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CORRELATION BETWEEN TRANSMISSION AND STRUCTURE IN AVIAN CILIARY GANGLION SYNAPSES

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SUMMARY

1. Extracellular responses from post-ganglionic axons of pigeon and chick isolated ciliary ganglia were elicited by stimulation of the presynaptic nerve. Intracellular recordings were also obtained from newly hatched pigeon and chick ganglion cells. The fine structure of ganglia from pigeons of various ages was examined with the electron microscope.

2. In ganglia from chick embryos and pigeons up to 10 days old, the extracellular response was unimodal with a long latency and could be blocked by the addition of D-tubocurarine (D-TC) or hexamethonium to the bathing solution. A bimodal extracellular response appeared in pigeons about 10 days after hatching. Only the second peak of the response could be blocked by D-TC or hexamethonium. The response recorded from 22 to 26-day-old pigeons was similar to that seen in the adult.

3. The intracellular recordings from ganglion cells of 2-week-old pigeons exhibit two post-synaptic potentials elicited by presynaptic stimulation. The first post-synaptic potential appears to be due to current flow through the ganglion cell during the presynaptic action potential. The second is chemically mediated. In pigeons from 1 to 6 days old, only the second post-synaptic potential is observed.

4. The presynaptic terminals in the 4-day-old birds were in the form of calyces. In pigeons 7 days old or older, boutons appeared. The boutons were presumably formed as a result of cleavage of calyciform nerve terminals. Myelin was seen first in the 7-day-old pigeon, was well developed in the 16-day-old bird, and persisted in the adults.

5. In adult ganglia, the first component of the extracellular response decreased and was finally abolished after 10–12 hr of superfusion with

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Tyrode solution. The second component of the response increased concomitantly. The only anatomical change noted in the ganglia after soaking was the disruption and separation of the myelin lamellae from each other and from around the ganglion and presynaptic terminals.

6. It is concluded that the myelin is necessary for electrical transmission in the pigeon ciliary ganglion.

INTRODUCTION

Martin & Pilar (1963*a*) described the joint occurrence of electrical and chemical transmission in certain synapses of the chick ciliary ganglion. It had been shown that in these synapses, synaptic contact is made through large calyciform presynaptic terminals which provide extensive areas of apposition between presynaptic and post-synaptic membranes (Carpenter, 1911; Terzuolo, 1951; de Lorenzo, 1960). Therefore, one factor which was thought to be significant for electrical coupling was a high resistance between the synaptic cleft and the extrasynaptic extracellular space, provided by the large area of synaptic contact. Such a high resistance pathway might force a significant portion of the current generated by the presynaptic action potential to flow through the post-synaptic membrane, thus giving rise to the observed electrical coupling. However, if a high cleft resistance is important for electrical coupling, it appears that it is not due to the large areas of membrane apposition produced by the calyciform presynaptic terminals, because in the adult chicken the calyces are broken up into small terminal boutons (Hámory & Dyachkova, 1964; Hess, 1965), while electrical coupling appears to improve with age (Martin & Pilar, 1963*b*). Hess (1965) suggested that the myelin lamellar envelope which is known to ensheath the synaptic apparatus may be of significance for the electrical coupling which occurs in the avian ciliary ganglion.

The anatomical changes and electrical properties of ganglia were studied during ageing and during prolonged superfusion experiments to investigate the possible relation between various anatomical structures and the occurrence of electrical coupling in the ciliary ganglion. This investigation has established a relationship between myelination and the appearance and persistence of electrical coupling in avian ganglionic synapses. A brief report of these findings has appeared elsewhere (Pilar, Weakly & Hess, 1968).

METHODS

Street pigeons (*Columba livia*) ranging in age from 1 day to adult were used. Chicks (*Gallus domesticus*) were employed in some experiments. Pigeons were used more than chickens because the softness of their bone and connective tissue allowed an easier dissection of the ciliary ganglion than in chickens of comparable age.

Physiology

Dissection was performed under brief ether anaesthesia. The isolated ganglion, including presynaptic (oculomotor) and post-synaptic (ciliary) nerves, was placed in a plastic chamber and superfused with oxygenated Tyrode solution at room temperature (22–24° C). A peristaltic pump (Holter Co.) kept the flow of solution steady at 3 ml./min. The preganglionic and post-ganglionic nerves were placed in separate compartments filled with mineral oil, and platinum electrodes were used for extracellular stimulation and recording. Extracellular responses were recorded from the ciliary nerves. Glass micropipettes filled with 3 M-KCl and having resistances of 40–60 MΩ were used to record responses intracellularly from ganglion cells which were identified by antidromic stimulation of the ciliary nerves. Details of the technique and apparatus employed for intracellular and extracellular recording and stimulation have been previously published (Martin & Pilar, 1963*a*, *b*).

The composition of the pigeon superfusion solution (Tyrode) was based on plasma analyses from six birds varying in age from 6 to 10 months. The pigeon Tyrode solution contained 150 mM-Na^+, 3 mM-K^+, 3 mM-Ca^{2+}, 1 mM-Mg^{2+}, 20·5 mM-HCO_3^-, 140·5 mM-Cl^-, and 17 mM glucose. The solution was buffered by bubbling with 5% CO_2 in O_2. The pH ranged from 7·3 to 7·4. The total tonicity of the solution measured by cryoscopy was found to be the same as pigeon plasma (330 m-osmole/l.). Based on an anlysis of chicken plasma by R. K. Ferguson & R. A. Wolbach (personal communication), the composition of the chick superfusion solution was 142 mM-Na^+, 3 mM-K^+, 1·5 mM-Ca^{2+}, 0·5 mM-Mg^{2+} ,129·5 mM-Cl^-, 20·5 mM-HCO_3^-, and 12 mM glucose. This solution was also buffered by bubbling with 5% CO_2 in O_2. The pH was 7·4. D-Tubocurarine chloride (D-TC, Eli Lilly and Company) and hexamethonium chloride were sometimes added to the bathing solution in concentrations described in the text.

