divalent cation binding in a manner similar to that for pCMB[13].

[13] This investigation was supported by Grant No. GM18819-02 from the National Institutes of Health and Grant No. NGL 05-003-024 from the National Aeronautics and Space Administration.

[14] I wish to thank Dr. GEORGE L. ELLMAN for several of the sulfhydryl reagents and for discussions relating to the present study. I also wish to thank Mr. THIAN VUI MARK LEE for his capable assistance during the course of this investigation.

*Zusammenfassung.* Nachweis an Lebermikrosomen der Ratte, dass Sulfhydryl Reagenzien *p*-Chloromercuribenzoat (pCMB) und *p*-Chloromercuribenzol Sulfonat (pCMBS) die Bindung von Magnesium und Calcium ungefähr um 20% vermindern.

H. SANUI[14]

*Department of Physiology, University of California, Berkeley (California 94720, USA), 16 October 1974.*

## An Ultrastructural Study of the Synaptic Glomeruli in the Intermediolateral Nucleus of the Rat

After the early studies of the intermedio-lateral nucleus (ILN) by conventional light microscopy (see RÉTHELYI[1] for references), major contributions were made by RAMÓN and CAJAL[2] using the Golgi silver impregnation technique and later by DAHLSTRÖM and FUXE[3] by means of fluorescence microscopy. But studies of the ILN at the ultrastructural level appears to be very limited. The ultrastructure of the ILN of the cat has been reported by RÉTHELYI[1] and that of rats after 6-hydroxydopamine induced experimental degeneration by WONG and TAN[4]. It was during the latter study that the axons and dendrites in the ILN of rat were noted to be organized into large numbers of synaptic complexes. This paper describes the organization of these complexes and discusses the significance of the findings.

Male adult albino rats which were apparently healthy and weighing between 200–250 g were anaesthetized with ether and perfused through the left cardiac ventricle with a solution containing 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 *M* cacodylate buffer[4]. The fixative also contained 0.5 mg/l $CaCl_2$. After 20 min of perfusion, the thoracic cord was dissected out and thin slices were cut with razor blade and immersed in similar fixative for a further 2 h or overnight at 4 °C. The tissues were post-fixed in osmium tetroxide[5] for $1^1/_2$–2 h, after which they were dehydrated in a graded series of acetone and embedded in Araldite. Semi-thin sections of 0.5 µm thick were cut with glass knives and stained with methylene blue. The ILN was identified and the block was retrimmed. Ultra-thin sections of silver-gray interference colours were cut in the frontal plane with a Porter-Blum ultramicrotome and double-stained on grid with uranyl acetate[6] and lead citrate[7]. The sections were viewed through an Hitachi HS-8 electron microscope.

The axon terminals in the ILN of rat appear as large, medium sized or small boutons; they may contain either round, flattened, ellipsoidal or pleomorphic vesicles.

Large boutons are observed to contain only round vesicles (LR boutons). Large boutons with flattened vesicles have so far not been observed to be present. Medium-sized and small boutons, however, may contain either round or flattened vesicles. Some of these profiles with round vesicles may possibly be part of an LR bouton.

The LR boutons are usually invaginated and contacted by dendritic shafts and spines or small axon terminals (Figure 1). Their vesicles, which are spherical and agranular, are distributed throughout the bouton but are more closely packed adjacent to the presynaptic membrane. Large granular vesicles (LGV) have not been observed in the LR boutons. Sometimes, however, the contour of the LR bouton may be more regular and not so heavily indented by postsynaptic profiles (Figure 5).

The medium-sized (Figures 2 and 4) and small boutons (Figures 2 and 4) have more regular contours and may be somewhat rounded, oval or crescent in shape. Their vesicles may be round or flattened. Some of these terminals may contain a few LGV (Figure 3).

