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# The Synaptic Ribbons of the Guinea-Pig Pineal Gland under Normal and Experimental Conditions*

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*Summary.* "Synaptic" ribbons (SR), functionally enigmatic structures of mammalian pinealocytes, were studied electron microscopically with regard to number, intracellular localization and topographical relationships, both under normal and experimental conditions. Pineal glands of guinea-pigs serving as controls contained 1.75 ribbon fields/unit area in the males and 2.58 in the females. In animals subjected to continuous illumination for 64 days the number of ribbon fields increased 20-fold in the males and 9-fold in the females. Continuous darkness (26 to 70 days) had varying effects; in some animals SR increased either strongly or moderately, in others they appeared unchanged. Under continuous illumination a higher percentage of ribbon fields bordered the cell membrane than in the controls. Moreover, paired ribbon fields occurred. The topographical analysis revealed that 98 % of the ribbon fields bordering the cell membrane lay opposite another pinealocyte and the remainder opposite nerve fibres, blood vessels and collagenous fibres. It is suggested that SR of mammalian pinealocytes do not represent non-functioning phylogenetic relics but true organelles possibly involved in coupling adjacent pinealocytes functionally.

*Key words:* Synaptic ribbon — Synapse — Pineal gland — Guinea-pig.

*Zusammenfassung.* In der vorliegenden Studie wurden die funktionell unklaren "synaptic ribbons" (SR) der Säugerzirbeldrüse hinsichtlich Zahl, intrazellulärer Lokalisation und topographischer Beziehungen unter normalen und experimentellen Bedingungen untersucht. In der Zirbeldrüse von Meerschweinchen, die als Kontrollen dienten, wurden bei Männchen 1,75 und bei Weibchen 2,58 Ribbonfelder/Flächeneinheit gefunden. Nach 64tägigem Aufenthalt der Tiere in ständiger Helligkeit zeigten die Männchen eine 20-, die Weibchen eine 9fache Zunahme dieser Strukturen. Nach Aufenthalt in ständiger Dunkelheit (26—70 Tage) waren die Resultate uneinheitlich: einige Tiere zeigten eine starke bzw. mäßige, andere keine Zunahme der SR. Nach Dauerbelichtung fand sich ein höherer Prozentsatz von Ribbonfeldern in unmittelbarer Nachbarschaft der Zellmembran als bei Kontrollen. Außderdem kam es zum Auftreten von paarigen Ribbonfeldern. 98 % der an Zellmembranen angrenzenden Ribbonfelder lagen benachbarten Pinealocyten, der Rest Nerven, Blutgefäßen und Bindegewebe gegenüber. Es wird angenommen, daß die SR der Säugerzirbeldrüse keine funktionslosen phylogenetischen Relikte darstellen, sondern echte Zellorganellen sind, die möglicherweise benachbarte Pinealzellen funktionell koppeln.

## Introduction

In the mammalian pineal gland "synaptic" ribbons (vesicle-crowned rodlets, vesicle-crowned lamellae) are typical, yet functionally enigmatic components of pinealocytes (Kappers, 1969, 1971a). Synaptic ribbons are not restricted to the pineal gland. In other organs (e.g. the retina: Sjöstrand, 1958; Dowling and Boycott, 1966; organ of Corti: Smith and Sjöstrand, 1961; vestibular organ: Wer-

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säll, Flock and Lundquist, 1965; electric receptor organs of teleost fishes: Szamier and Wachtel, 1970; Szabo and Wersäll, 1970; pineal organs of lower vertebrates: Collin, 1971; Oksche, 1971) they form part of synapses which link either receptor cells and postsynaptic dendrites or two neurons, and there can be no doubt that in these organs synaptic ribbons play an integral role in synaptic transmission.

To attribute a synaptic function to the "synaptic" ribbons of the mammalian pinealocyte, though tempting, poses certain problems. To begin with, the mammalian pineal gland lacks centripetal (pinealofugal) nerve fibres (Kappers, 1960, 1969, 1971a) to which the pinealocytes, and the "synaptic" ribbons they contain, could be functionally related. Furthermore, a considerable number of "synaptic" ribbons are found at some distance from the cell membrane (Wolfe, 1965), where synaptic transmission is very unlikely to occur. Finally, to ascribe to the "synaptic" ribbons a synaptic function linking adjacent pinealocytes (Hopsu and Arstila, 1965) is at variance with the commonly accepted concept of the mammalian pinealocyte which maintains that this cell is an exclusively secretory one (Kappers, 1969, 1971a).