Morphology

The ganglia were fixed in either 1% osmium tetroxide in phosphate buffer, in 2·5% glutaraldehyde followed by osmium tetroxide, or in Dalton's fixative. The latter provided the most consistently successful fixation, although all the fixatives were useful. The tissues were dehydrated, embedded in Maraglas, sectioned, mounted on bare or Formvar-coated copper grids, stained with lead citrate and/or uranyl acetate, and examined with the electron microscope. To determine the position and orientation of the sections, thick plastic sections were stained in a mixture of methylene blue and azure II and studied by light microscopy. The morphological findings presented in this report are all from the so-called large cell portion (Carpenter, 1911; Terzuolo, 1951) of the population of ganglion cells. The synaptic regions in the small cell population have neither calyces nor myelin lamellae at any stage of development (Hess, 1965).

RESULTS

Physiology

Extracellular recordings

In ganglia isolated from adult pigeons, the response of the post-ganglionic nerve to preganglionic stimulation was a double peaked compound action potential as illustrated in Fig. 1, upper rows of columns *A* and *B*. These responses, resembling those obtained in the chick by Martin & Pilar (1963*b*), imply that the mode of transmission in the pigeon ciliary ganglion is identical to that of the chick. Based on this similarity, the first peak, with a latency of 0·3–0·5 msec, was assumed to be associated with fibres whose

ganglion cells were activated by electrical coupling. The second peak with a latency of 1·4–2·0 msec was thought to represent ganglion cells in which the transmission was chemically mediated. Further evidence of a dual mechanism of synaptic transmission, electrical and chemical, was obtained when two different ganglionic blockers were used. Figure 1*A* shows the effect of D-TC on the bimodal post-ganglionic response. The upper record is a

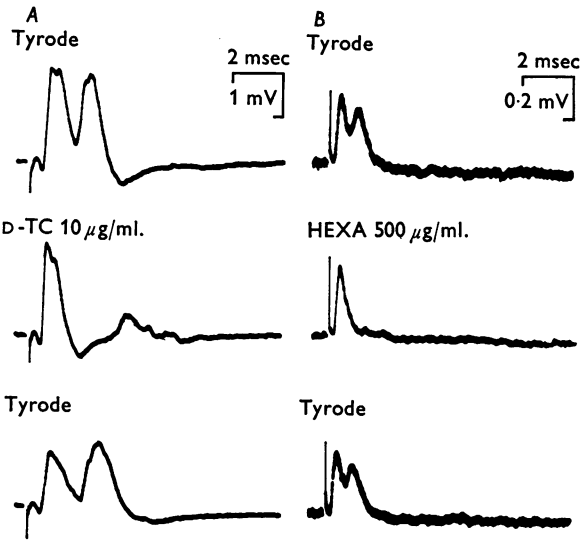

Fig. 1. Responses from the post-ganglionic nerves in two different adult pigeon ganglia (*A* and *B*) elicited by electrical stimulation of the presynaptic nerve. Each vertical column of potentials shows the effect of a blocking agent: *A*, D-tubocurarine (D-TC); *B*, hexamethonium (HEXA). The upper record of each column is the control; the middle record was obtained during the superfusion of the blocking agent; the lower record is the response after a washout period.

control taken before the addition of the drug. After superfusion with Tyrode solution containing 10 μg/ml. of D-TC for 10 min, the second component was almost completely abolished but the first component was hardly affected. The bottom record shows a later response after 4 hr of superfusion with drug-free Tyrode solution. The D-TC was partially washed out as evidenced by the reappearance of the second component. However, even after this long washout period, some residual block was present, and the amplitude of the first component decreased slightly as discussed below. Experiments were also carried out using hexamethonium (Fig. 1*B*). The results were similar to those illustrated in Fig. 1*A*, although the recovery time was considerably shorter than with D-TC.

Since the ganglion cells send only one axon to the ciliary nerve (Carpenter, 1911; Terzuolo, 1951; Hess, 1965), and since the axon diameters of these cells are unimodally distributed (Pilar & Vaughan, 1969), the area under each component of the extracellular response is

proportional to the number of ganglion cells discharged either electrically (first peak of the extracellular response) or chemically (second peak of the extracellular response). Assuming that all the cells of the large cell population are activated, either chemically or electrically, in response to supramaximal preganglionic stimulation, changes in the extracellular response can be related to changes in the mode of synaptic transmission in ganglion cells.

In the chicken, electrical coupling is bidirectional (Martin & Pilar 1963*a*, *b*). In contrast, in the pigeon only orthodromic electrical transmission was found. It is possible that, because of the large size of the oculomotor nerve, antidromic responses occurred but were masked by the recording noise. Even when the responses to 100 antidromic stimuli applied to the post-ganglionic nerve were summed with a computer of average transients, no antidromic response was detected from the presynaptic nerve.

Intracellular recordings

An intracellular response of a ganglion cell from a 14-day-old pigeon can be seen in Fig. 2. An action potential of 100 mV is elicited by orthodromic stimulation. The spike is preceded by an 8 mV depolarization of short time course. By passing hyperpolarizing current through the recording pipette (Figs. 2*B*, *C*, *D*), spike initiation is blocked, and an excitatory post-synaptic potential (EPSP) preceded by the small depolarization can be observed (Fig. 2*D*). The amplitude of the small depolarization did not change with increased membrane potential. In addition, the small depolarization was not dissociated from the EPSP by fine gradation of the stimulus. Therefore, it seems safe to conclude that the small amplitude, rapidly decaying potential with short latency represents the current flow in the post-ganglionic cell (coupling potential) produced by the presynaptic action potential. These records are similar to those obtained from the chick (Martin & Pilar, 1963*a*), where it was shown that the two post-synaptic potentials observed intracellularly correspond to the bimodal response recorded extracellularly from the post-ganglionic nerve.

