in most insects (Anderson, 1972). Even in the last case, there are various opinions about the products of those cell masses except for the anterior cell mass, which gives rise to the frontal ganglion.

In *Sialis*, the middle cell mass provides the recurrent nerve and hypocerebral ganglion as in the case of Ullmann (1967), Rempel *et al.* (1977) and Kobayashi and Ando (1983), and the ganglion-like swelling occurred from the posterior mass seems to correspond with the ventricular ganglion, mentioned by those authors.

References

Anderson, D. T., 1972. The development of holometabolous insects. *In* S. J. Counce and C. H. Waddington (eds.), Developmental Systems: Insects, Vol. 1, 165-242. Academic Press, London, New York.

Ando, H., 1962. The Comparative Embryology of Odonata with Special Reference to a Relic Dragonfly *Epiophlebia superstes* Selys. Jpn. Soc. Promot. Sci., Tokyo.

————, K. Miyakawa and S. Shimizu, 1985. External features of *Sialis mitsuhashii* embryo through development (Megaloptera, Sialidae). *In* H. Ando and K. Miya (eds.), Recent Advances in Insect Embryology in Japan, 191-201. Arthropod. Embryol. Soc. Jpn. (ISEBU Co. Ltd., Tsukuba).

Baden, V., 1936. Embryology of the nervous system in the grasshopper, *Melanoplus differentialis* (Acrididae; Orthoptera). *J. Morphol.* 60: 159-188.

Du Bois, A. M., 1938. La détermination de l'évauche embryonnaire chez *Sialis lutaria* L. (Megaloptera). *Rev. Suisse Zool.* 45: 1-92.

Eastham, L. E. S., 1930. The embryology of *Pieris rapae*. Organogeny. *Phil. Trans. Roy. Soc. Lond., Ser. B* 219: 1-50.

Kobayashi, Y. and H. Ando, 1983. Embryonic development of alimentary canal and ectodermal derivatives in the primitive moth, *Neomicropteryx nipponensis* Issiki (Lepidoptera, Micropterygidae). *J. Morphol.* 176: 289-314.

Miyakawa, K., 1974. The embryology of the caddisfly, *Stenopsyche griseipennis* MacLachlan (Trichoptera, Stenopsychidae). III. Organogenesis: ectodermal derivatives. *Kontyû* 42: 305-324.

————, 1979. Embryology of the dobsonfly, *Protohermes grandis* Thunberg (Megaloptera: Corydalidae). I. Changes in external form of the embryo during development. *Kontyû* 47: 365-375.

————, 1980. Embryogenesis of the pleuropodia and the abdominal filaments (tracheal gills) in *Protohermes grandis* Thunberg (Megaloptera: Corydalidae). *XVI Int. Congr. Entomol, Abstracts*, 50.

Nelson, J. A., 1915. The Embryology of the Honey Bee. Princeton Univ. Press, Princeton.

Okada, M., 1960. Embryonic development of the rice stem-borer, *Chilo suppressalis*. *Sci. Rep. Tokyo Kyoiku Daigaku, Sec. B* 9: 243-296.

Rempel, J. G., B. S. Heming and N. S. Church, 1977. The embryology of *Lytta viridana* Le Conte (Coleoptera: Meloidae). IX. The central nervous system, stomatogastric nervous system, and endocrine system. *Quaest. Entomol.* 13: 5-23.

Springer, C. A., 1967. Embryology of the thoracic and abdominal ganglia of the large milkweed bug, *Oncopeltus fasciatus* (Dallas), (Hemiptera, Lygaeidae). *J. Morphol.* 122: 1-18.

————, and C. W. Rutschky, 1969. A comparative study of the embryological development of the median cord in Hemiptera. *J. Morphol.* 129: 375-400.

Strindberg, H., 1915. Hauptzüge der Entwicklungsgeschichte von *Sialis lutaria* L. *Zool. Anz.* 46: 167-185.

Suzuki, N., S. Shimizu and H. Ando, 1981. Early embryology of the alderfly, *Sialis mitsuhashii* Okamoto. *Int. J. Insect Morphol. Embryol.* 10: 409-418.

Tanaka, M., Y. Kobayashi and H. Ando, 1985. Embryonic development of the nervous system and other ectodermal derivatives in the primitive moth, *Endoclita sinensis* (Lepidoptera, Hepialidae). *In* H. Ando and K. Miya (eds.), Recent Advances in Insect Embryology in Japan, 215-229. Arthropod. Embryol. Soc. Jpn. (ISEBU Co. Ltd., Tsukuba).

Ullmann, S. L., 1967. The development of the nervous system and other ectodermal derivatives in *Tenebrio molitor* L. (Insecta, Coleoptera). *Phil. Trans. Roy. Soc. Lond., Ser. B* 252: 1-25.

Authors' addresses: Dr. N. Suzuki
Japan Women's College of Physical Education, Kitakarasuyama, Setagaya, Tokyo 157, Japan

Mr. S. Shimizu and Prof. H. Ando
Sugadaira Montane Research Center, University of Tsukuba, Sanada, Nagano 386-22, Japan