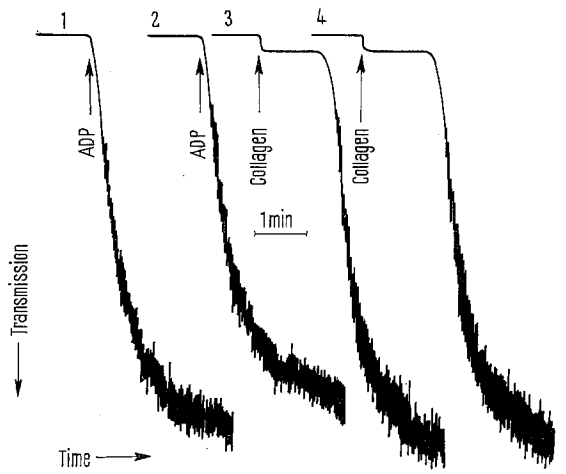
Influence of atractyloside on the release of serotonin and of nucleotides by human platelets, induced by ADP or collagen

Concentration of atractyloside (M)	Release of serotonin (%)			Release of nucleotides (%)		
	ADP	Collagen	No agent	ADP	Collagen	No agent
0	59	60	0	33	44	0
10^{-6}	61	60	0	40	55	0
5×10^{-6}	61	61	0	37	48	0
2×10^{-5}	58	60	0	40	58	0
10^{-4}	59	61	0	41	53	0
5×10^{-4}	60	60	0	41	56	0



Recorder tracings of the transmission curves of stirred human PRP, induced to aggregate by $10^{-5}M$ ADP or 20 µg/ml collagen. Curve 1: ADP — control. Curve 2: ADP + $10^{-4}M$ atractyloside. Curve 3: collagen-control. Curve 4: collagen + $10^{-4}M$ atractyloside.

The release of nucleotides was estimated by measuring the difference of extinction at 260 nm between the perchloric acid extracts of treated and untreated sedimented platelets[13]. This method is justified by the similarity between the absorption spectra of ADP and perchloric acid extracts of human platelets and of the material released by them[14].

Results. The Figure shows some of the curves of the transmission change of stirred PRP during aggregation by ADP and by collagen, with and without addition of $10^{-4}M$ atractyloside. The curves of the samples containing 10^{-6} to $5\times10^{-4}M$ atractyloside were very similar to those shown in the Figure. The compound had no effect on the aggregation, in accordance with the finding of ABDULLA[2]. Atractyloside alone caused no aggregation or change in the transmission of the sample.

The Table shows that atractyloside had no influence on the release of serotonin and of nucleotides under the conditions of our experiments at concentrations of up to $5\times10^{-4}M$. This supports the current view that nucleotides and serotonin are released together by a mechanism which is different from the translocation of nucleotides through the mitochondrial membrane. Since the platelet release reaction has many characteristics in common with other release reactions[15], these findings may be of general importance[16].

Zusammenfassung. Atractylosid hat bei menschlichen Blutplättchen keinen Einfluss auf die Aggregation und auf die Freisetzungsreaktion durch ADP und Kollagen in vitro.

P. MASSINI

Theodor Kocher Institute, University of Berne, CH-3012 Berne (Switzerland), 27 April 1970.

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Differential Neuronal Radiosensitivity as a Tool for the Study of Short Connections

The purpose of this note is to present a new experimental approach to the study of short neuronal connections that will complement the rather sparse methodology now available for that purpose. The rationale behind the method here presented is the possibility of provoking considerable and rather selective damage to a given neuronal population, that of the small interneurons, while almost sparing – at least from a connectional view point – all other neuronal elements. For this purpose, advantage was taken of the differential neuronal radiosensitivity[1,2] that for a given dose and time of evolution prevails between granule cells and all other neuronal components of the olfactory bulb.

Body shielded rats were irradiated on the head with a dose of 20,000 r X-rays. 24 h post irradiation, the brains were excised after perfusion with osmic or glutaraldehyde solutions. The olfactory bulbs were processed for electron microscopy observation. Degenerating terminal endings of the electron dense type were observed at the level of the plexiform layer, in synaptic contact with ramifications of the accessory dendritic branches of the mitral cells (Figure 1). In some sections the synaptic site and the synaptic vesicles at the mitral side of the contact were easily identified. The terminals exhibited graded alterations from crowding of synaptic vesicles and disruption of mitochondria to an extreme densification of the whole synaptic sac. However, in some of the latter terminals, mitochondria, although extremely electron