The records shown in Fig. 2 were selected because it is approximately at this age that electrical coupling first appears (see below). Intracellular records obtained from ganglion cells of pigeons, 1–6 days in age, exhibit only post-synaptic potentials which can be blocked by D-TC. Intracellular records from adult ganglia were also obtained; however, the number of successful impalements is insufficient to evaluate the detailed mechanism of electrical coupling.

Changes in mode of synaptic transmission with age

The records illustrated in Fig. 3 were obtained from four birds; 1, 5, 14 and 26 days after hatching. Stimuli were applied to the oculomotor nerve, and the post-ganglionic responses were recorded extracellularly from the ciliary nerve. In the 1-day-old bird (Fig. 3*A*), a unimodal response with

a 7 msec latency was elicited, and this could be blocked by D-TC. In Fig. 3*B*, taken from a 5-day-old bird, the response was still unimodal but had a shorter latency. The small deflexion between the stimulus artifact and the large response in Fig. 3*B* may represent a slight degree of electrical coupling in this ganglion. In Fig. 3*C* from a 14-day-old pigeon, two peaks are clearly distinguishable in the post-ganglionic response, and only the

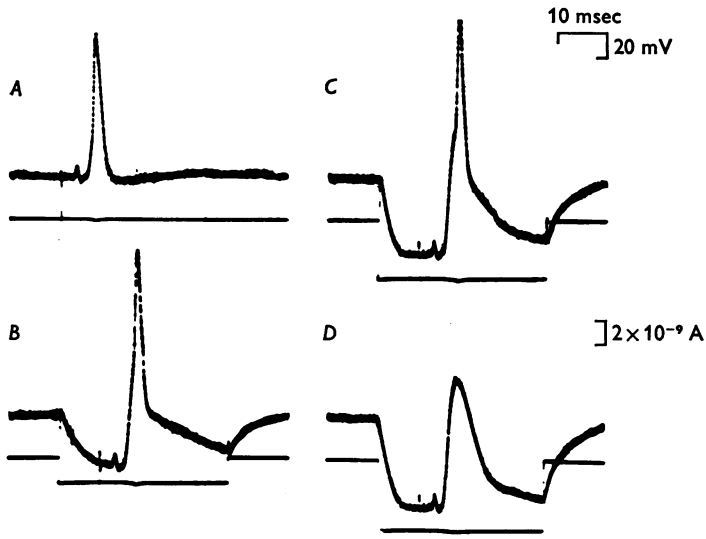

Fig. 2. Intracellular record showing the responses to preganglionic stimulation of a ganglion cell from a 14-day-old pigeon. Records were obtained in the absence (*A*) and presence (*B*, *C* and *D*) of membrane hyperpolarization produced by passing current pulses through the recording micropipette. Upper trace of each record, transmembrane voltage transient; lower trace, applied current pulses. Note: record *D* shows failure of ganglion cell action potential leaving the post-synaptic potential (EPSP). In all records the EPSP and action potential is preceded by a rapidly decaying potential change (coupling potential). Resting membrane potential, 50 mV.

second peak could be abolished by D-TC. The responses obtained from the 26-day-old pigeon are similar to those recorded in the adult (Fig. 1*A*, *B*, upper row). It is concluded that only chemical transmission is present in the 1-day-old bird and presumably in the 5-day-old bird. By 14 days, some synapses are electrically coupled, and by 26 days, half of the ganglion cells exhibit electrical coupling. The difference in the measured latencies of the chemical components in the response from 1-, 5- and 14-day-old pigeons may be due in part to a slower nerve conduction velocity in the younger birds.

Thus, electrical coupling improves with age in the pigeon. The temporal course of this change is illustrated in Fig. 4 which shows the results

obtained from ganglia isolated from forty-six pigeons ranging from 1 to 2000 days in age. The relative proportions of electrically and chemically induced responses are plotted along the ordinate.

Electrical coupling appears about 10 days after hatching, and after an additional 10–15 days the number of electrically coupled synapses reaches a proportion which is maintained during the rest of the bird's life. The dispersion of the values between 10 and 20 days may be related to different growth rates of the various birds at this early stage.

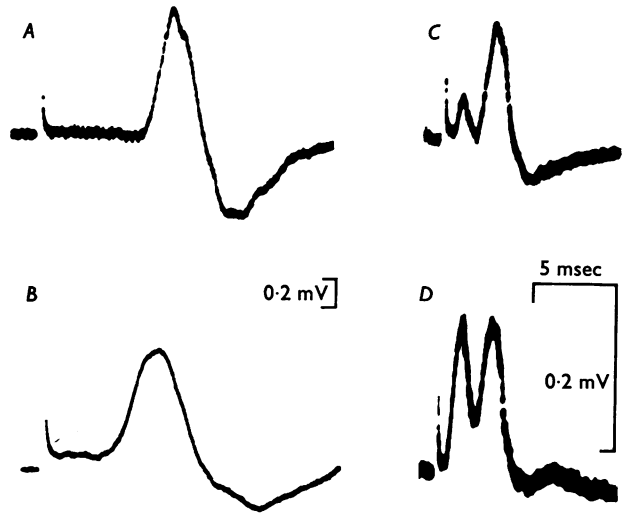

Fig. 3. Extracellular post-ganglionic responses from four pigeons: 1-day-old (*A*), 5 days old (*B*), 14 days old (*C*) and 26 days old (*D*). In *A* and *B*, the response is unimodal; in *C* and *D*, the response is bimodal and similar to the post-ganglionic response obtained in adult pigeons (see Fig. 1). Records *A* and *C* taken at same gain as *D*. Sweep speed same for all records.

