j. taxi editor

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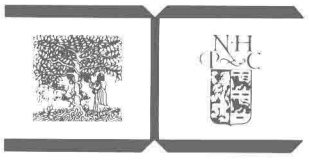

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Professor R. Couteaux

PREFACE

When Professor Couteaux retired in the autumn of 1978,some of those indebted to him for help received in various ways for their scientific work wondered how to approprately honour this eminent scientist,who is also endowed with exceptional human qualities, rendering his contact so pleasant and enriching.

Knowing Professor Couteaux's repulsion for formal celebrations, it seemed that the impetuous growth in recent years of works bearing on the developing and functional mechanisms of the peripheral synapses justified an attempt to synthesize these matters at the same time as giving this useful occasion the significance of a homage to Professor Couteaux,who has devoted all his scientific life to the cytology and cytochemistry of peripheral synapses,and especially the neuromuscular junction.

His achievements started with his medical thesis (1941) on the ontogenesis of muscle cells and motor end-plates.It hardly needs to be recalled that a crucial point in our knowledge of the vertebrate neuromuscular junctions was his discovery of the subneural apparatus by postvital staining with Janus Green B.This method requires a great savoir-faire and indeed very few people,if any, were able to reproduce the results published in his science thesis appeared in 1947.Thus there was a period of controversy on the exact significance of his pictures,until the demonstration of the subneural apparatus became simplified by using Koelle's histochemical method for cholinesterase activity.

I remember at the time of my arrival in Couteaux's laboratory the echos of these often sharp discussions,and in the first meeting in which I participated this old colleague saying that it seemed incredible to him that so large an organelle had escaped all histologists having studied the motor end-plate for over fifty years.What an involuntary homage it was!

Some years later,there was the great revolution of cytology

brought on by the electron microscopy.The beginning of that new era was difficult due to the rarity of this expensive tool and the time required to handle the ancillary techniques,which were far from their present reliability.In the 60's his knowledge of the biological material led Professor Couteaux to obtain images strongly suggesting a possible way for the neurotransmitter liberation in the frog motor end-plate.He thereby stimulated a serie of investigations on this topic which is not yet closed,as would appear from this symposium.

The proposition of a symposium has been well accepted by the qualified instances of the CNRS and INSERM,the financial support of which was an absolute requirement for organizing the meeting. To tell the truth,the CNRS and INSERM are also indebted to Professor Couteaux,as reminded by the scientific director of the CNRS A.Berkaloff at the opening session of the meeting,for many years of participation in the commissions and life of both organisations.

Finally those who have kindly accepted to participate in the meeting,ensuring his success and the publication of this book,have to be acknowledged ,as well as the large audience having regularly attended and animated the sessions.

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ONTOGENESIS AND MAINTENANCE OF GANGLIONIC SYNAPSES

Ontogenesis and Functional Mechanisms of Peripheral Synapses
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ON STUDYING THE MOLECULAR DETERMINANTS OF SYNAPSE FORMATION

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The establishment of specific synaptic connections between neurons and their target cells is a prerequisite for the function of the nervous system. Synaptogenesis can also be viewed as one of the most striking examples of cellular interaction and recognition in biology. Thus it is not surprising that the problem of how neuronal processes reach the correct location and then form synapses on the appropriate target cell has attracted much attention over the years. A major effort in this field has involved ablation or transplantation of various parts of the nervous system to see if connections can be made under novel circumstances. Such phenomenological studies are essential in establishing the ground rules of what is possible with embryonic or regenerating neurons, and the most provocative experiments have suggested possible mechanisms of guidance, recognition, or competition during synapse formation. In an effort to take the analysis a step further and examine the molecular determinants of these phenomena, recent advances in immunological and cell biological techniques are being applied to the problem. The goals are first, to determine if there are molecules that are specifically involved in synapse formation (as opposed to other aspects of differentiation, growth or metabolism), and second, if such molecules exist, to characterize their chemical nature and their functional roles. This review will consider some of these newer approaches and discuss a few of the issues that will be raised by these studies.* The first problem to be dealt with is the fundamental problem of definitions.