Looking at the problem from an evolutionary point of view, it emerges (Collin, 1971; Kappers, 1971a; Oksche, 1971) that the mammalian pinealocyte is derived from the neurosensory photoreceptor cells of pineal organs of lower vertebrates. According to Wurtman, Axelrod and Kelly (1968) this phylogenetic relationship is clearly indicated by the presence of synaptic ribbons in both the pineal receptor cells of lower vertebrates and the mammalian pinealocyte. Kappers (1971b) refers to the "synaptic" ribbons of the mammalian pinealocytes as "possible reminders of the original neurosensory function of this cell". On purely theoretical grounds there is therefore a possibility that "synaptic" ribbons represent non-functioning phylogenetic relics. That this, however, is probably not so has recently been suggested by pilot experiments which showed that the "synaptic" ribbons of the guinea-pig pineal gland increased in number and changed in shape when the animals had been kept under continuous illumination, or darkness, for up to 70 days (Lues, 1971).

In view of the growing body of evidence that the mammalian pineal gland is a "neuro-endocrine transducer" (Wurtman and Anton-Tay, 1969) of importance, even though its function is only partly known, it was felt that a thorough quantitative study of "synaptic" ribbons, both under normal and experimental conditions, should be carried out. It was hoped that these studies would not only contribute to a better understanding of the function of "synaptic" ribbons themselves but also to that of the pineal gland as a whole. The guinea-pig was chosen as the experimental animal for this investigation because of Lues' (1971) positive preliminary results.

## Materials and Methods

In the present study a total of 39 sexually mature guinea-pigs, weighing between 300 and 400 gm, were used. Eight animals (4 ♂, 4 ♀) kept under normal laboratory conditions with a lighting regimen of 14 hrs. illumination (7.30 a.m. to 9.30 p.m.) and 10 hrs. darkness, served as controls. Sixteen guinea-pigs (10 ♂, 6 ♀) were subjected to continuous illumination (provided by a 100 W bulb placed over the cages at a distance of approx. 1 m) for 64 days. Ten animals were kept under continuous darkness for 26 days (1 ♂), 33 days (1 ♀), 45 days

(1 ♂), 55 days (3 ♂, 2 ♀) and 70 days (1 ♂, 1 ♀) respectively. Five pregnant guinea-pigs were studied at 16, 30, 37, 54 and 58 days of gestation respectively. All animals were killed by decapitation under ether anaesthesia between 10.00 and 12.00 a.m.

The pineal glands were rapidly removed, fixed for 2 hours in 3 % buffered (0.1 M phosphate buffer, pH 7.4) glutaraldehyde, with the addition of 2.5 % sucrose, and subsequently divided into three pieces of equal length, referred to as proximal, intermediate and distal parts respectively. The proximal part represents the head of the gland which is intimately related to the habenulae. After post-osmication (1 % buffered osmium tetroxide) and dehydration the tissue was embedded in Araldite. Sections were cut on an LKB Ultrotome III with glass knives, mounted on formvar-coated or uncoated copper grids, stained in uranyl acetate saturated in methanol and investigated under an AEI 6B electron microscope[1].

For the quantitative assessment of "synaptic" ribbons the following procedure was used. Thin sections were taken from each of the three areas of the gland and the "synaptic" ribbons lying inside 10 grid apertures were counted. Since "synaptic" ribbons lie in groups of one, two, three or more (Lues, 1971) both the number of these groups, as well as the number of ribbons they comprise, was recorded. In the present study the term "ribbon field" (RF) is used to include all the various types of ribbon grouping. The data obtained are expressed as means $\pm$ standard error per unit area (UA) or as means/UA. The unit area corresponds to an area of tissue covering 10 grid apertures measuring 42 $\mu$m by 42 $\mu$m, thus comprising a total area of 17640 $\mu m^2$. — The data obtained from the proximal, intermediate and distal regions of the gland were first used to determine whether statistical differences existed between the various regions; they were then pooled and divided by 3 to obtain data representative of an individual gland. — For the statistical analysis of the data the Wilcoxon test (Pfanzagl, 1968) was used.

Parts of sections lying over some grid apertures which were smaller than normal due to faulty manufacture were not used for assessment. Counting the same structures twice was avoided by using only one section out of a series. If sections covered less than 10 grid apertures additional counts were made from sections obtained from considerably deeper levels of the block. The incidence that parts of ribbon fields were covered by grid bars was extremely rare. If this happened to be the case then the "synaptic" ribbons of the uncovered parts of the fields were counted as well and included in the data. — For a detailed description of the guinea-pig pineal gland and its "synaptic" ribbons see Lues (1971).

