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An Electron Microscope Study of the Locus Coeruleus in the Rabbit, with Special Reference to Direct Hypothalamic and Mesencephalic Projections

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Summary. Fine structures of the locus coeruleus of the rabbit, in particular the synaptic organization, were studied with the electron microscope.

Synaptic contacts occurred much more frequently on the dendritic profiles than on the neuronal somata. No axo-axonal synapses were encountered. Many synaptic bags contained a large number of closely packed round agranular vesicles; synaptic bags filled only or chiefly with flattened vesicles were less common. The synaptic densities usually were symmetrical in the synaptic terminals with flattened vesicles. A few dense-cored vesicles of 500–1,000 Å diameter were often found within these synaptic bags; these vesicles, however, were almost always more or less apart from the presynaptic membrane. On rare occasions, a few dense-cored vesicles were also found in the neuronal somata. The nucleolus-like bodies in the subsynaptic areas, the synaptic vesicles aggregated in a crystal-like array, and the subjunctional bodies were also observed.

On the third or fourth day after making electrolytic lesions in the hypothalamus, the mesencephalic central gray or the mesencephalic dorsal tegmentum, electron-dense degenerated synaptic bags were found in the locus coeruleus ipsilateral to the lesions. The degenerated synaptic bags usually contained round vesicles and were in contact with large or medium-sized dendritic profiles. A few degenerated synaptic bags containing somewhat elongated vesicles were also encountered in the locus coeruleus of the rabbits with lesions involving the mesencephalic central gray and the mesencephalic dorsal tegmentum. No degenerative changes could be found in the axosomatic synaptic terminals.

Possible functional meanings of the hypothalamic and mesencephalic projections to the locus coeruleus were discussed.

The locus coeruleus is characterized by melanin-pigmentation in man and higher primates. The homologous nucleus has also been described in the dorsolateral portion of the rostral metencephalic tegmentum of subprimate species, although the melanin pigment is lacking in the locus coeruleus of these animals (SANO, 1941; CROSBY and WOODBURN, 1943; RUSSELL, 1955).

Based on physiological data, several possible functions have been assigned to this nucleus; respiratory regulatory functions (JOHNSON and RUSSELL, 1952; BAXTER and OLSZEWSKI, 1955), regulation of micturition (KURU and YAMAMOTO, 1964) and triggering of the paradoxical sleep (JOUVET and DELORME, 1965). On the other hand, the locus coeruleus was shown to be rich in monoamine oxidase (SHIMIZU et al., 1959; HASHIMOTO et al., 1962), and to be made up almost entirely of catecholamine-containing cell bodies (DAHLSTRÖM and FUXE, 1964) and nerve terminals (FUXE, 1965a); the amine has been demonstrated to be noradrenaline (HILLARP et al., 1966; DESCARRIES and DROZ, 1968; CORRODI et al., 1970; FUXE et al., 1970). Thus the locus coeruleus has

been recognized as one of the nuclei of the catecholamine-containing system in the brain. There have also been presented morphological data indicating that the locus coeruleus neurons give axons to the hypothalamus as well as the cerebral and cerebellar cortices (ANDÉN et al., 1966; LOIZOU, 1969; OLSON and FUXE, 1971; MAEDA and SHIMIZU, 1972).

In the present study, in an attempt to shed more light on the regulatory mechanisms of the rostral portions of the brain upon the locus coeruleus, degeneration of descending afferent terminals in the locus coeruleus was investigated electron microscopically after placing experimental lesions in the rostral brain structures. A preliminary account of the present findings has been presented (MIZUNO and NAKAMURA, 1970).

Material and Method

In nine young adult rabbits of 2–3 kg body weight, experimental lesions were made electrolytically or surgically under general anesthesia (sodium pentobarbital; 30 mg/kg i.v.). Unilateral electrolytic lesions in the diencephalon or the mesencephalon were produced stereotactically by using a slender stainless steel needle electrode which was completely insulated except at the tip; the diameter of the shaft of the electrode was less than 0.5 mm. After vertical insertion of the electrode through a small trephine opening in the skull, an anodal current of 1–2 mA was applied for 10–20 sec; the tongue or the upper canine tooth was connected to the cathode. Unilateral cortical ablation was performed in one animal by thermocauterization. All animals were treated postoperatively with antibiotics (mycillin).