Experiments were also performed on chick embryos 3 to 7 days before hatching. Figure 5 is an example of the response obtained from an excised ganglion of an embryo about 7 days prior to hatching (Stage 40 of Hamburger & Hamilton, 1951). Figure 5*A* shows a unimodal wave with a 4 msec latency which represents the post-ganglionic response elicited by oculomotor nerve stimulation. When one 50 μl. drop of D-TC (3 mg/ml.) was added to the bath, the response was almost completely abolished (Fig. 5 *B*). Figure 5*C* is the response obtained after D-TC was washed out for 50 min. When the recording electrodes were placed on the preganglionic nerve trunk, and stimulation was applied to the post-ganglionic nerve, no antidromic response was elicited (Fig. 5*D*). These records indicate that electrical coupling is not present in the chick embryo. Its appearance coincides with the time of hatching (Martin & Pilar, 1963*a*, *b*).

In conclusion, similar changes were observed in the mode of synaptic transmission in the ciliary ganglia of pigeons and chicks during ageing. However, some electrical coupling occurs in all chicks at the time of hatching (Martin & Pilar, 1963*a*, *b*), whereas in the pigeon, electrical transmission does not appear until about 2 weeks after hatching.

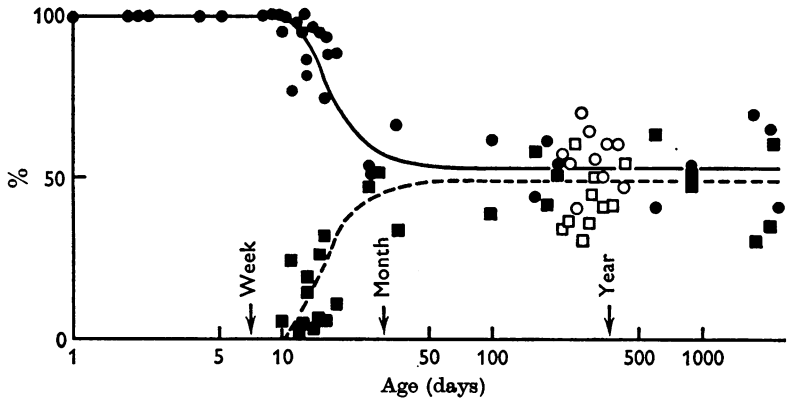

Fig. 4. Changes in the mechanism of synaptic transmission with age. The graph combines data from forty-six experiments similar to the one shown in Fig. 3. The total area under the response from each ganglion was considered to be 100%. The ordinate represents the fraction of the area of each peak to the area of the total response. Squares represent the electrically coupled synapses; filled circles represent chemically mediated synapses; open symbols represent data from adult pigeons ranging in age from 6 months to 1 year. Since the exact age in these birds was unknown, the points were distributed arbitrarily over the range of ages indicated. Lines indicate the average time course of the change in the transmission mechanism. Continuous line, chemical transmission; dashed line, electrical coupling. Lines fitted by eye. All responses were obtained under comparable procedures within 60 min after dissection was initiated.

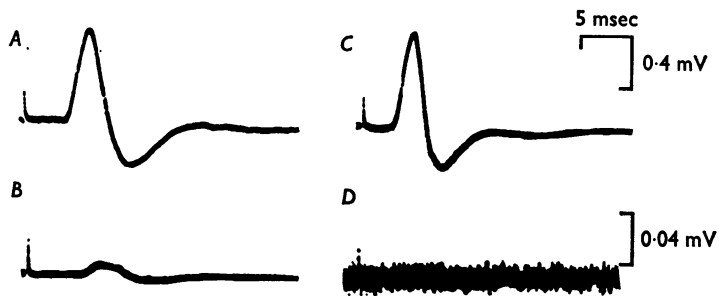

Fig. 5. Extracellular responses from the ganglion of a chick embryo approximately 7 days before hatching. *A* shows a unimodal post-ganglionic response with a 4 msec latency. *B* shows the response 3 min after the addition of D-TC to bathing solution. *C* shows the recovery after 50 min of drug washout. The decreased latency in *C* may be due to an increased stimulus strength used at this time. *D* illustrates the absence of a response to antidromic stimulation.

Morphology

Structural changes in the synaptic apparatus during ageing

In chicken ciliary ganglia it has been demonstrated that during maturation myelin lamellae appear on the perikarya and changes occur in the structure of the synapses (Hámory & Dyachkova, 1964; Hess, 1965). In the present study similar alterations were seen in the pigeon, and an approximate quantitative estimation of these changes was made using ganglia from four different age groups: 4 days, 7 days, 16 days after hatching and adult (1 year and 6 years).

4-*day-old pigeon.* Most of the presynaptic nerve cells in the 4-day-old pigeon ciliary ganglion terminate in calyces. These are large terminals which partially envelop the ganglion cell body. Extensive areas of apposition of presynaptic and post-synaptic cell membranes are present (Pl. 1*A*). Desmosomes or thickening of the membranes of the nerve terminal and the post-synaptic neurone could be seen frequently along the synaptic junction. Often in the calyx, synaptic vesicles accumulated near the desmosomes.

The ganglion cell in the 4-day-old pigeon is surrounded by more than one Schwann or capsule cell. Schwann cells also cover the calyx (Pl. 1*A*). However, since the layers of Schwann cells are irregular in thickness and not orderly arranged, they cannot be classified as myelin lamellae.

7-*day-old pigeon.* In the 7-day-old pigeon, it is relatively difficult to find a calyx although some are present. Most of the terminals found on the neurones are rounded and have the appearance of large boutons. The terminal no longer enclasps the ganglion cell.

