Electron Microscopic Observations on the Olivary Projections to the Cerebellar Nuclei in the Cat

Studies with the experimental silver impregnation methods have shown that spinocerebellar[1–3] and olivocerebellar tracts[1,4] send their collaterals to the cerebellar nuclei. The former projection has been also confirmed by an electron microscopic study[5]. These collaterals are regarded as excitatory input to the cerebellar nuclei, while the cerebellar corticonuclear fibres are known to be inhibitory input to the nuclei[6,7]. According to our previous study with the Nauta method, the olivocerebellar tract (OCT) fibres of the cat terminate in all the cerebellar nuclei on both sides, but mainly on the contralateral side (see reference[4] for review). In fact, there is an electrophysiological report showing that stimulation of the inferior olivary complex evokes monosynaptic excitatory responses in the contralateral dentate nucleus[7]. However, no electron microscopic study has thus far been made on the projections from the inferior olive to the cerebellar nuclei. The present study was undertaken to confirm the results of our experimental silver impregnation study and to investigate the mode of termination of OCT fibres.

Material and methods. Electrolytic lesions of the inferior olivary complex were made in 6 adult cats. The procedure of surgery is the same as described elsewhere[4]. On 4–5 days after operation, the animals were perfused under deep pentobarbital anesthesia with 1500–2000 ml of the fixative composed of 4% paraformaldehyde and 0.5% glutaraldehyde buffered with Millonig's phosphate at pH 7.3 or 7.4. Subsequently, small blocks were taken from the cerebellar nuclei of both sides and postfixed with 2% osmium tetroxide buffered with phosphate. Ultrathin sections were cut from these blocks and stained with uranyl acetate and lead citrate for electron microscopy. Examination of the Weil stained sections through the medulla oblongata revealed that in 4 of 6 animals the lesions were limited to the inferior olivary complex, but in 2 of them they invaded further part of the paramedian reticular nucleus; 5 cats were examined as controls.

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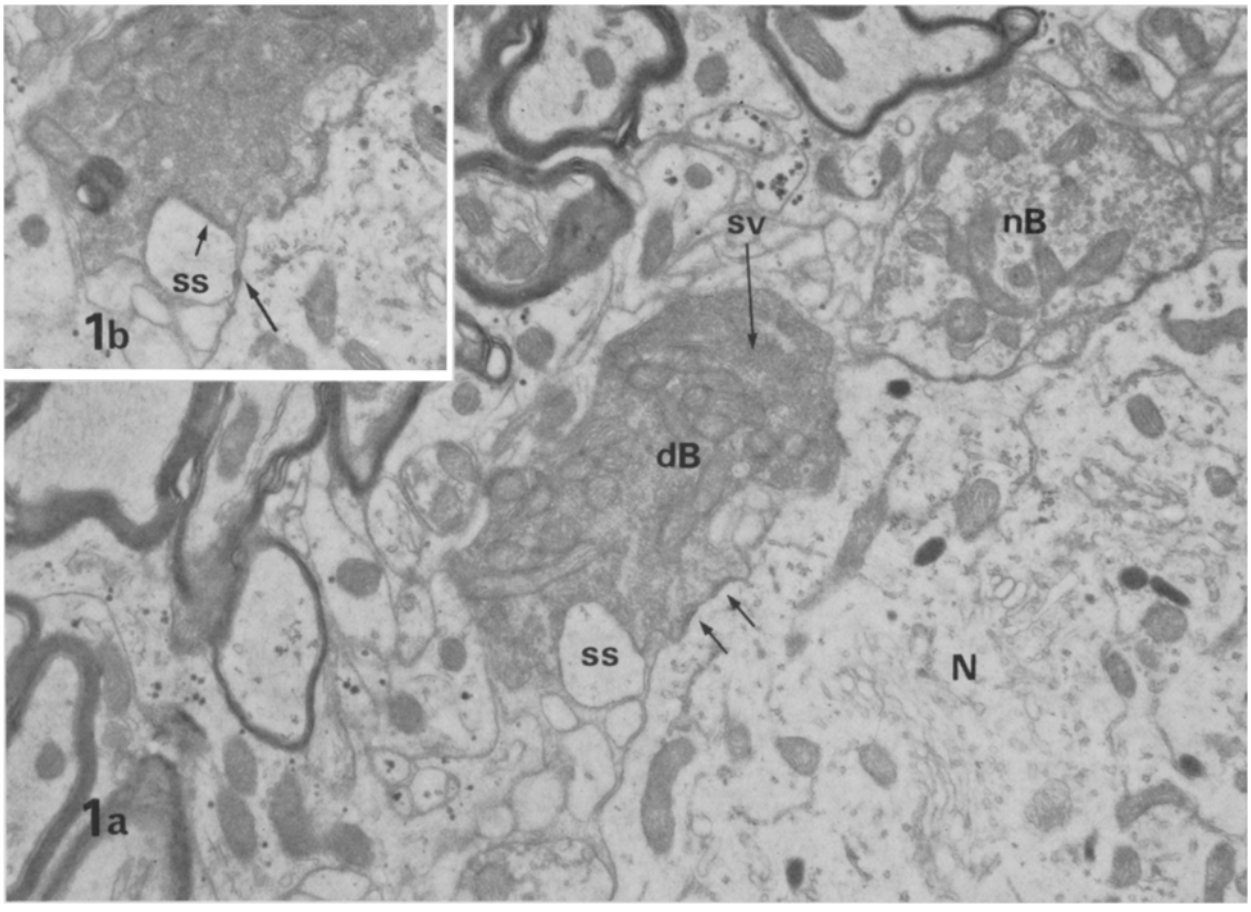