The LR boutons form Gray Type 1 synapses[8] with a primary dendrite or with a dendritic spine (Figure 1). No spine apparatus, however, has been observed in the latter. In addition, the LR boutons are often contacted by small axonal profiles forming axo-axonal synapses (Figures 1 and 2). When such synapses are present, the smaller profiles are always presynaptic to the LR bouton, while the latter is in turn presynaptic to a dendrite. The small presynaptic axon terminal usually contains round vesicles (Figures 1 and 2).

The neural elements which establish synaptic contact with each other are often enclosed in a prominent glial lamina (Figures 1, 2, 4 and 5) to form synaptic glomeruli. The number of elements in each glomerulus varies. These glomeruli may be tentatively classified into 3 types. Type 1 (Figure 1), in which there is a prominent central LR bouton surrounded and contacted by dendrites and small axon terminals. Type 2 (Figure 4), in which the prominent central profile is a large dendrite surrounded and contacted by medium-sized and small axon terminals. Sometimes other dendritic profiles may lie adjacent to the large dendrite, but no synapse has been observed between them although desmosomal types of contact may be present. Type 3 (Figure 5), in which the central profiles consist of an LR bouton contacted by a large dendrite. They are surrounded and contacted by other axons and dendrites.

The present study has shown that glial encapsulated synaptic glomeruli constitute a prominent feature in the ILN of rat, although RÉTHELYI[1] did not describe them in the cat. Such synaptic complexes are now a well-established feature in many areas of the central nervous system e.g. lateral geniculate nucleus[9,10], medial geniculate nucleus[11,12], pulvinar[13,14], ventrobasal nucleus of the thalamus[15,16], cuneate nucleus[17], nucleus gracilis[18], and

[1] M. RÉTHELYI, Brain Res. *46*, 203 (1972).
[2] S. RAMÓN and CAJAL, in *Histologie du Système Nerveux de l'Homme et des Vertèbres* (A. Maloine, Paris 1909–1911), vol. 1.
[3] A. DAHLSTRÖM and K. FUXE, Acta physiol. scand. *64*, Suppl. *247* (1965).
[4] W. C. WONG and C. K. TAN, Experientia *30*, 1455 (1974).
[5] A. J. DALTON, Anat. Rec. *121*, 281 (1955).
[6] M. L. WATSON, J. biophys. biochem. Cytol. *4*, 475 (1958).
[7] E. S. REYNOLDS, J. Cell Biol. *17*, 208 (1963).
[8] E. G. GRAY, J. Anat., Lond. *93*, 420 (1959).
[9] A. PETERS and S. L. PALAY, J. Anat., Lond. *100*, 451 (1966).
[10] E. V. FAMIGLIETTI and A. PETERS, J. comp. Neurol. *144*, 285 (1972).
[11] K. MAJOROSSY and M. RÉTHELYI, Expl Brain Res. *6*, 306 (1968).
[12] E. G. JONES and A. J. ROCKEL, Z. Zellforsch. *113*, 44 (1971).
[13] K. MAJOROSSY, M. RÉTHELYI and J. SZENTAGOTHAI, J. Hirnforsch. *7*, 415 (1965).
[14] L. H. MATHERS, J. comp. Neurol. *146*, 15 (1972).
[15] E. G. JONES and T. P. S. POWELL, Proc. R. Soc., ser. B *172*, 153 (1969).

substantia gelatinosa[19]. Such arrangements of neuronal elements would provide excellent means for convergence and divergence of impulses to and from various sources. The many synaptic relationships established between the various neural elements within the glomeruli would provide an opportunity for the interaction and integration of impulses and make these glomeruli important tools for information processing[20].

The large axon terminals containing flattened vesicles described by RÉTHELYI[1] in the cat have not been observed by us in the rat. So far we have observed that all the large axon terminals contained only round vesicles. It is, however, possible that the flattening of the vesicles of the large boutons observed by RÉTHELYI[1] could have been the result of primary aldehyde fixation[21]. Furthermore, KORNELIUSSEN[22] had shown that vesicles tended to become osmotically more sensitive after primary aldehyde fixation and that this could contribute to the flattening effect of some of the vesicles.