On occasion, myelin lamellae sometimes surround the neurones. In this case, the Schwann cell cytoplasmic layers on the neuronal surface are arranged in an orderly fashion. Most ganglion cells in the 7-day-old pigeon, however, appear like those of the 4-day-old pigeon and are covered by one or perhaps two layers of Schwann cells not organized into myelin lamellae.

16-*day-old pigeon.* The 16-day-old pigeon presents essentially the same picture as the adult, which will be described in detail.

Adult (1- *and* 6-*year-old*) *pigeon.* The synapses in adult pigeons do not have calyces or extensive areas of continuous contact between nerve terminal and post-synaptic neurone. The calyx is broken up into boutons (Pl. 1*B*). The boutons apparently result from altered calyciform terminals since the largest mass of fragments, now boutons, are frequently accumumulated at one pole of the cell in a location formerly occupied by the calyx. The boutons contain large masses of closely packed synaptic vesicles. Thickenings of the membranes between the bouton and the post-synaptic neurones occur frequently with accumulations of synaptic vesicles near them. Small, short processes of the post-ganglionic neurone seem to

evaginate and insert themselves between the boutons. Also, small processes may extend from boutons into the ganglion cell body. These evaginations of boutons and ganglion cells usually have several membrane thickenings on them. Spaces are seen frequently between adjacent boutons. The surrounding Schwann cell does not separate the boutons from each other.

The ganglion cells of adult pigeons, like those in the 16-day-old pigeon, are ensheathed by myelin (Pl. 2*A*). Almost every ganglion cell has myelin lamellae along its surface and covering the boutons. The lamellae can be either loose with cytoplasm intercalated between them, semicompact with no cytoplasm but still distinctly separated from one another, or compact with fusion of adjacent lamellae and the formation of a dark line and very dense appearance in electron micrographs. As described in the chicken, one kind of myelin can blend into another and then back again as it courses around the nerve cell, and all the types of myelin can appear ensheathing a single post-synaptic neurone.

Finally, 'tight' junctions were not observed in ganglia from either adult or baby pigeons (cf. Takahashi & Hama, 1965; de Lorenzo, 1966; Koenig, 1967; Hámory & Dyachkova, 1964; Hess, 1965).

Correlation of synaptic structures with electrical coupling

Randomly taken thin sections of pigeon ciliary ganglia were examined with the electron microscope, and 100 neurones were studied from each age group for the presence or absence of boutons, calyces, and myelin lamellae in the synaptic region. The percentage of these elements observed in the ganglia of the different age groups is presented in Table 1 and Fig.6. The presynaptic terminals exist either as boutons or calyces; therefore, in any one age group, the sum of the percentage of terminals which are in the form of boutons and the percentage in the form of calyces must equal 100 %. Thus, in 4-day-old pigeons (Table 1), of 100 ganglion cells examined, eighty-two had presynaptic endings in the form of calyces, eighteen had terminals in the form of boutons. Table 1 also shows that in 4-day-old pigeons, twelve of 100 ganglion cells examined had myelin lamellae, twenty-six of 100 had myelin lamellae in the 7-day-old group etc. Figure 6*A* shows the time course of the appearance and persistence of electrically coupled synapses in the ganglia from Fig. 4. From a comparison of Fig. 6*A* and Fig. 6*B* it is apparent that calyces are present without the existence of electrical coupling at first, and no calyces are present when electrical coupling is well established in the adult stage. As expected, the proportion of boutons is inversely related to the presence of calyces (Fig. 6*C*) since the boutons appear to be formed by the cleavage of the large terminals.

Comparison of Fig. 6*A* with Fig. 6*D* indicates that electrical coupling

correlates well, temporally, with the presence of the myelin sheath around the synaptic apparatus. There is no change in the proportion of myelin lamellae throughout the adult stage. This correlation suggests that the presence of myelin is involved in the electrical coupling mechanisms.

Further support linking the presence of myelin lamellae around the post-synaptic neuronal perikarya to the presence of electrical coupling was obtained in prolonged perfusion experiments. After bathing an adult pigeon ciliary ganglion in Tyrode solution for a few hours, the incidence of electrical coupling was reduced, and the number of chemically mediated synapses increased.

TABLE 1. Percentages of occurrence of anatomical structures in 100 cells from each age group in pigeons of various ages

Age	Presynaptic terminal		Myelin lamellae
	Calyces	Boutons	
4 days	82	18	12
7 days	8	92	26
16 days	12	88	92
1 year	0	100	92
6 years	6	94	86

Such changes are shown in Fig. 7. The initially recorded response to orthodromic stimulation is shown in Fig. 7*A*. Sixty percent of the response corresponds to efficient electrically coupled synapses. The remainder corresponds to chemically coupled synapses. Figure 7*B* shows the response obtained from the same ganglion after superfusion with Tyrode solution for 12 hr. It can be seen in Fig. 7*B* that after the superfusion the initial component of Fig. 7*A* is abolished, and the second component is increased. This latter component was completely blocked by the addition of 20 μl. D-TC (3 mg/ml.) to the superfusate. Thus, it appears that at the end of the superfusion period almost no synapses were electrically coupled, and the proportion of chemically coupled synapses was increased. The area of the total response remained constant during the course of this experiment and ten similar experiments. This observation implies that the same neural elements are involved in both components of the response and demonstrates that no physiological deterioration occurred in synaptic transmission and nerve conduction during prolonged superfusion. After long superfusion electrical coupling was insufficient to discharge most ganglion cells, and then transmission was mediated chemically in almost all of the cells.