Fig. 1. a) Electron-dense, degenerated bouton (dB) making synapses (arrows) at the 2 sites with the soma (N) of a large cell in the interpositus nucleus. Numerous pleomorphic synaptic vesicles (sv) are seen. A normal bouton with the less dense matrix is located in the vicinity (nB). In one of the serial sections (b) the bouton is seen synapsing (arrow) with a somatic spine (ss) of the same cell. Large arrow points to a connecting portion between the soma and the spine. Side contralateral to the lesions in the inferior olivary complex, 5 days postoperatively. a) ×16,200; b) ×17,300.

Results and discussion. On both sides of all the cerebellar nuclei, a number of degenerated boutons were observed synapsing with the cell bodies and proximal dendrites of large and medium-sized cells, and also with peripheral dendrites and dendritic spines. Among degenerated boutons, electron-dense, degenerated boutons were most numerous. As described by ANGAUT and SOTELO[8], some semi-opaque boutons were observed in normal materials. However, they could be easily distinguished from the electron-dense, degenerated boutons. Figure 1a shows that a large, electron-dense, degenerated bouton with pleomorphic vesicles forms synapses with the soma of a large cell in the contralateral interpositus nucleus. In one of the serial sections it was found that this bouton also contacts a somatic spine arising from the same cell (Figure 1b). Figure 2 shows that a small, electron-dense, degenerated bouton makes an asymmetrical synapse with a small dendrite profile in the ipsilateral medial nucleus. It is markedly dense but vesicle-like structures can still be recognized. Boutons exhibiting the filamentous degeneration were also found; they were usually associated with the electron-lucent matrix, but some were with the electron-dense matrix. As in Figure 3, a large bouton containing many neurofilaments shows a slight increase in the electron density of the matrix, and forms asymmetrical synapses both on the shaft of the dendrite and on a spine arising from it. In addition, glycogen-particle rich, degenerated boutons were frequently observed on the peripheral dendrites and dendritic spines. Although glycogen particles were contained in a certain type of normal boutons, they appeared to be more numerous in degenerated boutons. For example, a spherical vesicle bouton, which forms an asymmetrical synapse on a dendrite shows an increased electron density of the matrix and the presence of clustered glycogen particles (Figure 4).

From the present observations it can be concluded that fibres from the inferior olivary complex terminate upon the somata of large cells and the dendrites of cells in the cerebellar medial, interpositus and lateral nuclei. The termination of OCT fibres could not be found on small cells, which were identified as interneurons[9], because they had rarely axosomatic synapses. The results of the present study are in good agreement with the light microscopic[4] and electrophysiological findings[7,10] that suggest direct connections between the inferior olivary complex and the cerebellar nuclei.