## Results

### *Control Animals*

Quantitative assessment of ribbon fields in guinea-pigs kept under a lighting regimen of 14 hrs illumination and 10 hrs darkness revealed that pineal glands of males contained $1.75 \pm 0.64$ RF/UA (ribbon fields/unit area) and those of females $2.58 \pm 1.04$ RF/UA. These differences are not statistically significant. The frequency distribution of "synaptic" ribbons per ribbon field is given in Fig. 1. It can be seen that, in sections, more than half of the ribbon fields contained one "synaptic" ribbon only. Furthermore it is apparent that an inverse relationship exists between the frequency of ribbon fields and the number of ribbons they contain. — The mean number of ribbons per field was 1.80.

As regards the shape of the ribbons it was found that most of them were straight (Fig. 2), a few were slightly curved.

Examination of the proximal parts of the glands resulted in 1.2 RF/UA in the male and 3.0 RF/UA in the female. The intermediate areas contained 3.0 RF/UA (♂) and 3.5 RF/UA (♀) respectively and the distal areas 1.0 RF/UA (♂) and 1.2 RF/UA (♀). The differences are not statistically significant.

1 Provided by the Medical Research Council, London.

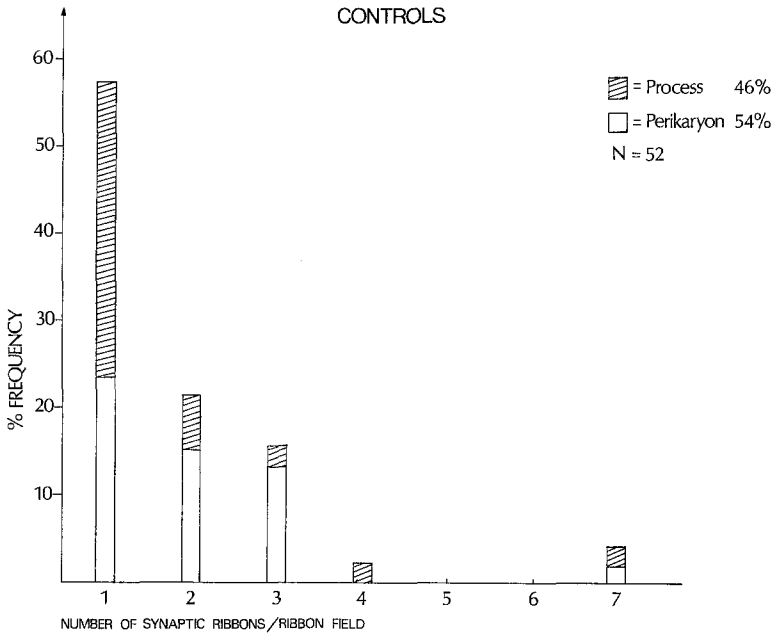


Fig. 1. Frequency distribution of ribbon fields comprising one, two, three and more "synaptic" ribbons. The incidence of ribbon fields present in perikarya and processes of pinealocytes is indicated by the white and the hatched parts of the columns respectively. It can be seen that, in electron microscopic sections, the majority of ribbon fields consist of one "synaptic" ribbon only. Control guinea-pigs kept under a lighting regimen of 14 hrs illumination and 10 hrs darkness. *N* number of ribbon fields studied

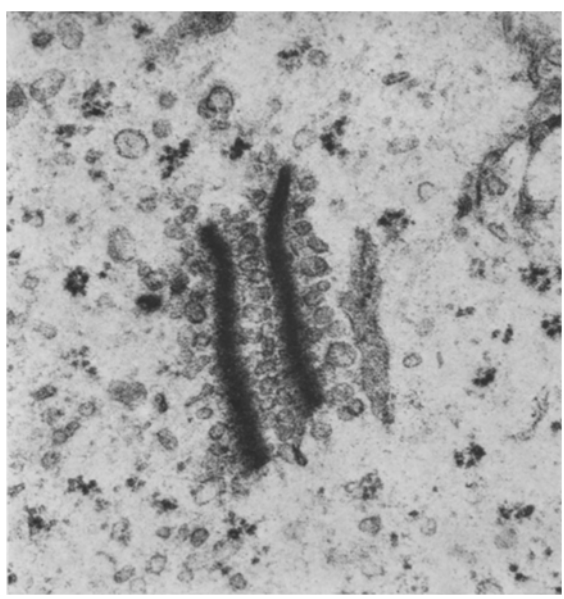

Fig. 2. Electron micrograph of a ribbon field containing two "synaptic" ribbons and possibly a third one in the process of being formed. It can be seen that the electron-dense rodlets of the ribbons are fairly straight and that they are surrounded by electron-lucent vesicles. Control guinea-pig. Magnification × 61000