After survival periods of three (rabbits 8 and 9) or four (rabbits 1–7) days the animals were anesthetized with pentobarbital, and perfused through the ascending aorta with 800–1,200 ml of a mixture of 4% paraformaldehyde and 0.5% glutaraldehyde buffered to pH 7.2–7.4 with Millonig-buffer. The perfusion was extended over a period of 15 minutes, from a height of approximately 1.2 m. Two additional rabbits without lesions were also perfused as controls. The brains were then dissected free, and transverse coronal slices taken from the dorsal part of the mediotlateral pontine tegmentum were postfixed for 1.5–2 hrs in chilled Dalton's fixative containing 2% osmium tetroxide (pH 7.2–7.4). The slices were embedded in Epon after dehydration in a graded series of ethanol.

Semi-thin sections cut from each block were stained with 0.5% toluidine blue in 1% borax. On the basis of these light microscopical sections, the locus coeruleus was identified and trimmed for electron microscopy. Ultrathin sections were cut with glass knives on a Porter-Blum Ultra-microtome MT-1 stained with lead acetate, and examined with a Hitachi-HU-12, a HS-7 or a HS-8 electron microscope.

Portions of the brain containing the lesions were also fixed in 10% neutral formalin after the perfusion, and then embedded in celloidin. The location and extent of the lesions were verified in 30 μ serial sections stained by the Nissl or the Weil method.

Results

Normal structure

In the rabbit the locus coeruleus extended from the level of the dorsal tegmental

nucleus caudally to the middle level of the motor nucleus of the trigeminal nerve. It was recognizable as a triangular or semilunar nuclear mass medial or ventromedial to the mesencephalic nucleus of the trigeminal nerve (Fig. 1).

The region was characterized by rather compactly arranged nerve cell bodies of small and medium size; 15–25 μ and 25–35 μ in diameter, respectively. The cells were mainly oval or fusiform in shape and had a modest amount of perinuclear cytoplasm, upon which a satellite oligodendroglia was often observed to abut. There were occasionally seen a prominent dendrite stretching out from a pole of the cell body (Fig. 2). Some of the cell bodies were located very closely to blood vessels (Fig. 3). On rare occasions, neuronal somata were in close apposition to each other, leaving one or more thin glial layers between them (Fig. 4).

A round or oval nucleus of the neurons was located in the center of the cytoplasm

Fig. 1. Light micrograph of an Epon-embedded frontal section of dorsolateral portion of the rostral metencephalic tegmentum, stained with alkaline toluidine blue. Arrows demarcate the locus coeruleus medial or ventromedial to the mesencephalic nucleus of the trigeminal nerve (*M*). *CG* central gray, *V* fourth ventricle. $\times 73$

Fig. 2. Electron micrograph of a basal portion of a dendrite stretching out from the perinuclear cytoplasm. Three synaptic bags are seen. *G* Golgi apparatus. $\times 13,000$

Fig. 3. Electron micrograph of a neuron located close to a blood vessel (*B*). A solid arrow shows the cytoplasmic membrane of the neuron. Open arrows indicate the deep infoldings of the nucleus (*N*). Note clusters of vesicles contained in each infolding. $\times 7,200$

Fig. 4. Electron micrograph of two closely apposed neurons with a few intervening glial layers. Arrows indicate the cytoplasmic membrane of the neurons. *N* nucleus, *G* Golgi apparatus. $\times 12,000$

Fig. 5. Electron micrograph of a portion of a neuron contacted with two axosomatic synaptic bags (open arrows). Two solid arrows point to dense-cored vesicles in the perinuclear cytoplasm. A double arrow points to the membrane of the nucleus (*N*). *G* Golgi apparatus. $\times 16,000$

and contained a prominent nucleolus. The nuclear envelope exhibited usually one or more infoldings or indentations, which sometimes contained several vesicular structures of $0.15\text{--}0.5\mu$ diameter (Fig. 3). The perinuclear cytoplasm contained diffusely distributed mitochondria, a small number of lysosomal bodies, a prominent