The ganglion, from which the recordings shown in Fig. 7 were taken, was fixed in osmium tetroxide, embedded in Maraglas, and thin sections were observed with the electron microscope (Pl. 2*B*, *C*). There were no obvious changes in the ganglion cells. Their nuclei appeared normal; their mito-

chondria were distributed as usual and appeared unswollen; their ribosomes, Nissl bodies, and endoplasmic reticula showed normal structure and distribution. Boutons were seen synapsing on the ganglion cell as in the normal ganglion (Pl. 1*B*, Pl. 2*A*). The boutons were dense in the electron micrograph and were filled with masses of synaptic vesicles. Membrane thickenings occurred between bouton and perikaryon. The only histological

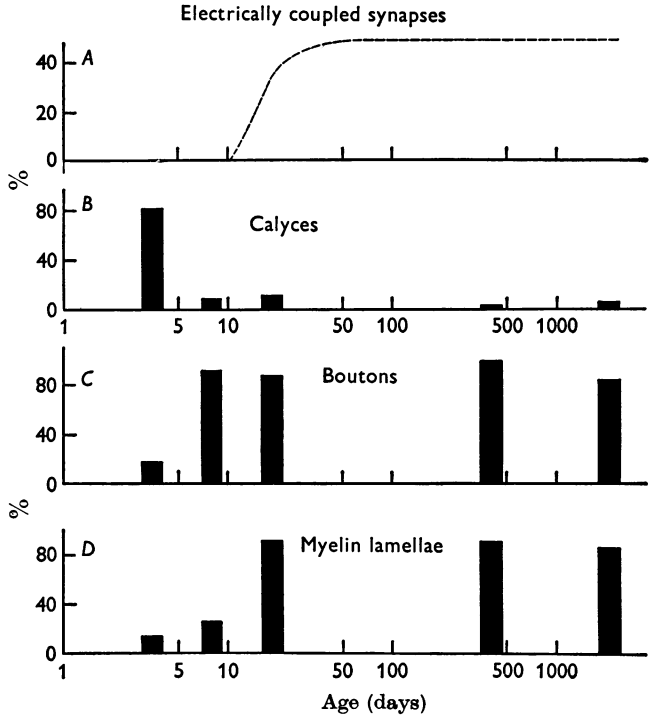

Fig. 6. Occurrence of calyces, boutons, and myelin lamellae in synaptic regions of pigeon ciliary ganglia. One hundred synapses from ganglia taken from 4-, 7- and 16-day-old and 1- and 6-year-old birds were investigated by electron microscopy. The ordinates in bar graphs *B*, *C* and *D* represent the percentage of each element. The relationship between the development of calyces, boutons, and myelin lamellae is compared to the appearance of electrically coupled synapses as shown in *A* (taken from Fig. 4).

element disturbed was the myelin sheath around the post-synaptic cell body and the boutons. The myelin lamellae lifted off from the cell body and boutons, and large spaces, not usually seen, appeared between the ganglion cell and its myelin sheath (Pl. 2*B*, *C*). In addition, the myelin lamellae seemed to separate from each other, and large spaces occurred between lamellae. The myelin lamellae were no longer smooth and parallel to each other. These disturbances were seen in virtually all of the ganglia examined

after prolonged soaking. Furthermore, myelin figures, seen occasionally in normal ganglia in the Schwann cell (Hess, 1965), were encountered more frequently in these soaked ganglia. The myelin lamellae on nearly all the nerve fibres in the area were intact; only one or two nerve fibres were seen with disrupted lamellae.

Hence, it appears that the loss of electrical coupling in these experiments may be related to the disturbance of the myelin lamellae around the synapses in the ganglion.

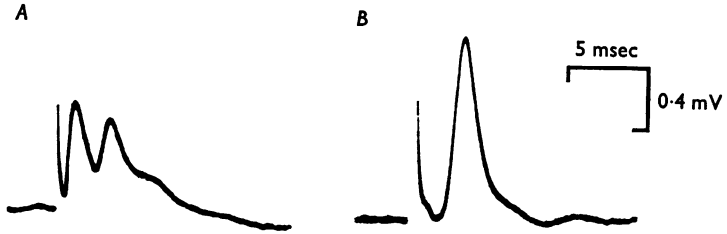

Fig. 7. Extracellularly recorded post-ganglionic responses from an adult pigeon ganglion. *A* was taken within 60 min after removal of the ganglion from the bird. *B* shows the response after superfusion for 12 hr. The proportion of electrically coupled synapses had decreased markedly while that of the chemically mediated synapses had increased (see text).

DISCUSSION

The structural entity responsible for the presence of electrical coupling in the ciliary ganglion is not the large contact area between calyx and ganglion cell body as indicated by the following findings: electrical transmission occurs without the presence of calyces in adult pigeons, and electrical transmission is virtually absent even though calyces are present in baby pigeons.

Certain observations seem to indicate that the myelin lamellae are important for electrical transmission in the avian ciliary ganglion. First, the initial appearance of myelin lamellae is correlated temporally with the initiation of electrical coupling. Secondly, the persistence of electrical coupling throughout the adult life of the bird is associated with the continued presence of myelin lamellae. Thirdly, the apparent decrease in electrical coupling observed after prolonged superfusion is associated with disruption of the myelin lamellae.

The changes in the myelin lamellae after prolonged superfusion are reminiscent of similar alterations in frog nerve fibres produced by exposure to a hypotonic solution (Robertson, 1958). The changes in the frog fibres were characterized by the disruption of Schwann cells accompanied by axon destruction. In the present study, the presynaptic and post-synaptic elements appeared to be well preserved anatomically and presumably were undamaged physiologically. It is possible that the thinner, more delicate myelin lamellae on the ganglion cells are more susceptible to prolonged soaking than the more organized, thicker myelin sheath typical of peripheral nerve fibres.

