tic senile change; whereas in accordance with Ts'o and Friedman[7], we think that they are a normal feature of the pigment epithelium, independent of the age of the animal and the region of the optic cup. The absence of mitoses, demonstrated by means of semithin serial sections, suggests that multinucleated cells may be formed during the foetal period as the result of partial and casual amitotic processes, and then persist unmodified during the life of the animals, with few and improbable changes in their number and size.

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The smooth endoplasmic reticulum as a possible storage site for dendritic dopamine in substantia nigra neurones[1]

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Summary. In dendrites of the substantia nigra neurones the monoamine marker 5-hydroxydopamine injected intracerebrally was localized inside of smooth endoplasmic reticulum cisterns. This observation opens the possibility of the existence of an alternative site for dopamine storage in dendrites as opposed to the well-known vesicular storage.

There is morphological[5,6] as well as biochemical evidence[7] that dopamine is stored in dendrites of rat substantia nigra neurones. A dopamine release from this brain area has been demonstrated in vitro[7–9] and in vivo[10,11]. Since this release is dependent on the presence of Ca^{++} in the superfusion medium[7–9] it is probable that an exocytotic mechanism is involved[12]. These observations would suggest the existence of a vesicular component for the storage and release of the dendritic dopamine. In the present study we used 5-hydroxydopamine (5-OHDA) as a monoamine marker[13,14] for the ultrastructural identification of the possible storage sites for dopamine in the dendrites of the substantia nigra neurones.

For the intranigral injections of 5-OHDA, male adult Wistar rats, weighing 280–350 g, were anaesthetized with equithesin and positioned in a Kopf stereotaxic apparatus. A 30-gauge cannula was implanted in the pars compacta of the substantia nigra in accordance with the co-ordinates from the Pellegrino and Cushman stereotaxic atlas[15]. A total volume of 1.2 µl of a 0.9% saline solution containing 120 µg of 5-OHDA and 0.1% ascorbic acid was delivered at a rate of 0.5 µl/min using a Braun perfusion pump. 40 min after the 5-OHDA injection, the animals, still under anaesthesia, were fixed by intracardiac perfusion with a solution of glutaraldehyde:formaldehyde (2.5%:2.5%) in 0.1 M phosphate buffer, pH 7.4. The brains remained overnight in 0.1 M phosphate buffer, pH 7.4, containing 5% sucrose, at 4 °C. The substantia nigra at intermediate levels from the pons to the diencephalon was dissected out from 400 µm thick slices with small knives, under direct microscopic observation. The tissue was then postfixed in 1% osmic acid in the same buffer and flat embedded in epoxy resin. Sections, 1 µm thick, were cut and stained with 1% toluidine blue for the cytoarchitectonic recognition of the substantia nigra pars compacta and pars reticulata. Areas from the medial aspects of the pars compacta and half of the adjacent pars reticulata were trimmed and selected for thin sectioning. The grids were stained with lead citrate and observed and photographed using a Philips 300 electron microscope at 60 kV.