Fig. 6a. Round agranular vesicles within an axodendritic synaptic bag are arranged in a highly ordered crystal-like array (a solid arrow with longer shaft). Solid arrows with shorter shaft indicate dense-cored vesicles. A synaptic bag (*F*) is filled with flattened vesicles. $\times 30,000$
b. One synaptic bag contains round agranular vesicles arranged in a crystal-like array (a larger arrow), and another contains a dense-cored vesicle (a smaller arrow). $\times 20,000$ **c.** A large axodendritic synaptic bag filled with round agranular vesicles surrounds a small, spine-like dendritic profile (*S*). A synaptic terminal (*F*) is filled with flattened vesicles and shows a symmetrical synapse. $\times 22,000$ **d.** A synaptic bag envelops a small dendritic profile (*S*), making a synaptic contact upon it. $\times 19,000$

Golgi apparatus and well-developed granular endoplasmic reticulum, which was often arranged in Nissl bodies (Fig. 4–6).

On rare occasions, the nucleolus-like bodies were found in the subjunctional areas (see Fig. 2 of MIZUNO and NAKAMURA, 1970), as observed in the hypothalamic areas (SHIMIZU and ISHII, 1965; LEBEUXS, 1971; LEBEUX et al., 1971) and in the intermedio-lateral nucleus of the spinal cord of the cat (to be published).

On somata of the neurons, the synaptic connections appeared to occur rather infrequently. The axosomatic synaptic bags contained usually round and/or oval agranular vesicles (Fig. 2, 5); synaptic bags filled only or mainly with flattened vesicles were less common (see Fig. 2 of MIZUNO and NAKAMURA, 1970).

The axodendritic synaptic bags were most commonly found on the dendritic profiles of large or medium size (Fig. 2, 6a, c). Less commonly, small, spine-like dendritic profiles attached or surrounded by a synaptic bag were also encountered (Fig. 6c, d). These axodendritic synaptic bags most frequently contained groups of round and/or oval clear vesicles about 300–500 Å in diameter. Axodendritic synaptic bags filled only or chiefly with flattened vesicles were also seen (Fig. 6a, c), but they represented a small population compared with synaptic bags with round vesicles. The synaptic densities of the synaptic terminals with flattened vesicles were usually symmetric. The synaptic bags often contained a large number of closely packed round agranular vesicles. These aggregated agranular vesicles were rarely observed in a highly ordered crystal-like array (Fig. 6a, b), as observed in tissue cultures of rat spinal cord (BUNGE et al., 1965), in the spinal cord of the cat (HARREVELD and KHATTAB, 1967) and in the spinal trigeminal nucleus, red nucleus and lateral reticular nucleus of the cat and rabbit (to be published). Some synaptic bags on small dendritic profiles were enveloped by concentric glial lamellae (Fig. 7). The postsynaptic dense “subjunctional bodies” (AKERT et al., 1967; MILHAUD and PAPPAS, 1966) were rarely observed, especially in narrow neck-like dendritic profiles lying between a pair of synaptic bags (Fig. 8). Additionally, honeycomb-like tubular structures were found in a few dendritic profiles (Fig. 9). Although the synaptic bags were often in

Fig. 7a, b. Concentric glial lamellae surrounding a synaptic terminal on a small dendritic profile (S). $\times 19,000$

close apposition to each other, no axo-axonal synapse could be found.