Although RÉTHELYI[1] did not describe any axo-axonal contacts in the ILN of cat, we have observed many such contacts in the rat. Such a synapse may represent the anatomical substrate for presynaptic inhibition[23,24]. If this is the case, then presynaptic inhibition may be an

[16] J. SPACEK and A. R. LIEBERMAN, J. Anat., Lond. *117*, 487 (1974).
[17] C. K. TAN and A. R. LIEBERMAN, J. Anat., Lond., in press.
[18] A. RUSTIONI and C. SOTELO, J. comp. Neurol. *155*, 441 (1974).
[19] M. RÉTHELYI and J. SZENTAGOTHAI, Expl Brain Res. *7*, 258 (1969).
[20] J. SZENTAGOTHAI, in *The Neurosciences. 2nd Study Programme* (Ed. F. O. SCHMITT; Rockefeller Univ. Press, New York 1970).
[21] F. WALBERG, Acta anat. *65*, 224 (1966).
[22] H. KORNELIUSSEN, J. Neurocytol. *1*, 279 (1972).
[23] J. C. ECCLES, Ergebn. Physiol. *51*, 299 (1961).
[24] E. G. GRAY, Nature, Lond. *193*, 82 (1962).

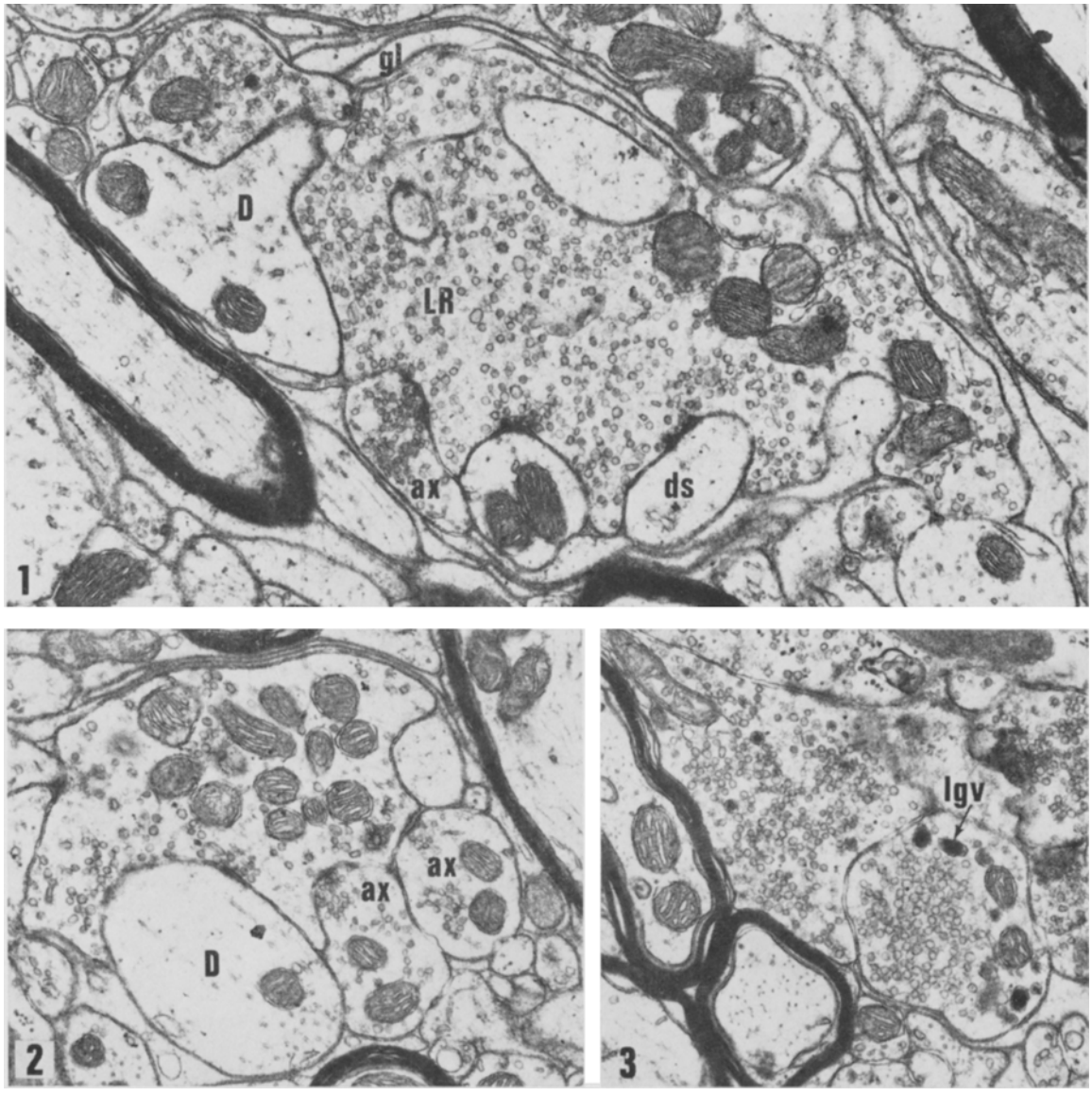
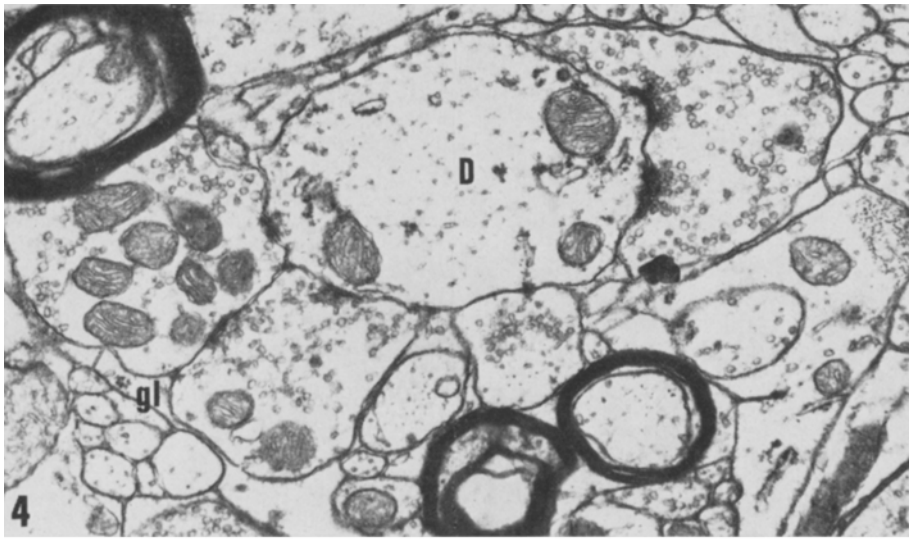



Fig. 1. A synaptic glomerulus in which a large bouton containing round agranular vesicles (LR) makes contact with dendritic spines (ds) and itself is contacted by a small axonal terminal (ax). The synaptic glomerulus is enclosed by a glial lamina (gl). D, dendritic shaft. ×27,500.

Fig. 2. A synaptic glomerulus in which a medium sized bouton (possibly part of an LR bouton) makes contact with a dendrite (D) and itself is contacted by small axonal terminals (ax). ×27,500.

Fig. 3. Two medium sized boutons containing large granular vesicles (lgv) in addition to numerous small agranular vesicles. ×27,500.