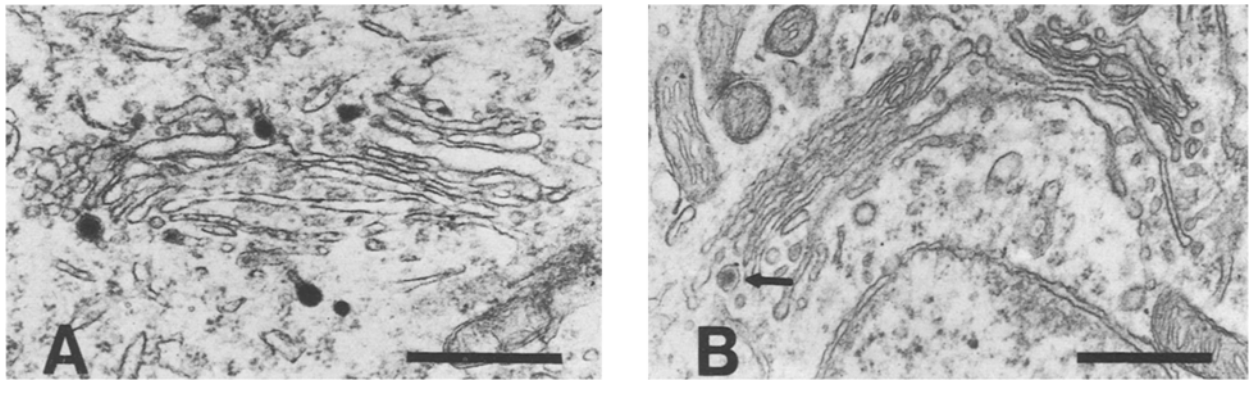
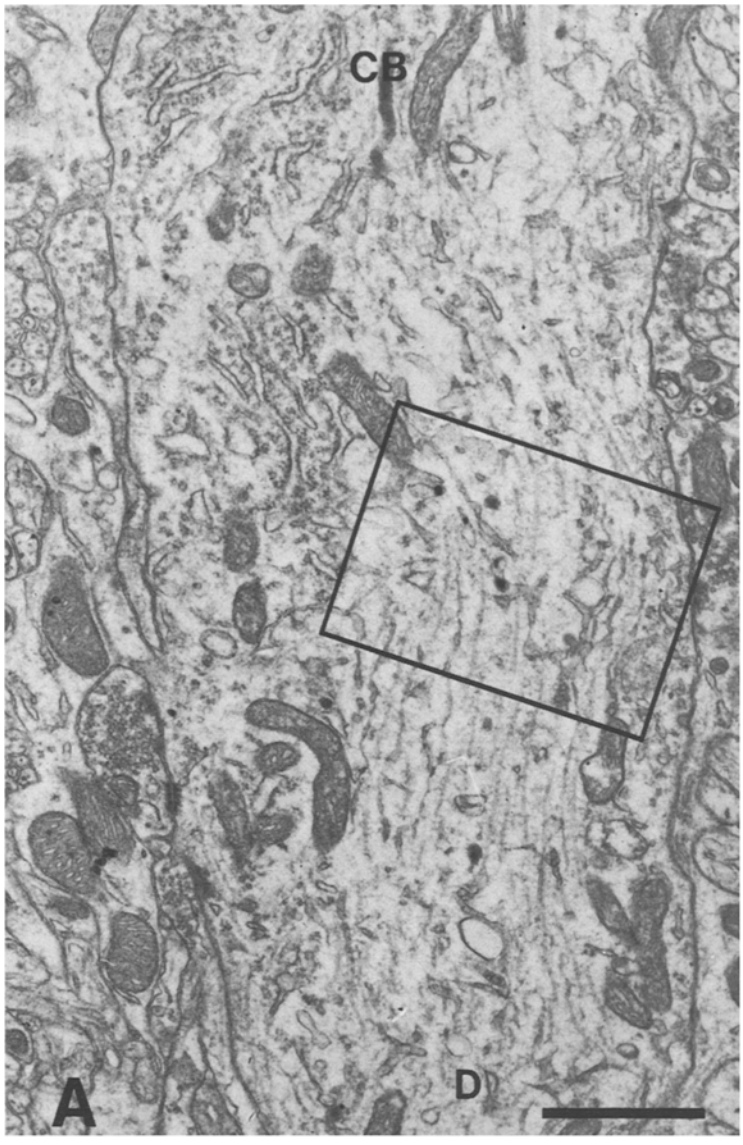