One of the characteristic features of the neuropil of the locus coeruleus was that the majority of the synaptic bags, both axodendritic and axosomatic, contained a few

Fig. 8a, b. A pair of solid arrows indicate a row of postsynaptic dense bodies seen in a narrow neck-like portion of dendritic profile (a), or in a spine-like process arising from a large dendritic profile (b). a: $\times 35,000$. b: $\times 24,000$

Fig. 9a, b. Two kinds of honeycomb-like tubular structures (solid arrows) in dendritic profiles. $\times 30,000$

granular or dense-cored vesicles of 500–1,000 Å diameter (Fig. 6). These vesicles were located almost always at some distance from the presynaptic membrane. Similar vesicles were also seen, though only infrequently, in the perinuclear cytoplasm (Fig. 5). On rare occasions, somewhat larger dense-cored vesicles of 1,300–1,700 Å in diameter were observed in a cluster within synaptic bags or preterminal axons (Fig. 10a, b); these looked similar to the neurosecretory granules (cf. BARGMANN, 1966).

Fig. 10a, b. Clusters of large dense-cored vesicles are seen in an axodendritic synaptic bags (a) and in a preterminal axonal profile (b). Compare the large vesicles (solid arrows) with usual dense-cored vesicles (open arrows). $\times 20,000$

Degeneration study

In rabbits 1, 2 and 3 the lesions were placed in hypothalamic areas (Fig. 11), and subsequent degenerative changes in the locus coeruleus were observed with the electron microscope. The lesion of rabbit 1 involved chiefly the lateral and the dorsal

Fig. 11. Projection drawings of the lesions in the hypothalamic areas of rabbits 1, 2 and 3. The largest area of the lesion is presented in the upper figure of each case; the lower shows the most caudal level of the lesion. In these animals degenerative changes were found in the locus coeruleus ipsilateral to the lesion. C crus cerebri, CI capsula interna, M corpus mamillare, MT tractus mamillothalamicus, ST subthalamus, T thalamus, TP tractus opticus, V ventriculus, X fornix.

hypothalamus and extended to the rostralateral aspect of the posterior hypothalamus. The lesion of rabbit 2 involved the anterior, the lateral and the medial hypothalamic areas and extended caudally to the premammillary area. The posterior hypothalamus and the supramammillary area were involved in the lesion of rabbit 3. In these

Fig. 12. Degenerative changes in the locus coeruleus ipsilateral to the lesion of rabbit 1. Solid arrows indicate electron-dense degenerated bags filled with round agranular vesicles; a few dense-cored vesicles are also seen in these synaptic bags. An open arrow with shorter shaft points to a multivesicular body in a large dendritic profile. *n* normal synaptic bag, *L* perinuclear cytoplasm of locus coeruleus neuron. $\times 14,000$

Fig. 13a, b. Electron-dense bodies in the locus coeruleus ipsilateral to the lesion in rabbits 3 (a) and 8 (b). **a:** $\times 30,000$. **b:** $\times 17,000$

animals degenerative changes of synaptic terminals were found in the locus coeruleus ipsilateral to the lesion.

Among previously described features of the degeneration of synaptic terminals (cf. GRAY and GUILLERY, 1966), increased osmiophilia of nerve-ending ground substance with concomitant aggregation of synaptic vesicles was most commonly encountered in the present study, as observed in other areas of the brain (MIZUNO and NAKAMURA, 1971a, b; NAKAMURA and MIZUNO, 1971; MIZUNO et al., 1972). In view of the critiques presented by COHEN and PAPPAS (1969), the absence of dark neurons in the locus coeruleus was confirmed in the carefully studied controls, and electron-dense profiles filled with aggregated vesicular structures were considered as degenerative features of the synaptic bags only when they were associated with the post-synaptic membrane specialization.

The electron-dense synaptic bags found in the present study were commonly in

Fig. 14. Lesions of rabbits 4-9. The marks (-) attached to 4, 5 and 9 indicate that no degenerative changes were found in the locus coeruleus of these animals. In other animals (6-8) degenerative changes were found in the locus coeruleus ipsilateral to the lesion. *A* velum medullare anterius, *B* brachium conjunctivum, *C* crus cerebri, *D* decussation of brachium conjunctivum, *F* fasciculus longitudinalis medialis, *G* corpus geniculatum laterale, *H* tractus habenulointerpeduncularis, *I* colliculus inferior, *L* lemniscus medialis, *M* corpus mamillare, *N* nucleus niger, *O* nucleus oculomotorius, *P* nucleus interpeduncularis, *R* nucleus ruber, *S* colliculus superior, *ST* subthalamus, *T* thalamus, *V* ventriculus.

contact with large or medium-sized dendritic profiles, and filled usually with nearly circular vesicles (Fig. 12). One or more dense-cored vesicles were often recognizable in these electron-dense synaptic bags. The electron-dense synaptic bags could be found neither on the nerve cell bodies, nor on the small, spine-like dendritic profiles. In association with the electron-dense synaptic profiles, dense bodies enveloped or engulfed by neuroglial profiles were also observed (Fig. 13).