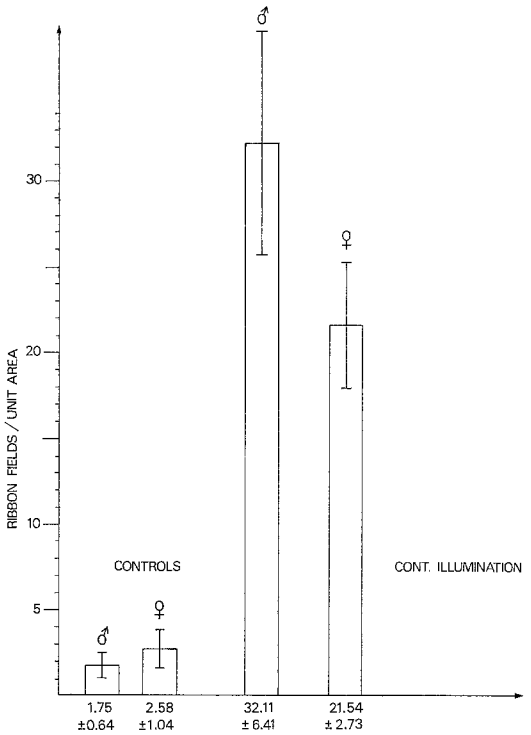


Fig. 3. Number of ribbon fields present in pineal glands of guinea-pigs kept under a lighting regimen of 14 hrs illumination and 10 hrs darkness (controls) and 64 days of continuous illumination respectively. Note the strong increase in number of the ribbon fields after continuous illumination

As regards the intracellular localization it was found that 54 % of the ribbon fields were located inside perikarya of pinealocytes and 46 % in cytoplasmic processes of these cells.

As can be seen from Fig. 1, ribbon fields containing only one ribbon predominate in cell processes, while fields with 2 and 3 ribbons are more numerous in perikarya than in processes.

In the perikarya 27 % of the ribbon fields were found in the immediate vicinity of the cell membrane, 73 % were more centrally located, some of which were found close to cell nuclei. In the cell processes 41 % were found lying close to the cell membrane while 59 % showed a more central localization.

Since "synaptic" ribbons in the retina and in pineal organs of lower vertebrates are intimately related to centripetal nerve fibres, it was pertinent to examine the topographical relationships of ribbon fields bordering the cell membrane with regard to neighbouring structures. In the present material no ribbon fields were found lying opposite nerve fibres or blood vessels. They all faced adjacent pinealocytes. Further analysis revealed that, in the perikarya, 75 % of the ribbon fields bordering the cell membrane were orientated towards

