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Morphological Changes of Rat hipposampal Neurons after Noradrenergic Depletion

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In developing rodent brain, the noradrenergic projections are among the earliest to be formed (Lauder et al. 1974, Seiger et al. 1973). The adult pattern of noradrenergic innervation in all areas of the hippocampus was shown to be established by the postnatal 10th day (Loy et al. 1980). During this early postnatal period, the existence of an intact noradrenergic system is believed to be associated with developmental plasticity (Kasamatsu et al. 1979). Early manipulation of noradrenergic projections significantly alter the normal course of neuronal development. Neonatal treatment with either 6-hydroxydopamine (6-OHDA) or N-(2-Chloro-ethyl)- N-ethyl-2-bromobenzylamine (DSP-4) causes a permanent noradrenergic hypoinnervation of forebrain regions an a hyperinnervation of the brain stem (Gustafson et al. 1987). In the present study we investigated the effects of noradrenergic depletion by neonatal injection of DSP-4 on the hippocampal neurons by electron microscopy.

Neonatal pups of Long Evans hooded rats were given subcutaneously DSP-4 in a dosage of 100 μg/g, dissolved in 0.9% saline, once within 12 hr after birth. Some litermates were used as control animals receiving an equal volume/body weight of saline. Animals were sacrificed and processed for immunohistochemistry to reveal noradrenergic innervation and for electron microscopy to examine ultrastructural changes in the hippocampus 4-6 months after injection.

By applying a polycolonal antiserum made to dopamine β-hydroxylase, an synthetic enzyme for norepinephrine and a reliable marker of noradrenergic innervation, immunohistochemical staining revealed a substantial decrease of noradrenergic fibers in the hippocampus. At the light microscopic level, heavily stained neurons were found in the dentate gyrus, the hilar region and the hippocampal CA1 area, but rarely seen in the CA1 area, after toluidine blue-staining of 1 μm epoxy sections. These neurons had a dark appearance of both cytoplasm and nucleus and were termed "dark cells" in contrast to the light staining of normal neurons. Electron microscopic examination revealed a gradient of neuronal darkness, from normal light, grayish, gray, to completely dark. The increased darkness were due to an accumulation of fine granules in both cytoplasm and nucleus (Fig. 1). Compared with normal neurons, the dark cells barely maintained typical processes. In addition to the increase of fine granules and shrunk shape, certain cytological changes were also noted. The nuclear envelope of dark cells was rich in invagination (Fig. 2). Stacks of mostly smooth endoplasmic reticulum and polyribosomes did not aggregate to form Nissl bodies. Instead, they appeared to be abundant and distributed over the neuronal soma (Fig. 3). Even in the primary dendrites, while the normal neurons did not have many organelles, the dark cells were found to be packed with mitochondria, ribosomes and cisternal structures (Fig. 4).

Most of dark cells had an invaginated nucleus containing a normal nucleolus with increased fine granules but no increase of heterochromatin (Fig. 2). They either had stacks of long slender endoplasmic reticulum (Fig. 3) or were packed with short tortuous and enlarged reticulum (Fig. 5). In addition to abundant free ribosomes, mostly polyribosomes, many lysosomes were found to be in the form of lipofuscin granules (Figs. 3 and 5). Enlargement of several membranous structures, including Golgi apparatus, endoplasmic reticulum and nuclear envelope, and enrichment of polyribosomes and residual bodies were typical features in these cells.

Based on these ultrastructural characteristics, the dark cells appeared to be metabolically active. The morphological features of their soma are similar to those of chromatolytic perikarya induced