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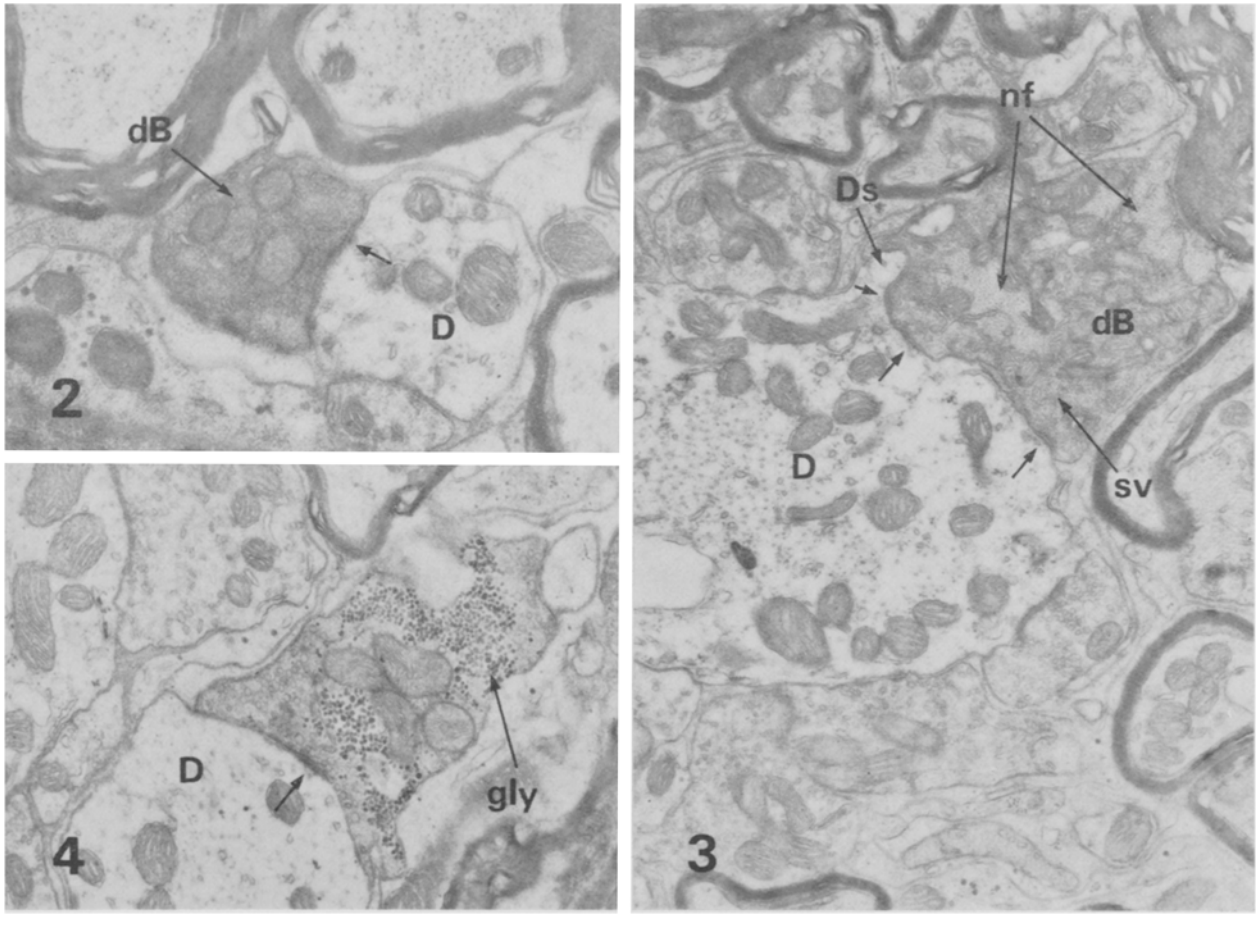

Fig. 2. An electron-dense, degenerated bouton (dB) synapsing (arrow) with a small dendrite (D) in the medial nucleus. Synaptic vesicles are still discernible. Side ipsilateral to the lesions in the inferior olivary complex, 4 days postoperatively. ×23,000.
Fig. 3. A filamentous, degenerated bouton synapsing (arrows) with both on the dendrite shaft (D) and a dendritic spine (Ds) in the interpositus nucleus. Cross section of neurofilaments (nf) is seen in the electron-dense matrix with pleomorphic synaptic vesicles (sv). Side contralateral to the lesions in the inferior olivary complex, 4 days postoperatively. ×15,000.
Fig. 4. A degenerated bouton synapsing (arrow) with a dendrite profile (D) in the lateral nucleus. A number of glycogen particles (gly) are seen inside the densified bouton. Side contralateral to the lesions in the inferior olivary complex, 5 days postoperatively. ×18,000.