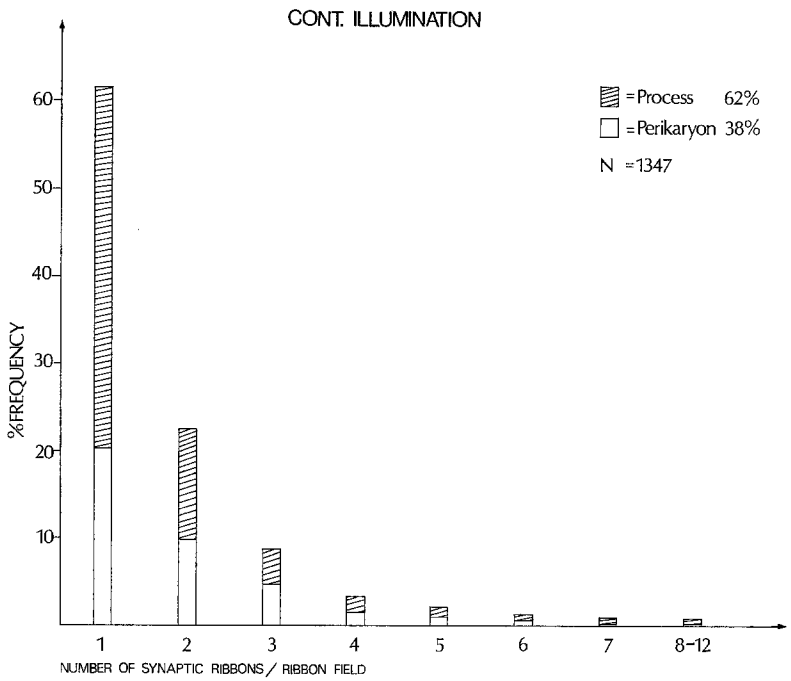


Fig. 4. Frequency distribution of ribbon fields comprising one, two, three and more "synaptic" ribbons after continuous illumination for 64 days. The comparison with Fig. 1 shows that, although there is a striking increase in number after continuous illumination (see Fig. 3), the different groups of ribbon fields appear to be almost equally affected by the increase in number. The white and the hatched parts of the columns indicate the incidence of ribbon fields found in perikarya and processes of pinealocytes respectively. *N* number of ribbon fields examined

neighbouring perikarya and 25 % towards adjacent pinealocyte processes. In the pinealocyte processes, 60 % faced adjacent perikarya and 40 % neighbouring pinealocyte processes.

### *Continuous Illumination*

Since light and darkness are regarded as being the appropriate inhibitory and excitatory stimuli of the rat pineal gland (Wurtman, Axelrod, and Kelly, 1968) it was of interest to study the effects of different lighting conditions on the structures in question. In the present study long-term rather than short-term exposure was applied because of Lues' (1971) results and since it seemed possible that "synaptic" ribbons might respond to extreme lighting conditions only.

Pineal glands of guinea-pigs kept under continuous illumination for 64 days showed a striking increase in the number of ribbon fields. As can be seen from Fig. 3 this increase was about 20-fold in males and some 9-fold in females. The differences between control and experimental animals are statistically significant (99 %). The frequency distribution of ribbons/ribbon field is shown in Fig. 4. The comparison with Fig. 1 reveals that the different groups are almost equally affect-

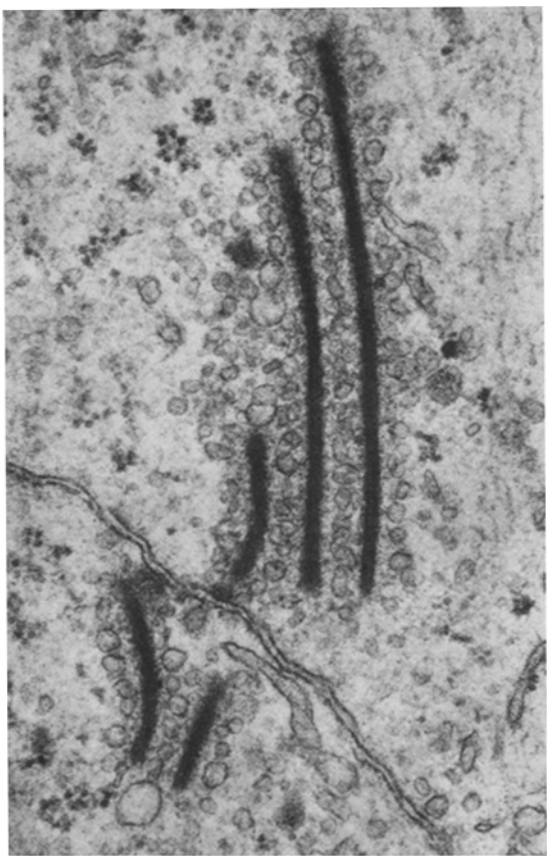

Fig. 5. Electron micrograph of an arrangement of ribbon fields termed paired ribbon fields. For details see text. Magnification × 61000

ed by the increase in number. The mean number of ribbons per field was found to be 1.68 as compared to 1.80 in normal animals.

The proximal part of the gland contained 31.9 RF/UA in the male and 22.0 RF/UA in the female, the intermediate area 32.3 RF/UA (♂) and 23.8 RF/UA (♀) and the distal region 31.9 RF/UA (♂) and 17.5 RF/UA (♀). No statistically significant differences have been detected either between males and females or between the different regions of the gland.

As regards the intracellular localization it was found that, under continuous illumination, 38 % (54 % in the controls) of the ribbon fields were located in perikarya. The remainder were found lying in cell processes. Further analysis revealed that in the perikarya 59 % of the ribbon fields were located in the immediate vicinity of the cell membrane as compared to 27 % in the controls. The respective figure for the cell processes was 60 % as compared to 41 % in the controls.