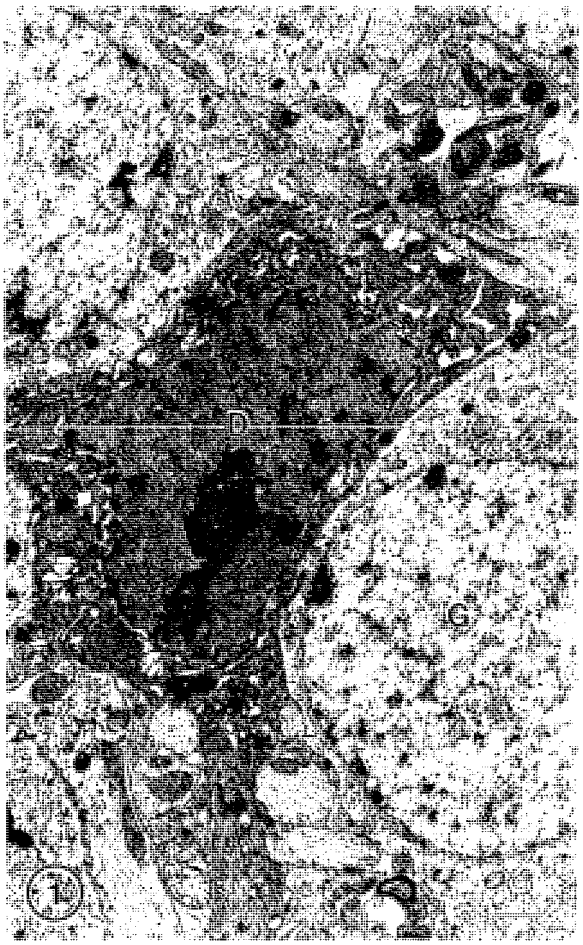

Fig. 1. Cellular elements in the dentate gyrus of DSP-4 treated rats. Normal granule cells (G) reveal light appearance of nucleus and cytoplasm. A dark cell (D) within tie granule cell layer is characteristic of intensive electron density throughout nucleus, cytoplasm and processes except enlarged cisternal structures. Scale bars: 1 μm.

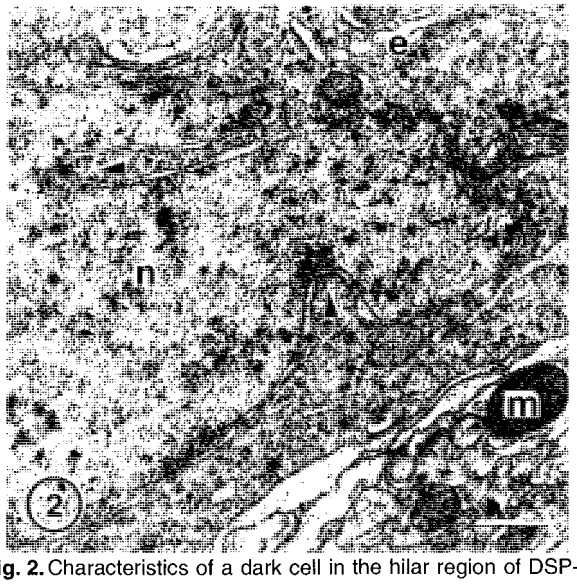

Fig. 2. Characteristics of a dark cell in the hilar region of DSP-4 treated rat. Note the increase of invagination (arrowheads) of the nucleus (n), polyribosomes, endoplasmic reticulum (e), mitochondria (m), and abundance of fine granules in both nucleus and cytoplasm. No increase of heterochromatin was seen. Scale bars: 0.5 μm.

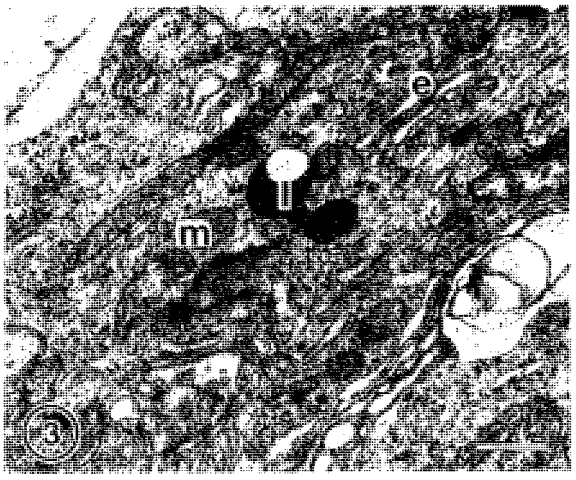

Fig. 3. Cytoplasm of a dark cell in the hilar region of DSP-4 treated rat. Except the increased polyribosomes, endoplasmic reticulum (e), mitochondria (m) and fine granules, more lysosomes (l) were seen as a form of residual body containing lipofuscin granules. Scale bars: 0.5 μm.

by axotomy. Electron microscopic studies of chromatolytic neurons have shown fragmentation of Nissl bodies in which the aggregates of granular endoplasmic reticulum decreased markedly in size with a concomitant increase of polyribosomes (Lieberman 1974). Because of the appearance of invaginated nuclear envelope, an enlarged Golgi apparatus and increased numbers and complexity of lysosomes, the chromatolytic response to axonal damage was considered to be rather a restorative process aimed at reconstituting the neurons than a degenerative phenomenon (Mathews et al. 1972). Similarly, the dark cells could be in an active state of cytoplasmic protein synthesis. The accumulation of fine granules in the nuclei and the cytoplasm could have resulted from an increased nuclear RNA synthesis, as observed in the cytochemical analysis of chromatolytic perikarya (Watson 1965 1978). Since the amount of lipofuscin granules had increased in the dark cells, accumulated fine granules could