In the next step, degenerative changes of the synaptic terminals were studied electron microscopically in the animals with lesions in the mesencephalic tegmentum and/or the mesencephalic central gray (rabbits 6, 7 and 8) (Fig. 14). In these animals degenerative changes, as observed in rabbits 1, 2 and 3, were found in the locus coeruleus ipsilateral to the lesion. The degenerative changes, however, were less marked in rabbit 7 than in rabbits 6 and 8. Degenerated synaptic bags in these animals were rarely found to contain somewhat flattened vesicles (Fig. 15, 16).

For the control purpose, lesions were placed in the cerebral cortex (rabbit 4), thalamus (rabbit 5) or inferior colliculus (rabbit 9) (Fig. 14). No degeneration could be found in the locus coeruleus in these animals.

Fig. 15. Two electron-dense axodendritic synaptic bags (solid arrows) in the locus coeruleus ipsilateral to the lesion in rabbit 8. The larger degenerated synaptic bag contains a large vacuole and somewhat flattened vesicles. An open arrow indicates the symmetric synapse of the larger degenerated synaptic terminal. A normal synaptic terminal with round agranular vesicles (*n*) shows an asymmetric synapse. $\times 18,000$

Fig. 16. An electron-dense axodendritic synaptic bag in the locus coeruleus ipsilateral to the lesion in rabbit 7. The degenerated synaptic bag contains a dense-cored vesicle (a solid arrow) and somewhat elongated vesicles. An open arrow points to the symmetric synapse of the degenerated synaptic terminal. A normal synaptic terminal with round vesicles (*n*) shows an asymmetric synapse. $\times 23,000$

Discussion

The posterior portion of the nucleus lateralis tegmenti (dorsolateralis) of sub-primate species is generally considered as a structure homologous with the nucleus locus coeruleus of primates (CROSBY and WOODBURN, 1943). This region in the rabbit

borders upon the medial surface of the mesencephalic nucleus of the trigeminal nerve (cf. SANO, 1941; MEESSEN and OLSZEWSKI, 1949; RUSSELL, 1955).

The present findings about the normal fine structure of the locus coeruleus in the rabbit were in consistent with those described in the rat (LENN, 1965; FUXE et al., 1966; SHIMIZU and IMAMOTO, 1970). One of the characteristic features was the frequent occurrence of large dense-cored vesicles of 500–1,000 Å diameter within the synaptic bags. SHIMIZU and ISHII (1964), WOOD (1966) and BAK et al. (1969) have reported that the large dense-cored vesicles might contain biogenic amines, while AKERT and SANDRI (1968) have indicated the existence of large granular vesicles in possible cholinergic neurons. According to BLOOM and AGHAJANIAN (1968), such dense-cored vesicles might represent protein-laden organelles and tend to be present in a relatively high proportion of the synaptic bags in brain regions rich in monoamines. The exact nature of the large dense-cored vesicles, however, could not be considered as finally settled (cf. HÖKFELT, 1968). In the present study almost all of the large dense-cored vesicles within the synaptic bags were seen more or less apart from the presynaptic membrane. Therefore, even if they might have some relationship with chemical transmission, they might not be in their final or active forms.