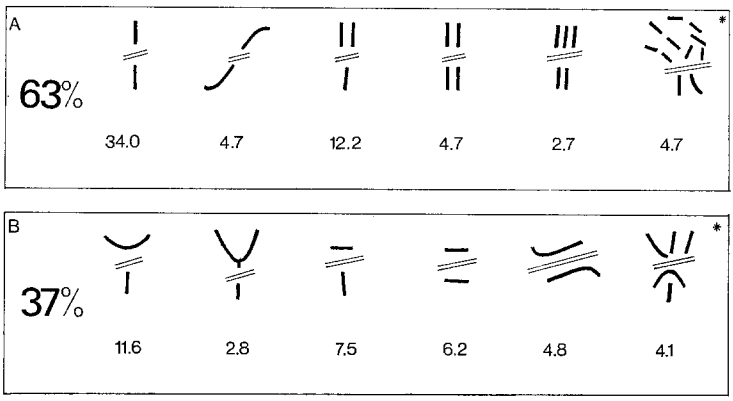


Fig. 6. Diagrammatic representation of different types of paired ribbon fields found in pineal glands of guinea-pigs kept under continuous illumination for 64 days. Group A includes fields where all the ribbons are arranged perpendicularly to the cell membrane, Group B those in which at least one ribbon lies parallel to the cell membrane. * denotes that this group represents a collection of various types present in small numbers

The topographical analysis of the ribbon fields bordering the cell membrane revealed that about 98 % faced adjacent pinealocytes and only a negligible number other structures. Two of the 16 ribbon fields in this category were found lying opposite non-myelinated nerve fibres, 6 opposite blood vessels and 8 opposite collagenous fibres.

The ribbon fields bordering the cell membrane and facing adjacent pinealocytes can be subdivided into two main groups: paired and unpaired ribbon fields.

*Paired Ribbon Fields:* A striking feature, not observed in control animals, was the occurrence of ribbon fields juxtapposed in adjacent pinealocytes and bordering on their respective cell membranes (Fig. 5). Frequently paired ribbon fields seemed to represent a single complex since in this situation "synaptic" ribbons often appeared to be traversing the narrow intercellular space. This latter observation could however not be substantiated in sections where the cell membranes had been cut in an exactly transverse manner. Apposing "synaptic" ribbons often exhibited a mirror image conformation (Fig. 6). Paired ribbon fields amounted to 22 % of the ribbon fields observed under experimental conditions. 56 % of the paired ribbon fields were found "linking" adjacent cell processes, 29 % a cell process and a perikaryon and 15 % adjacent perikarya. No statistically significant differences were observed in the number of paired ribbon fields between males and females or between different regions of the gland.

From a morphological point of view two main groups of paired ribbon fields could be distinguished, based on the arrangement of their "synaptic" ribbons in relation to the cell membrane (Fig. 6). In the first group, amounting to 63 %, all the "synaptic" ribbons were arranged perpendicularly to the cell membrane. The majority consisted of only two synaptic ribbons, one on either side of the cell membrane. The incidence of paired ribbon fields containing more than two synaptic ribbons is shown in Fig. 6. The second group included paired ribbon

fields where at least one "synaptic" ribbon was found lying parallel to the cell membrane. The ribbons lying parallel to the cell membrane were either straight or slightly curved. Occasionally they had the shape of a boomerang. The different types of paired ribbon fields included in this group and their frequencies are also shown in Fig. 6. It has not been possible to relate any of these groups or types to any particular region of the gland or to any particular type of cell contact (i.e. contacts between two perikarya, a perikaryon and a cell process or two cell processes). This is perhaps not surprising especially if one assumes that these structures represent sections through ribbons at different levels and that the ribbons are plate-like rather than rod-like (see Bayrhuber, 1972).

*Unpaired Ribbon Fields.* A topographical analysis of the unpaired ribbon fields bordering the cell membrane revealed that in the perikarya 44 % lay opposite an adjacent perikaryon and 56 % opposite a cell process; in the processes 74 % faced an adjacent process and 26 % a perikaryon.

The ratios of unpaired to paired ribbon fields varied considerably from animal to animal and ranged from 1.0 to 19.2. Further analysis showed that, with regard to the varying ratios, possibly two populations of animals existed. The majority of animals (9 males and 3 females) exhibited ratios between 1.0 and 7.0 and the remainder (1 male and 3 females) ratios between 13.1 and 19.2.