In the model proposed by Martin & Pilar (1964) to explain electrical coupling in the chick ciliary ganglion, one of the requirements necessary was a high resistance between the synaptic cleft and the extrasynaptic extracellular space. A large area of contact between calyx and post-synaptic cell body now appears inadequate to provide this high resistance. Rather, the myelin lamellae, known to produce insulation of nerve fibres elsewhere (see Tasaki, 1959), seem to be responsible for the relatively high cleft resistance since they ensheath the synaptic apparatus and thereby reduce the current leakage. If the myelin electrical seal around the synaptic apparatus in the ciliary ganglion has a resistance value similar to that of the myelin around peripheral nerve axons, the so-called saltatory conduction of the nerve impulse that occurs in myelinated nerve fibres also might occur through the ciliary ganglion (cf. Hess, 1965). That is, the synaptic apparatus with its myelin envelope may behave as an 'internode' allowing the action potential to jump from the terminal segment of the presynaptic fibre to the initial segment of the post-synaptic fibre. But, it remains to be determined whether different types of myelin, loose or compact, have similar resistances.

The results of the prolonged superfusion experiments are consistent with the idea that myelin normally seals the cleft resulting in significant current flow through the post-synaptic membrane and good electrical coupling; and, if this myelin be disrupted, current leakage out through the cleft occurs with a subsequent reduction in electrical coupling.

In several instances, electrical coupling has been related to close junctional membrane apposition (see Martin & Veale, 1967), which provides a low resistance pathway for current flowing across the junction. Such close membrane apposition has been described in the synapses of the chick ciliary ganglion by Takahashi & Hama (1965), de Lorenzo (1966), and Koenig (1967). However, Takahashi & Hama (1965) and Koenig (1967) have both commented on the rarity of occurrence of these close membrane appositions. In addition, Hámory & Dyachkova (1964), and Hess (1965) failed to find 'tight junctions' in the chick. Electrical coupling occurs in all chicks at the time of hatching (Martin & Pilar, 1963*a*, *b*) but Takahashi & Hama (1965) have reported that close apposition of synaptic membranes does not occur in newly hatched chicks. Therefore, it seems that the appearance of electrical coupling is better correlated with the presence of myelin lamellae than with the occurrence of 'tight junctions'. Although the present observations implicate the necessity of the myelin lamellae in electrical transmission, it is still possible that the 'tight junctions' play some role in this process.

The electrical insulation of the synaptic cleft by myelin does not necessarily mean that substances are prevented from diffusing in and out of the

synaptic region. The rate of diffusion of substances may, however, be slowed. The myelin envelope could possibly provide a diffusional barrier (cf. Bunge, 1968). For example, in the present investigation D-TC required several minutes to block transmission in adult ganglia during the first hour of experimentation. It would be expected that following prolonged superfusion, i.e. when the myelin envelope is disrupted, the time necessary to block transmission would be reduced. Experiments in progress (Pilar, G. & Weakly, J. N. unpublished observations) favour this interpretation.

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EXPLANATION OF PLATES

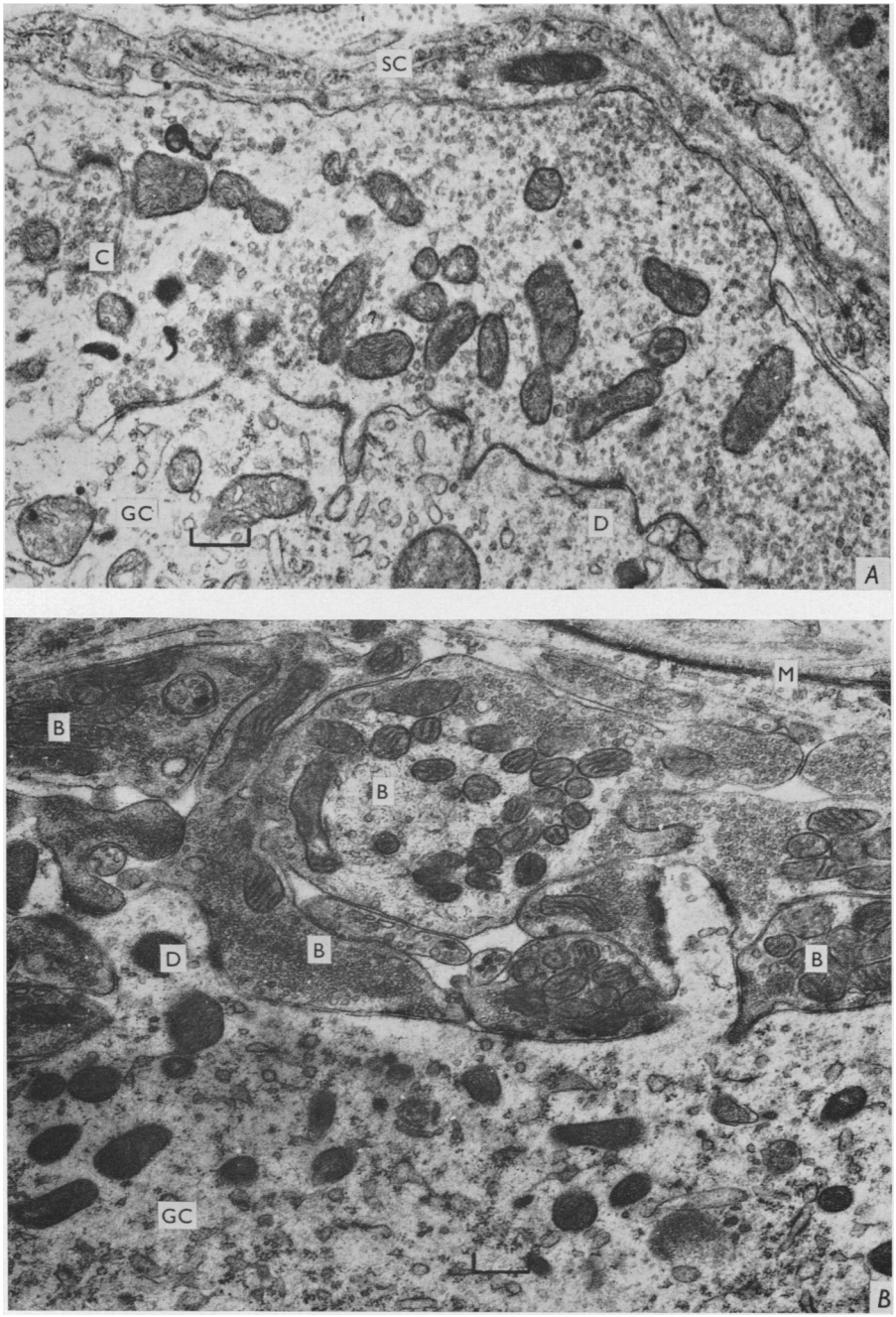
All illustrations are electron micrographs from pigeon ciliary ganglia. The scale mark on each photograph represents 0·5 μ. B, bouton; C, calyx; D, desmosome; GC, ganglion cell; M, myelin lamellae; SC, Schwann cell.