Fig. 1. *A* Electron-dense material in the interior of Golgi cisterns and vesicles of the substantia nigra pars compacta neurones following the intracerebral injection of 5-OHDA. Scale bar 500 nm. *B* Less dense material is normally present in the Golgi of neurones located in the contralateral noninjected side (arrow). Scale bar 500 nm.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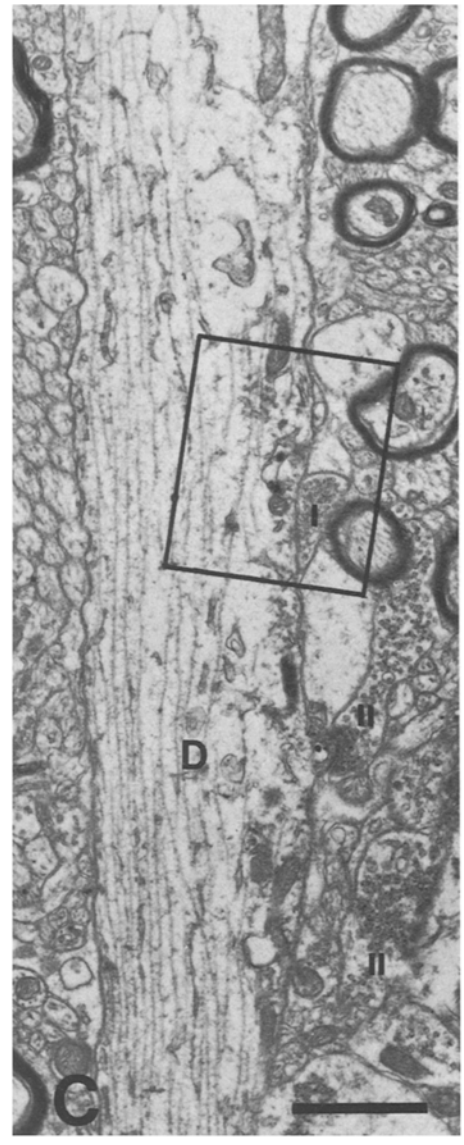
A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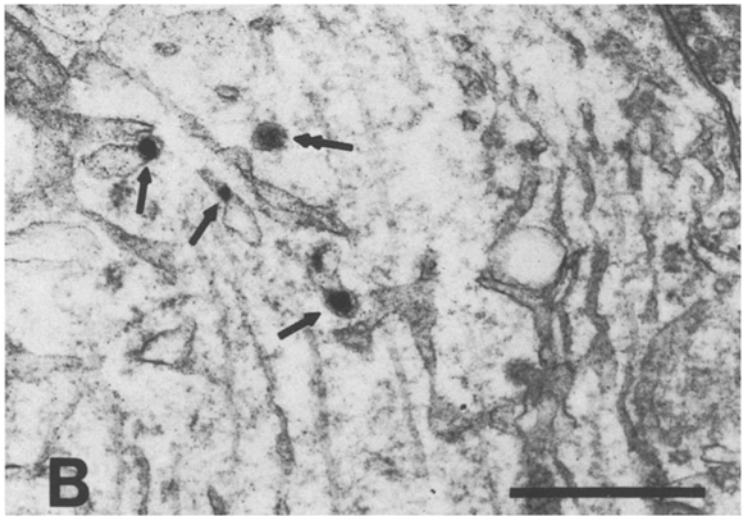
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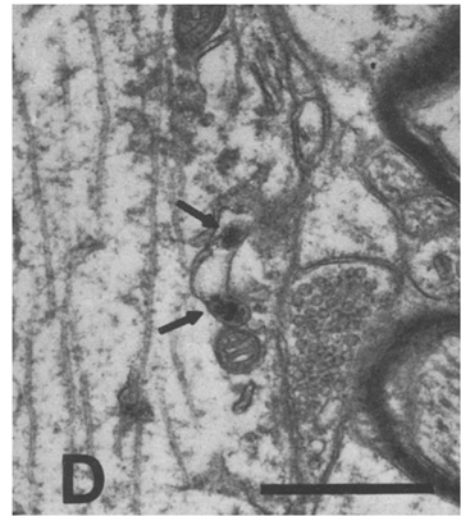
D

The intranigral injection of 5-OHDA resulted in the visualization of electron dense spots in cell bodies, dendrites and nerve terminals in the substantia nigra. Numerous nerve terminals displayed a dense core in small synaptic vesicles after this treatment. This was not observed in the control noninjected side. Those nerve terminals which did take up 5-OHDA showed the marker in practically all their synaptic vesicles, while those remaining unreactive showed no appreciable reaction in any subcellular components. The positive terminals may well correspond to the tryptaminergic innervation of the substantia nigra[16].

In the cell bodies, the electron dense product was observed in the interior of the Golgi compartment (figure 1, A), in small and large vesicles and in cisterns of the smooth endoplasmic reticulum. Large granular vesicles were also observed in the control noninjected side, although without the same degree of opacity (figure 1, B, arrow).

