Atypical Glial Cells in Avian Brains—Golgi Study—

By

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The identification and classification of glial cells has been established together with the development of various metal impregnation methods such as the Golgi, Cajal, and Rio-Hortega methods, etc., and current established classifications of the glial cells consist of astrocytes (both protoplasmic and fibrous), oligodendroglia, microglia and ependymal cells. Metal impregnation methods, however, have an inevitable weakness that they are not able to stain entire glial elements in a single material and, moreover, the images and types of silver-impregnated cells vary a great deal depending upon the methods used. Therefore, it is apparent that as to morphological studies on glial cells by metallic techniques various of methods should be employed and the pictures of glial cells respectively obtained should be discussed in comparison with each other. It is, however, questionable whether we should deny completely the existence of other types of glial cells, which have never been stained with the silver-impregnation methods.

Up to the present other types of glial cells than the four conventional types have been rarely noted in the mammalian brain and spinal cord at the light microscopic level (Andrew and Ashworth 1945, Klatzo 1952, Ramón-Moliner 1958). Inoue and coworkers (1971), however, observed an atypical glial element in the chicken brain, which could not be identified into any common types from the viewpoint of the conventional criteria of identification. In the present work it is intended to investigate the atypical glial cells mainly in the brain of several species of birds.

Materials and Methods

1. The brains of five species of adult birds; chicken (*Gallus gallus domesticus*), pigeons (*Columba livia domestica*), linnets (*Estrilda amandava*), lovebirds (*Uroloncha striata domestica*) and quails (*Conturnix*

conturnix japonica), were used.

2. The adult rat brains were used.

3. The brains of the young chick (one to three days old after hatching) and the chick embryo (Hamburger-Hamilton stage 37-45) were used.

4. The young rat (one to three weeks old) brains were used.

These brain tissues were stained with Golgi methods modified by Rio-Hortega (1928), Ramón-Moliner (1957), Colonnier (1961) and Stensaas and Stensaas (1968a) respectively. Available techniques for the present work were already described in detail in the previous paper (Inoue 1970).

Observation and Discussion

In the avian brains used in this study there were typical glial cells, which were easily identified as astrocytes, oligodendroglia, microglia or ependymal cells according to the conventional criteria for mammalian glial types (Jakob 1927, Penfield 1932, Glees 1955, Nakai 1963, Polak 1965). Besides these common types a different type of glial cells was found in materials stained with Golgi methods in all avian brains used (Fig. 1-5). The cell body of this type was round, oval or kidney-shaped and sometimes displayed a triangular- or sickle-shape due to the somal protrusions. The longer diameter of the cell body was 6 to 10 μ . The processes were, as a rule, characteristically very thin and numerous, extending in all directions and branching several times smoothly with an acute-angle bifurcation.

This atypical glial cells in the lovebird and linnet brain had similar or rather fewer processes than those of other birds (Fig. 3 and 4), but the manner of their branching and extending was identical with those in other avian brains.

This type of glial cell was distributed in all parts of the brain without any variation in their silver-impregnated images except in the molecular layer of the chicken cerebellum, where processes of this type had a tendency to extend in a direction parallel to the frontal axis, namely to parallel fibers of the granule cells (Fig. 6).

This atypical glial element somewhat resembled microglia in the contour of the cell body and tendency to sending thick primary processes from one pole of the cell body. However, no thorn-like spines, which were characteristic in microglia (Inoue 1971), were observed, and processes were much more numerous than those of microglia. This type of glial cell possessed no vascular feet which were characteristic of astrocytes (Inoue et al 1971). Moreover, processes of this type had a rather smooth surface and were very thin compared with those of protoplasmic astrocytes, and were also different from those

of fibrous astrocytes according to the manner of extending and branching the processes. Compared with oligodendroglia there were several differences in shape, number and the manner in which the processes branched, for processes of oligodendroglia were fewer and thinner, had nodular enlargements in the course of extension, sometimes showed a bead-like shape, and tended to branch at right angles (Inoue et al 1971).

The cell body or somal protrusion of this type was sometimes found in close contact with the nerve cell body as a perineuronal satellite (Fig. 4 and 7), with the dendrite of the nerve cell (Fig. 8) and the blood vessel as a perivascular satellite (Fig. 9), and these relationships were also observed in other types of glial cells such as astrocytes, oligodendroglia and microglia (Inoue 1971, Inoue, et al 1971).

At the light microscopic level the glial elements in the central nervous system have been investigated chiefly with metallic techniques. Since Cajal (1913a, b), Rio-Hortega (1919) and others (Penfield 1928, Tsujiyama 1963 and so on), however, developed the metal impregnation methods for frozen sections (especially using ammoniacal silver solutions), the Golgi method has much less frequently been applied to a morphological study of the glial cells in spite of its superiority to the frozen section methods in getting the complete external configuration (Rio-Hortega 1928, Silver 1945, Klatzo 1952, Ramón-Moliner 1957, 1958, Polak 1965, Stensaas and Stensaas 1968a, b, Millhouse 1971, Inoue, 1970, 1971, Inoue et al 1971). This atypical glial cell in the chicken brain has never been demonstrated by the frozen section methods, but only by the Golgi methods (Inoue et al 1971). In the adult mammals and other animal species no other types of glial cell than the four conventional types have been noted up to the present day even upon observation of Golgi materials, but if the Golgi methods had been used more often, other types of glial elements might have been demonstrated. Klatzo (1952) stained the adult cat brain with his modification of the Golgi method and observed an uncommon glial cell with numerous processes. This type, however, possessed processes branching at right angles, and gliosomes typical of oligodendroglia, and he concluded that they should be classified as a larger variety of oligodendroglia. The type was clearly to be distinguished from the avian atypical glial cells in the manner of branching of the processes.