Zusammenfassung. Elektronenoptisch wird gezeigt, dass bei der Katze die Bahnen von der unteren Olive zum Kleinhirn Kollateralen besitzen, welche an die Kleinhirnkerne ziehen und sowohl ipsilateral als auch kontralateral von einer Olive versorgt werden. Damit können elektrophysiologische Befunde sicher interpretiert werden.

M. IKEDA and M. MATSUSHITA

Department of Anatomy, Kansai Medical School, Moriguchi, Osaka 570 (Japan),
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Short Term Effects of Dimethylnitrosamine and Methylmethanesulphonate on Hydrolases of the Rat

The metabolic and biochemical effects, particularly those concerned with alkylation of nucleic acid bases, caused by the administration of dimethylnitrosamine (DMN) and methylmethanesulphonate (MMS) in experimental animals have been extensively reported in the literature[1-3]. The enzyme systems that have been mostly investigated include those associated with tissue oxidation and glycolysis[4-6]. The behaviour of acid hydrolases is less well documented and the available data mainly deal with changes that result from administration of either a single lethal dose or of repeated sublethal doses over a prolonged period[7,8]. Report of acute effects induced by these alkylating agents following a single sublethal dose are scant.

In view of the above, the short term effects of administration of single sublethal doses of DMN and MMS on 2 hydrolytic enzymes, namely acid phosphatase (EC 3.1.3.2) and nonspecific esterases (EC 3.1.1.1) were investigated in the rat. These 2 enzymes were selected also because of their known dynamic nature and responsiveness to changes in cellular environment[9].

Materials and methods. 3 groups, each consisting of 10 male adult Wistar rats, were administered single i.p. injections of DMN (10 mg/kg), MMS (90 mg/kg) and normal saline (control) respectively. Liver, kidney and testis obtained from these animals at 72 h were subjected to histochemical, electrophoretic and biochemical techniques for the study of acid phosphatase and nonspecific esterases as described previously[9]. Routine histological staining was also carried out.

Results. Scattered areas of centrilobular necrosis were evident in the liver of 3 DMN-treated rats. Vascular congestion was present, although no frank haemorrhages were observed. In histochemical preparations these areas appeared as enzyme deficient islands. The kidney and testis of the DMN- and MMS-treated rats showed no histological or enzyme histochemical changes. Esterase zymograms of the testis in 3 DMN-treated rats showed a characteristic suppression of the fastest cathodally migrating isozyme with α-naphthyl butyrate, while in the control and MMS preparations this band was prominent. No other changes in acid phosphatase of esterase zymograms were noted. The findings are summarized in Table I.

Biochemical assay of both the hydrolases exhibited wider variations in enyzme levels between the individual

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Table I. Morphological and enzymological (acid phosphatase and nonspecific esterases) changes at 72 hours following administration of single sublethal doses of DMN and MMS in the liver, kidney and testis of the rat

Organs	Morphology (H & E), (paraffin sections)	Enzyme histochemistry (cryostat sections, post-fixed)	Zymogram pattern (*Tris*/boric buffer system; pH 7.3)
Liver	Scattered areas of centrilobular necrosis and vascular congestion in three DMN-treated animals	Enzyme deficient islands, corresponding to necrotic areas, in both acid phosphatase and esterase preparations	Normal
Kidney	Normal	Normal enzyme distribution	Normal
Testis	Normal	Normal enzyme distribution	Suppression of the fastest cathodally migrating isozyme with α-naphthyl butyrate in three DMN-treated animals

Substrates employed for acid phosphatase were α-naphthyl phosphate and naphthol AS-MX phosphate with Red Violet LB as the diazonium salt. For esterases, α-naphthyl acetate, α-naphthyl propionate, α-naphthyl butyrate and naphthol AS acetate were used with Fast Blue BB as the diazonium salt. Simultaneous capture technique was employed for the histochemical and electrophoretic studies.