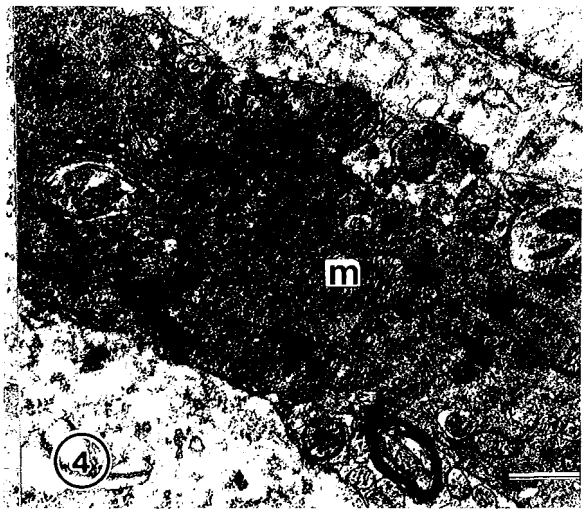

Fig. 4. Primary dendrite of a dark cell in the hilar region of DSP-4 treated rat. Like the soma, the dendrite is packed with great amount of polyribosomes, mitochondria (m) and fine granules. Scale bars: 0.5 μm.

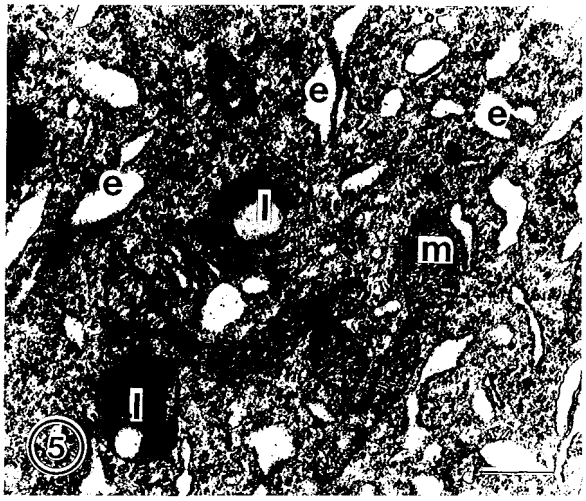

Fig. 5. Enlarged cisternae in the cytoplasm of a dark cell in the hilar region of DSP-4 treated rats. Some dark cells, like the one shown here, have enlarged cisternae (e) distributed with abundant polyribosomes, mitochondria (m), lysosomes (l) and fine granules. Scale bars: 0.5 μm.

also be a different pattern of lipofuscin accumula-'tion as found in pyramidal neurons of the aging brain (Braak 1984).

Concerning cytotoxic or neurotoxic effects of DSP-4 on developing brain, noradrenergic neurons and even other monoaminergic neurons degenerate or undergo plastic changes after drug treatment. In noradrenergic target areas, there was a dispute about neuronal loss in the neocortex after 6-OHDA injection (Ebersole 1981, Onteniente et al. 1980). Despite the presence of few disintegrated cells, no neuronal loss was observed in the hippocampus 4-6 months after injection in the present study. No ultrastructural features indicating degeneration processes, such as pyknotic cells and profiles of dense or lamellar bodies, were seen in the dark cells. Thus, most, if not all, of the dark cells are unlikely too be in a process of degeneration. Examination of specimens from drug-treated animals at several time points after injection revealed that no significant amounts of dark cells were present at light and electron microscopic levels until 2-3 months after injection. No significant changes in neuronal numbers of the hippocampal areas were found even in one-year-old norepinephrine-depleted rats. Furthermore, deep toluidine blue-stained pyramidal cells and granule cells have been reported in the early postnatal hippocampus of developing Long-Evans hooded rats before the postnatal 7th day (Fuh et al. 1990). Like the dark cells in this study, they contained fine granules in both nucleus and cytoplasma dn had a gray and dark appearance. These dark cells developed gradually into normal neurons of light appearance within a week after birth. In other system, particularly in the reproductive organs, cells of dark appearance are believed to be germ cells having mitogenic potency or newly formed cells from stem cells (Clemont et al. 1976, Cormack 1987). Although a significant increase in granule cells in the ventral dentate occurred 4 months after DSP-4 injection, no evidence of cell division was seen in the current observation. Nevertheless, dark cells may represent a population of neurons at a physiological status different from other cells.

In conclusion, neonatal injection of DSP-4 caused a substantial reduction of noradrenergic fibers and the appearance of dark cells in the hippocampus of rats aging between 4-6 months. The dark cells may have occurred as a result of long-term noradrenergic deficiency instead of direct cytotoxic or neurotoxic actions of DSP-4 on hippocampal neurons. The nature of the dark cells and their functional significance in norepinephrine-depleted rats are still unclear and require further investigation.

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