Consequently, it was not known precisely whether this atypical form of the glial cells in the adult avian brains was a new and specific type or belonged to some variety of the conventional glial forms. If this type was a specific form of the glial elements, not only the avian brains but also the brains of the lower animal species should be further investigated from the viewpoint of the comparative morphology, and the mammalian brains should be re-examined from materials stained

by the Golgi methods.

In the developing mammalian brains, however, an uncommon type of glial cell was demonstrated with the Golgi method by Ramón-Moliner (1958). In the young cat brain (one to two months old) he observed an undifferentiated type of glial cell with 'mossy or seaweed-like' processes and transitional forms in the direction of oligodendroglia at various stages, and this uncommon type of glial cell at certain stages was similar to the avian atypical glial cells, described above.

In the adult rat brain this atypical form of glial cells was not silver-impregnated, whereas in the young rat brain (one to three weeks old) an uncommon type of glial cell, which resembled the atypical one in the avian brains, was stained with Golgi methods (Fig. 10). This type had a round or oval soma, and sent out numerous, thin processes in all directions with frequent branching. Simultaneously another glial type, which Ramón-Moliner (1958) described as 'the undifferentiated type or mossy glia', was silver-impregnated (Fig. 11). The soma of this type gave off a few big trunks, which did not branch until they reached a certain distance from the cell body. They then branched frequently, giving off numerous, thin processes. The uncommon glial cells and 'mossy glia' in the young rat brain, however, often had reticulate-tubular structures on the tip of their processes (Fig. 12 and 13), and it was thought that these pictures might indicate a close association with the nerve fibers. Therefore, it was fairly probable that in the young rat brain this uncommon type of glial cell, some of which resembled the atypical one in the avian brain, might be transitional and immature forms developing in the direction of oligodendroglia.

In the young chick brain (one to three days after hatching) and in the chick embryo brain even at the earlier stages (Hamburger-Hamilton stage 37 to 45), 'mossy glia' like those found in the young cat and rat brain (Fig. 14) and atypical glial cells like those found in the adult avian brain (Fig. 15) were also observed together with immature forms of oligodendroglia, astrocytes and microglia. Some of these glial cells possessed reticulate-tubular structures on the tip of the processes, which appeared to enclose the neuronal axons (Fig. 16). Therefore, in the developing brain, on the one hand, it was certainly considered that immature types of oligodendroglia resembled the atypical glial element found in the mature brain, but in the adult avian brain, on the other hand, there were no morphological findings to confirm clearly that the atypical glial cell might be an undifferentiated form which could be transformed to oligodendroglia or other glial cells, for no transitional forms were silver-impregnated. Stensaas and Stensaas (1968b) investigated glial cells in the chicken brain and spinal cord with the Golgi method, and noted the glial element with

hair-like processes, which resembled the atypical glial cells in shape, described above. At the same time they pointed out the similarity of this type to the immature type of fibrous astrocytes in the chicks shortly after hatching, but they noted that it was not known whether this unknown type could be considered to indicate immature glial cells. Smart and Leblond (1961) inferred from an autoradiographical study that oligodendroglia might be transformed to astrocytes in the young mouse brain. This atypical glial element in the adult avian brains appeared to have rather distinctive shapes and numbers of processes, indicating an intermediate form between oligodendroglia and astrocytes. No clear continuity from the atypical glial element to oligodendroglia and astrocytes was, however, demonstrated from silver-impregnated images in normal and mature states.

Therefore, it was unknown whether this type might be a stem or undifferentiated form which could be transformed to other glial cells in the normal adult brain, though this type was found either in the chick and chick embryo brains at the earlier stages or in the adult chicken brains. On the other hand, it was possible to consider that the life-span of glial cells might be too long to observe easily the transitional forms in the normal and mature brains, even though the atypical glial element might be an undifferentiated form. Thus it remains a problem for the future to determine whether this type is capable of transformation to other glial elements or the performance of any other morphological changes under pathological or abnormal conditions.

Conclusion

1) In adult avian brains stained with the Golgi methods an atypical glial element was found, which was clearly different from the four conventional glial forms (astrocytes, oligodendroglia, microglia and ependymal cells). Since this type has not hitherto been demonstrated in adult mammalian brains, it should be further investigated in the brains of the lower animal species and mammals from the viewpoint of comparative morphology by means of the Golgi methods, which have been used less frequently than other silver-impregnation methods in studies on the glial cells and are the only techniques to have demonstrated this atypical glial cell.

2) On the other hand, this atypical glial element resembled immature oligodendroglia in young mammalian brains (the cat and rat) and also in the chick and chick embryo brains. In adult avian brains, however, there were no findings to lead us to presume that this type might be an undifferentiated form later to be transformed to other glial types. One possible reason for this phenomenon is that the life-

span of the glial cells might be too long to observe their transformation under normal and mature conditions. Therefore, morphological changes in glial cells should be further investigated under pathological conditions as a future topic for research.

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Explanation of figures

Plat I

- Fig. 1. An atypical glial cell in the chicken brain. 15×40.
Fig. 2. An atypical glial cell in the pigeon brain. 15×40.
Fig. 3. An atypical glial cell in the linnet brain. 10×40.
Fig. 4. An atypical glial cell in the lovebird brain. 15×40. N: the neuronal soma.
Fig. 5. An atypical glial cell in the quail brain. 10×40. a: astrocytes, N: the neuronal soma.
Fig. 6. The secondary processes of the atypical glial cell in the chicken cerebellum were extending parallel to the parallel fibers (p) of the granule cells. 15×40.
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Plate II

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