PLATE 1

A. 4-day-old pigeon. The elongated calyx is seen synapsing on the ganglion cell. The calyciform terminal contains relatively few synaptic vesicles. Only one or two processes of Schwann or capsule cells cover the nerve terminal ganglion cell. *B*. Adult pigeon (1 year). Boutons, developed from fragmented calyciform terminals, are seen synapsing on the ganglion cell, the number of synaptic vesicles is increased markedly, and the terminal itself appears more dense in the electron micrographs. Schwann cell layers on the neurones and over the free surface of the boutons are generally organized into myelin lamellae.

PLATE 2

A. Adult pigeon (1 year). Compact myelin lamellae cover the ganglion cell and nerve terminals. *B, C*. Adult pigeon (1 year). Ganglion bathed in Tyrode solution for 12 hr. The compact myelin lamellae have lifted away from the ganglion cell body and its boutons, and the lamellae are disrupted and separated from each other. The ganglion cell and boutons appear intact.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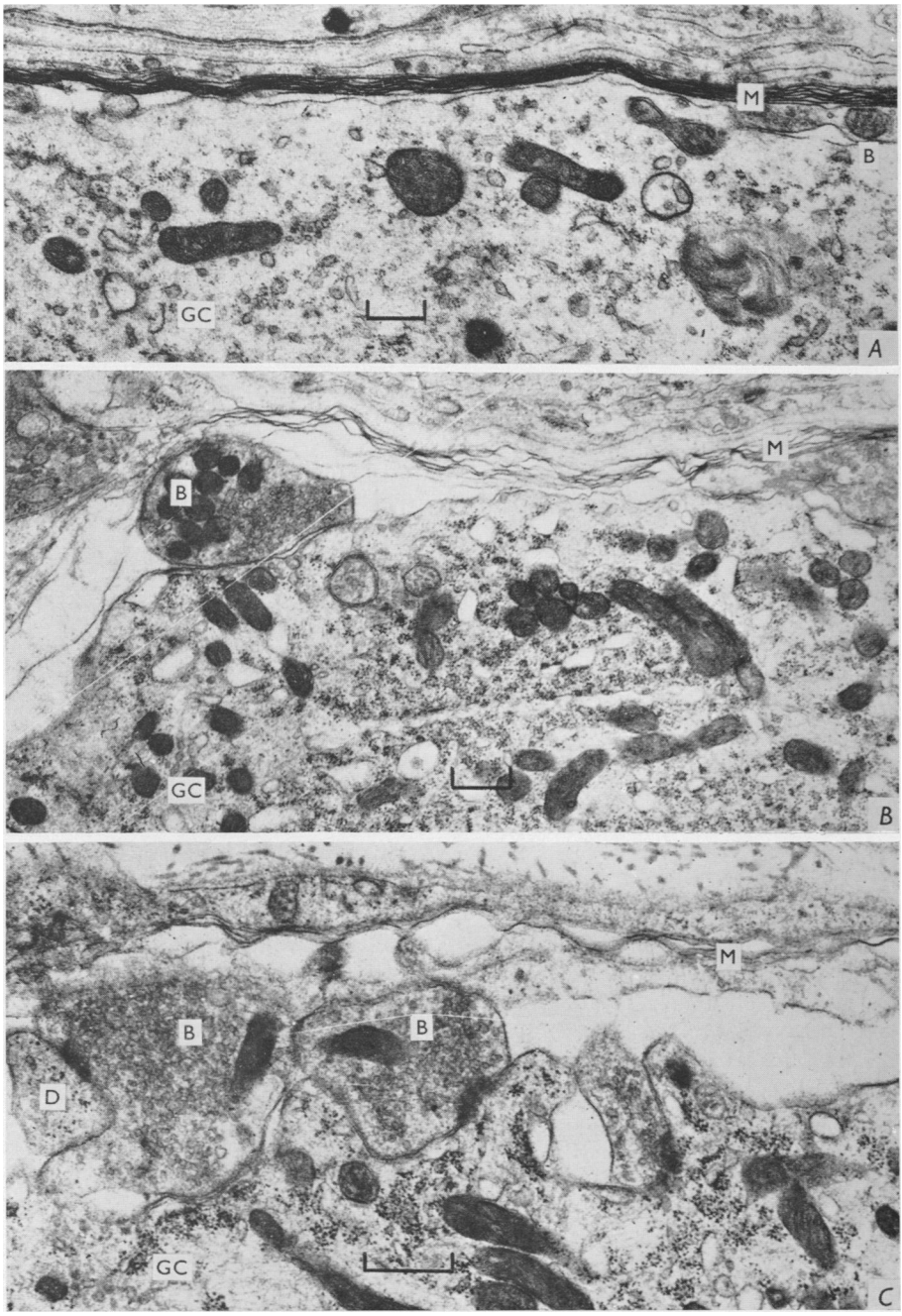

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