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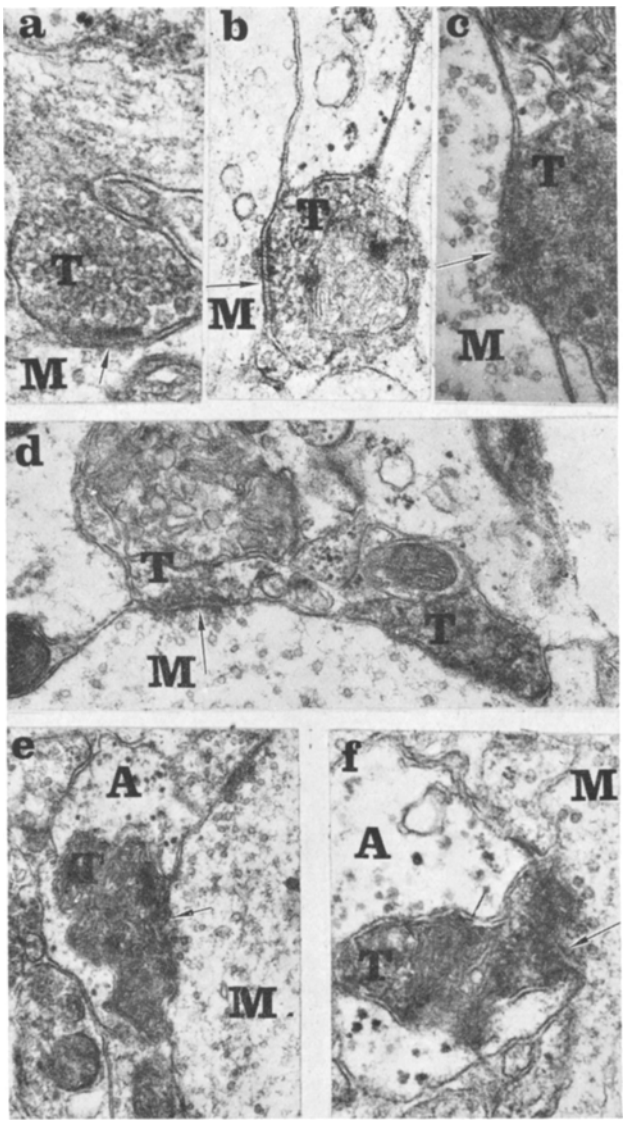
opaque, were still recognizable. Some of these profiles were surrounded by astroglial processes with abundant granules of glycogen. Because of their postsynaptic position towards the mitral cell dendrite, and taking into account the intense pyknosis of many internal granule cell perikarya found in these specimens, the degenerating profiles were identified as the dendritic granule terminals. These profiles are normally in reciprocal dendrodendritic synapses with dendritic branches of mitral cells. Degeneration of the granulo mitral reciprocal synapses then occur at the granular side of the contact due to the much higher radiovulnerability of granule cells than mitral neurones. This alteration is presynaptic in relation to the granulo-mitral pathway but postsynaptic if referred to the mitro-granular direction of the dendrodendritic synaptic complex.



Plexiform layer of the irradiated rat olfactory bulb; a) a granule dendrite terminal (T) with crowded synaptic vesicles is seen in synaptic contact with a mitral dendrite (M); b) degenerating granule terminal (T) with altered mitochondria in synaptic contact with mitral dendrite (M); c) very electron dense degenerating granule terminal (T) in synaptic contact with mitral dendrite (M); d) degenerating granule terminals in synapses with mitral dendrite (M); e) and f) electron dense degenerating terminals (T) in synapses with mitral dendrite (M) are seen surrounded by astrocytic processes with glycogen content (A). The arrows point to the synaptic sites.

The precise structural analysis of short neuronal connections always represented a formidable task for the neuroanatomist. This remains true despite the rapid increase in the number and variety of methods and techniques now composing the research armamentarium. Indeed, most of our knowledge on this topic is still dependent on the study of Golgi preparations to which some precision has undoubtely been added by electron microscopical correlations. In fact, conventional studies based on experimental degeneration have been almost exclusively directed towards the study of long connections both at the light and electron microscopical levels. One obvious major limiting factor for the experimental approach to short connections is the impossibility of performing lesions within the range of the processes of short axon neurones without producing at the same time and site confusing traumatic pathology. In particular these limitations have prevented our knowledge of short neuronal connections from having precision on the structural type and space arrangement of nerve endings attainable through electron microscopy. Remedies for this situation have been obtained by the performance of minute wounds of the CNS on precise loci of cerebral and cerebellar cortices[3-5], the study of residual connections after severance of the known long ones[6], and the study of the distribution and synaptology of the postsynaptic profiles pertaining to the dendritic arborizations of neurones undergoing retrograde degeneration elicited by a rather distant axonal sectionning[7].

It is believed that acute radiation injury in certain areas of the CNS, combined with electron microscopy, represent a directly valuable tool for the investigation of short neuronal connections.

Résumé. La radiolésion aigue du bulbe olfactif provoque des dommages sélectifs aux petits interneurons. L'examen au microscope électronique du tissu irradié a mis en évidence la présence de nombreuses terminales en dégénérescence, au niveau de la couche plexiforme. Elles ont été interprétées comme étant des dendrites appartenant aux granules radiolésées par leur position postsynaptique dans des synapses réciproques avec des dendrites mitrals. On propose d'utiliser cette méthode pour l'étude des connections interneuronales courtes dans certains endroits du SNC.

ROSITA F. DE ESTABLE-PUIG
and J. F. ESTABLE-PUIG[8]

Department of Pathology, Laval University, Québec 10 (Canada), 16 April 1970.

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