### *Continuous Darkness*

Pineal glands of guinea-pigs kept in continuous darkness showed inconsistent results as regards the number of ribbon fields. After 55 days of continuous darkness two animals showed a strong increase in the number of ribbon fields (46.2 RF/UA ♂, 17.2 RF/UA ♀), two males exhibited a moderate increase (9.6 RF/UA and 7.5 RF/UA respectively) and one female no change (1.3 RF/UA). A distinct increase (16.6 RF/UA) was observed in a male kept under darkness for 45 days. Low values were obtained from animals kept in darkness for respectively 26 days (1.3 RF/UA), 33 days (0.3 RF/UA) and 70 days (0.6 RF/UA and 1.0 RF/UA).

Paired ribbon fields were found only after 55 days of continuous darkness and only in three out of five animals. In these animals the ratio of singly-lying to paired ribbon fields was 2.6, 4.3 and 27.6 respectively.

### *Pregnancy*

Since pineal glands of pregnant guinea-pigs show signs of increased activity in the second half of gestation as revealed by enzyme histochemistry (Vollrath and Schmidt, 1969) and electron microscopy (Lues, 1971) it was relevant to enquire whether the ribbon fields were affected or not during pregnancy. The quantitative assessment showed that pineal glands of pregnant animals contained 1.4 RF/UA. No paired ribbon fields were observed.

## Discussion

As outlined in the introduction, a functional interpretation of the "synaptic" ribbons of the mammalian pineal gland is difficult, for various reasons. The results of the present study certainly do not solve this problem. However, the information obtained permits a more meaningful speculation about the function of the structures in question than has hitherto been possible.

To begin with, the results give a clear indication of the intracellular localization of the "synaptic" ribbons in relation to the cell membrane. It can be seen that two major groups of "synaptic" ribbons, or ribbon fields, according to the terminology of the present paper, have to be distinguished: 1. ribbon fields bordering the cell membrane, and 2. ribbon fields lying distinctly apart from it. That the second group really exists, and that it is not merely due to the plane of sectioning, is illustrated by the fact that some ribbon fields can be found in the immediate vicinity of a cell nucleus. From a functional point of view it would be most interesting to know the absolute sizes of each of the two groups. This kind of information can, however, only be obtained from a study of serial sections which we did not attempt in the present investigation. The interesting point that emerges from a comparison of the figures obtained is that the localization of the ribbon fields differs depending on the environmental lighting conditions of the animals. Thus it can be seen that, in continuous light, a higher percentage of ribbon fields are found bordering the cell membrane than in animals kept under a lighting schedule of 14 hrs illumination and 10 hrs darkness. This finding is of particular importance, especially if one assumes that, in analogy to e.g. the retina, it is the synaptic ribbons bordering the cell membrane which matter most.

The functional interpretation of the "synaptic" ribbons is also helped by the results of the topographical analysis. They show that about 98 % of the ribbon fields bordering the cell membrane face adjacent pinealocytes and only a negligible number face collagenous fibres, blood-vessels or nerve fibres. Thus it is clear that, if synaptic ribbons are functionally related to neighbouring tissue components at all, it would appear that it is another pinealocyte rather than anything else. This conclusion is substantiated by the fact that, under experimental conditions, paired ribbon fields occur in which each individual of the pair lies exactly opposite each other in different pinealocytes.

Perhaps the most important finding of the present study, from a functional point of view, is the observation that "synaptic" ribbons show characteristic changes under certain experimental conditions, as indicated by their 10 to 20-fold increase in number, the occurrence of paired ribbon fields and their change in localization. These findings, in our view, do not favour the concept that "synaptic" ribbons are non-functioning phylogenetic relics. They rather suggest that they are true cell organelles.

Without relevant physiological information it is certainly premature to be precise about the functional significance of the "synaptic" ribbons of the mammalian pinealocyte. For the retina, where synaptic ribbons form part of synapses, it has been suggested that they may serve as orienting structures to channel synaptic vesicles in an orderly, conveyor-belt fashion to the plasma membrane for transmitter release (Bunt, 1971). They would thus have a similar function to fulfill as the dense projections of brain synapses, namely to guide vesicles to a narrow strip of presynaptic membrane (Gray and Pease, 1971).

As far as a transport of vesicles directed to certain parts of the cell membrane is concerned, one can easily visualize a similar function for the "synaptic" ribbons of the pinealocytes, at least for those which are arranged perpendicularly to the cell membrane. These ribbons exhibit exactly the same orderly arrangement of vesicles as those of e.g. the retina. The crucial problem, however, is what the

chemical nature of the content of the vesicles is. Do they contain specific transmitter substances? As long as this question remains unanswered one can only speculate about the function of the "synaptic" ribbons. The fact that the ribbon fields which border the cell membrane lie almost exclusively opposite pinealocytes and that they are not related to blood vessels does not favour a concept that it is the primary function of these structures to release substances into the systemic circulation. In our view it is more likely that the vesicles of the "synaptic" ribbons contain, and release, substances whose targets are adjacent pinealocytes. According to this assumption "synaptic" ribbons bordering the cell membrane could represent a device for interconnecting neighbouring pinealocytes, a view which is in accordance with that of Hopsu and Arstila (1965).