Direct hypothalamic projection fibers to the locus coeruleus were degenerated most numerous in the rabbits with lesions involving the supramammillary area and/or the posterior hypothalamus. The hypothalamomesencephalic fibers could be roughly divided into three groups; fibers coursing towards the central gray, the dorsal, and the ventral tegmental regions (cf. MIZUNO et al., 1969b). The amount of degeneration in the locus coeruleus was larger in the rabbits (6, 8) with lesions involving a wider area of the dorsal tegmentum of the mesencephalon than in the rabbit (7) with its lesion mostly confined within the central gray. RAMÓNY CAJAL (1909) described that the locus coeruleus received a considerable number of collaterals from longitudinally running fibers in the reticular formation. Some of these fibers might be descending ones. It seems probable from these data that the hypothalamic projection fibers to the nucleus locus coeruleus run mainly through the dorsal tegmentum of the mesencephalon to enter the nucleus from its rostral and ventral aspects.

In the present study, terminal degeneration within the locus coeruleus was always found in the axodendritic synapses; no degenerated axosomatic synaptic bag was encountered. This seems to suggest that the neurons giving rise to presynaptic terminals of the axosomatic synapses in the nucleus locus coeruleus might localize in the nucleus proper or its vicinity, although the ascending nature of the axosomatic presynaptic terminals can not be excluded. By means of the fluorescence histochemical technique, DAHLSTRÖM and FUXE (1964) have found nerve cells of the catecholamine type in the locus coeruleus (group A6) as well as in hypothalamic areas such as the substantia grisea periventricularis, area supramammillaris, nucleus hypothalamicus posterior (group A11), nucleus arcuatus and nucleus periventricularis anterior (group A12). Noradrenaline-containing terminals were also found in the periventricular region of the hypothalamus as well as in the locus coeruleus (FUXE, 1965a, b). Recently, after placing lesions in the locus coeruleus of the rat, the disappearance of fluorescence of catecholamine-containing nerve fibers was observed in hypothalamic areas such as the mammillary areas, posterior hypothalamus (Loizou, 1969) and periventricular areas (Loizou, 1969; MAEDA and SHIMIZU, 1972). On the other

hand, JOUVET and DELORME (1965) presented evidence suggesting that the locus coeruleus was one of the essential structures for triggering the paradoxical sleep. Furthermore, the data reported by KAWAKAMI and SAWYER (1959) indicated that the paradoxical sleep could be induced by low-frequency stimulation of the mammillary nuclei, the medial and the lateral hypothalamic areas. These findings suggested the existence of close functional relationships between the locus coeruleus and the hypothalamus. Thus, the hypothalamic afferent fibers to the locus coeruleus found in the present study can be considered as a counterpart of the projection system of the locus coeruleus to the hypothalamus, and may provide a final link of the pathways for regulatory functions of the basal forebrain projection system (cf. MIZUNO et al., 1968, 1969a, b) upon the locus coeruleus.

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カイウサギ青斑核の電子顕微鏡的研究

——とくに視床下部および中脳よりの直接投射について——

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カイウサギ青斑核の微細構造，ことにシナプス構成を電子顕微鏡により実験形態学的に追究した。

軸索-細胞体シナプスは軸索-樹状突起シナプスに比べて少なく，軸索-軸索シナプスは見られなかった。扁平小胞を含むシナプス終末は円形小胞のみを含むシナプス終末に比べて少く，また前者では後者に比べ相称性シナプスの特徴を示す傾向が大であった。これらのシナプス終末には，多くの場合少数の直径 500 ないし 1,000 Å の含粒小胞が見られたが，前シナプス膜に接して存在する含粒小胞はまれに細胞体の中にも見られた。“nucleolus-like body,” “subjunctional body” および結晶様配列を示す円形シナプス小胞がまれに見られた。

視床下部，中脳中心灰白質または中脳被蓋背側部を脳定位的電気凝固により損傷した後，3日または4日生存させたカイウサギにおいて，傷と同側の青斑核内に電子密度の高い軸索-樹状突起性の変性終末が見られた。これらは通常円形シナプス小胞を含んでいたが，中脳損傷例ではやや扁平なシナプス小胞を含むものもまれに見られた。軸索-細胞体シナプス終末の変性は見られなかった。

視床下部および中脳より青斑核にいたる直接投射の機能的意義について考察した。

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