In the dendrites of the substantia nigra neurones, the most interesting finding was the association of the electron dense material with cisterns of the smooth endoplasmic reticulum. This dense material was present mainly in short cisterns, filling partially the intracisternal compartment. The electron dense material was more readily detected in the initial portion of the dendrites and was abundant in the somadendritic juctions (figure 2, A, B). In the furthest projections of the dendrites the electron dense products were seen more rarely in the interior of isolated short cisterns of the smooth endoplasmic reticulum (S.E.R.) (figure 2, C, D).

Although vesicular elements have been reported in substantia nigra dendrites[17,18], we failed in this study to detect any obvious vesicular compartment which could be regarded as synaptic-like vesicles. This absence of vesicular elements accounts in particular for the nigrostriatal neurones as identified by the horseradish peroxidase retrograde transport (Kanazawa and Cuello, unpublished). In a careful high resolution autoradiographic study after the intraventricular administration of ^{3}H-noradrenaline, Sotelo et al.[19] emphasized the lack of correlation between the silver grain deposition in substantia nigra neurone dendrites and any 'vesicular' elements. This fact has also been corroborated recently by electron microscopic radioautography following the intranigral injection of ^{3}H-dopamine[20]. The presence of reaction products both in vesicles and S.E.R. could indicate that these structures represent storage sites for endogenously contained dopamine in the substantia nigra cell bodies. It can be speculated that the granular vesicles will be exported towards the axonal processes, while the smooth endoplasmic reticulum will carry dopamine towards the dendrites. Alternatively, the S.E.R. cisterns may play some role in storing and/or transporting monoamines in axons and nerve terminals as well. In fact, Tranzer[21] gave some evidence that this may be the case for noradrenergic nerves of the rat vas deferens and Droz et al.[22] have provided evidence indicating an active role of the S.E.R. in axonal transport.

The S.E.R. may be considered as an alternative compartment for monoamine storage. It is not clear, however, how monoamines stored in this type of compartment may be available for neurotransmitter release. All our present understanding of the release of neurotransmitters is largely based on the concept of vesicular exocytosis[12,23,24]. It is possible that other mechanisms of release may be present in the central nervous system. In this regard, it is of interest to note that the release of dopamine in the substantia nigra differs from that observed in the corpus striatum. In vitro experiments showed for instance that there is a significantly higher spontaneous release of dopamine from the substantia nigra dendrites than from nerve terminals in the corpus striatum[8]. This may indicate a less efficient mechanism of storage of dopamine in the dendrites which could be attributed to the more 'immature' storage. In the same direction recently it has been shown that tetrodotoxin, a drug which blocks Na^+ channels in axons and nerve terminals, prevents the release of dopamine from corpus striatum nerve terminals but not from substantia nigra neurone dendrites[11], thus indicating that a differential release mechanism may exist in these cellular processes. Combined biochemical and morphological studies could clarify the significance of this differential situation for the storage and release of dopamine in the nerve terminals and dendrites of the dopaminergic neurones of the nigrostriatal pathway.

1 Acknowledgments. The authors would like to thank Dr L.L. Iversen for helpful criticism and reviewing the manuscript.
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Fig. 2. *A* Initial portion of a substantia nigra neurone dendrite (D). Note the presence of several smooth endoplasmic reticulum cisterns containing electron dense material after the injection of 5-OHDA. CB: cell body region of the neurone. Scale bar 1 μm. *B* Enlarged area corresponding to the square shown in *A*. Arrows indicate electron dense material detected in the interior of smooth endoplasmic reticulum cisterns. Double headed arrow points to a large granular vesicle. Scale bar 500 nm. *C* Dendritic shaft (D) in the substantia nigra pars reticulata injected with 5-OHDA. Labelled (II) and unlabelled (I) nerve terminals are present in this field. Scale bar 1 μm. *D* Enlarged area corresponding to the square shown in *C*. Arrows point to electron dense material present in smooth endoplasmic reticulum cisterns located subsynaptically. Scale bar 500 nm.