We realize that, for the time being, this interpretation can only be regarded as a working hypothesis. Certainly much more work (e.g. of enzyme histochemical and electrophysiological nature) would have to be carried out to prove, or to disprove, this hypothesis. In our view it should not be rejected merely because it is not in keeping with the concept that the mammalian pinealocyte is a secretory cell. After all, mammalian pinealocytes have been regarded as modified glial cells (Bargmann, 1967), modified nerve cells (Hopsu and Arstila, 1965; Wartenberg, 1968) and true secretory cells phylogenetically derived from photoreceptor cells of lower vertebrates (Kappers, 1969, 1971a). Moreover, there is ample evidence that intercellular communication is an important and common biological phenomenon, even between gland cells (Loewenstein, 1966).

If one accepts the concept that "synaptic" ribbons bordering the cell membrane act as functional links between adjacent pinealocytes then, on purely morphological grounds, it is tempting to distinguish two types: the uni-directional link, represented by a single ribbon field bordering the cell membrane, and the bi-directional link, represented by what in the present study is termed a paired ribbon field. The "synaptic" ribbons which lie distinctly apart from the cell membrane cannot, according to the present concept, be directly involved in intercellular communication. They could, however, play a role as reserve structures which could move to the cell membrane if required. "Synaptic" ribbons lying parallel to the cell membrane do not seem to be ideally arranged for the transport of vesicles to the cell membrane. Perhaps they are in the process of moving to a position at right-angles to the cell membrane, or, alternatively, they could be related to a cell membrane lying in a different plane of the tissue block.

Finally the question arises as to the possible significance of the "synaptic" ribbons for the functioning of the pineal gland as a whole. Looking at the architecture of the mammalian pineal gland it can be seen that the nerve fibres which are responsible for the regulation of pinealocytes function are restricted to certain areas of the gland and do not make direct contacts with all the pinealocytes. Here a special device for intercellular communication could be required to enable the gland to synchronize the function of its cells. Such a mechanism could become particularly apparent under extreme conditions, e.g. as applied in the present study, or when the functional activity of the gland changes abruptly. In this context it is interesting to note that "synaptic" ribbons disappeared in rat pineal glands cultured in vitro where the pinealocytes were no longer under the regulating influence of synpathetic nerve fibres (Arstila, Kalimo, and Hyyppä, 1971).

From a functional point of view it would be interesting to correlate the number of the ribbon fields with the functional state of the pineal gland. For the time being such a correlation appears to be premature. First, no relevant biochemical information is currently available for pineal glands of guinea-pigs kept under similar lighting conditions as used in the present study. Secondly, if one assumes that light and darkness have the same inhibitory and stimulatory effects on the pineal gland of the guinea-pig as they have on that of the rat then the striking differences in the number of ribbon fields between the control animals and those kept under continuous illumination seem to suggest that ribbon fields are particularly prominent when the functional activity of the gland is depressed. If this assumption were correct, then one could expect to find no ribbon fields, or only very few, after continuous darkness, when the activity of the gland is greatly enhanced. The results after continuous darkness, though inconsistent, show, however, an increase in the number of ribbon fields rather than a decrease. Certainly experiments of a more physiological nature, in conjunction with biochemical assay methods, would have to be carried out before a satisfactory answer to this problem can be expected.

However, one can also envisage a different functional implication of the ribbon field/cell membrane complexes with regard to the functioning of the pineal gland as a whole. It could be that these complexes are not engaged in spreading information which has reached the gland via the sympathetic nerve fibres. There is also the possibility that mammalian pinealocytes, in addition to their endocrine function, act like neurons and that "synaptic" ribbons are special devices by which as yet unknown neuronal circuits are established within the gland. These circuits could enable the gland to act not merely as a subordinate neuroendocrine transducer governed by impulses from sympathetic nerve fibres but to play a more active and independent role in neuroendocrine integration. Perhaps mammalian pinealocytes are neuroendocrine cells *par excellence* in so far as they combine both a distinct endocrine activity and other strictly neural functions.

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