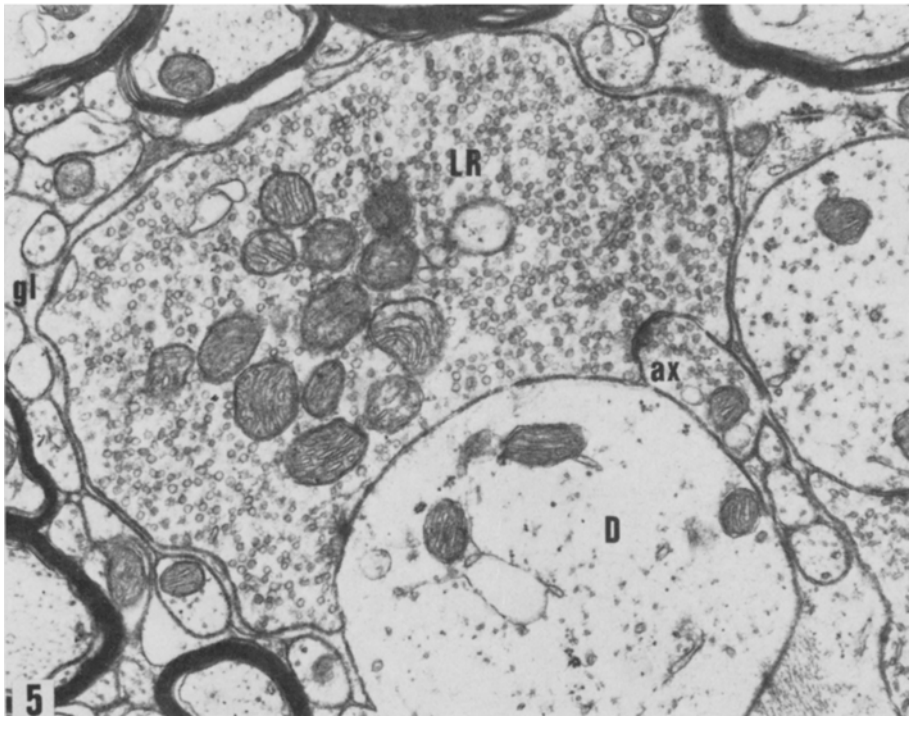




Fig. 4. A synaptic glomerulus in which a dendritic shaft (D) is contacted by small and medium sized boutons. The synaptic glomerulus is enclosed by a glial lamina (gl). ×27,500.
Fig. 5. A synaptic glomerulus in which an LR bouton makes contact with a large dendrite (D) and itself is contacted by a small axonal terminal (ax). The synaptic glomerulus is enclosed by a glial lamina (gl). ×27,500.

important mechanism by which negative feedback processes may operate in the ILN of rat.

*Résumé.* On a constaté que le neuroptile du noyau intermédio-latéral du rat contient de nombreux glomérules synaptiques gliaux encapsulés dans lesquels se trouve habituellement un dentrite ou axe terminal très visible surmonté par de petits boutons et des dentrites. Les glomérules sont accompagnés de synapses axo-axonaux qui peuvent être le corrolaire structural d'une inhibition présynaptique. La signification fonctionnelle des glomérules et des synapses axo-axonaux est discutée.

[25] It is a pleasure to acknowledge the technical assistance of Mr. H. L. Chan.

[26] Please direct all correspondence and requests for reprints to: Dr. W. C. Wong, Department of Anatomy, University of Singapore, Sepoy Lines, Singapore, 3.

C. K. Tan[25] and W. C. Wong[26]

*Department of Anatomy, University of Singapore, Sepoy Lines, Singapore 3, 30 September 1974.*

## Evidence for the Stimulant and Depressant Central Effects of L-α-Acetyl Methadol[1,2]

Most neurotropic drugs are thought to be either stimulants or depressants. Experimentally such a system of classification is justified on the basis of gross behavioral effects exerted by the drugs in question. Their responses represent for the most part an algebraic sum of the individual effects exerted by the drugs on the central nervous system (CNS) over a given period of time to which a subject is exposed to the drugs. Opiates and opiate derivatives