What is a Synapse?

Any study of synapse formation ought to begin with a firm idea of what the final product should be; what are the criteria for saying a synapse has or has not formed between two cells? Perhaps surprisingly, it is difficult to achieve a consensus on what properties a junction between two cells should display to be termed a synapse. This difficulty reflects both the variety of synapses found in the nervous system and the variety of techniques which have been used to characterize synapses. Sherrington coined the term "synapse" in the context of the cell theory, to emphasize the separation between the cells. "It seems therefore likely that the nexus between neurone and neurone in the reflex-arc...involves a surface of separation between neurone and neurone; and this as a transverse membrane across the conductor must be an important element in intercellular conduction. The characters distinguishing reflex-arc conduction from nerve-trunk conduction may therefore be largely due to intercellular barriers, delicate transverse membranes, in the former."[1]

Subsequently, anatomical methods have shown that these delicate membranes display many different configurations[2,3]. Presynaptic membranes possess

*This paper is a relatively brief overview of many overlapping areas of interest. The literature citations are intended only to be selected guides to further reading, and are not meant to be comprehensive.

specializations or fuzz or they may appear unmodified. Postsynaptic membranes may or may not exhibit conspicuous thickenings, and they can be smoothly and continuously apposed to their presynaptic partners, or they can be thrown into complex folds or even poke finger-like projections into the presynaptic terminal. Synaptic vesicles are usually clustered close to the junctional area, but in some cases they are scattered about the terminal as if ignoring the junction. The synaptic cleft itself can be electron lucent or filled with electron dense material. The width of the cleft can vary greatly as well; for instance, within the autonomic system the cleft can be as small as 20 nm or as large as 2,000 nm. Such variety in cleft width may contribute to the heterogeneity in latency, rise time, and duration of synaptic potentials seen in different target tissues[4]. In addition, each new morphological technique, such as freeze fracture, reveals further heterogeneity among synaptic types[3]. Clearly, there is no prototypical synaptic structure that can be generalized.

For many physiologists, the ultimate definition of a synapse is function. The structure of a brain might be perfect, but if there is no function, "it is no more than a statue."[5] In fact, normal appearing adrenergic synapses have been described by electron microscopy[6], but no synaptic interaction detected with microelectrode techniques, even though the presynaptic cell was known to be releasing catecholamines[7]. However, a definition based strictly on function also has its problems. Some "synaptic" interactions can be extremely different from that of the standard neuromuscular junction. For instance, how long a latency and how slow a rise time should a synaptic potential have before it is termed a hormone response? Developing systems offer especially difficult terminology problems. Freely moving growth cones have been shown to contain transmitter stored in synaptic vesicles[8], and can release it when electrically stimulated[9]. This release results in a normal appearing response that can be recorded in the muscle cell under the growth cone; should this response be termed a postsynaptic potential? This example can serve to move the discussion from the murky realm of nomenclature into the glaring light of experimental problems.

Experimental Approaches

Given the difficulties in generalizing about synapses on either a functional or a structural basis, perhaps a flexible and pragmatic outlook is the most logical one. One approach would be to (I) pick a particular system to work with and describe the various properties of the junctions between the mature cells in that system, (II) describe the development of each of these properties, and (III) perturb the system experimentally to get at the molecular mechanisms involved in forming the junctions. In asking whether a set of neurons can, under experimental conditions, form junctions with the characteristic properties they normally have, one sidesteps controversy over terminology. What are some of the factors to consider in each of these three steps?

(I) The choice of a system. Obviously any part of the nervous system may be used in this type of study, and there are numerous species to choose from as well. In making this decision, there are some theoretical considerations worth mentioning.

(a) Prior work on the system: the more that is already known about a particular set of synapses, the less groundwork that has to be done. Some of the better studied synaptic arrays, in terms of specificity and competition, are the retinal-tectal connections[10], the neuromuscular junction in skeletal muscle[11], and the preganglionic synapses on sympathetic neurons[12]. In the latter two cases, the neurotransmitters and morphology of the synapses have been well described, but much less is known about those properties in the