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The organization of dopaminergic and non-dopaminergic mesencephalo-cortical neurons in the rat

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The dopamine containing mesencephalo-cortical pathway was studied in the rat by means of a combined retrograde fluorescent tracing and catecholamine histofluorescence technique. After large injections of the fluorescent retrograde tracer, Evans blue, into the frontal cortex, many neural somata of the ventral midbrain tegmentum were retrogradely labeled; most of the retrogradely labeled neurons also showed catecholamine fluorescence. However, some labeled cells (10–15%) did not show any catecholamine fluorescence. The present findings confirm the existence of a non-dopaminergic (DA) mesencephalo-cortical pathway and describe the topographical interrelationships between its DA and the non-DA cell bodies of origin.

The ascending efferents of the mesencephalic ventral tegmental area of Tsai (VTA) are distributed over several areas of the telencephalon, such as the anterior limbic and frontal cortex, the caudate-putamen, the septum, the olfactory tubercle and the amygdala[2]. The VTA contains the dopaminergic (DA) cell bodies of the A10 cell group[7], and histofluorescence studies have described DA efferent projections of the VTA (see reviews in refs. 11 and 13). However, recent electrophysiological studies[14,19] suggested that the VTA efferents are in part derived from non-DA cell bodies. The introduction of the anatomical tracing technique by means of fluorescent compounds (ref. 10) raised the possibility of a simple simultaneous observation of retrograde labeling and catecholamine histofluorescence[5,17]. The present study was aimed at verifying by means of this combined technique the existence of non-DA VTA neurons which project to the frontal cortex.

The present results are based on a total of 12 rats, which were injected with the red fluorescent tracer, Evans blue (EB)[10]. The injections were made unilaterally in the frontal cortex with a Hamilton microsyringe, via several needle penetrations at a depth of 1 mm from the cortical surface, in order to deliver a total amount of approximately 1 μl of a 12% EB aqueous solution. After 2 or 3 days survival all the animals were perfused transcardially with an ice-cold phosphate-buffered (pH 7.4) 2% glyoxylic acid–1% formaldehyde solution and processed for catecholamine histofluorescence according to the glyoxylic acid procedure by Bloom and Battenberg[4]. In order to enhance catecholamine fluorescence, 4 h prior to the sacrifice animals were given i.p. a

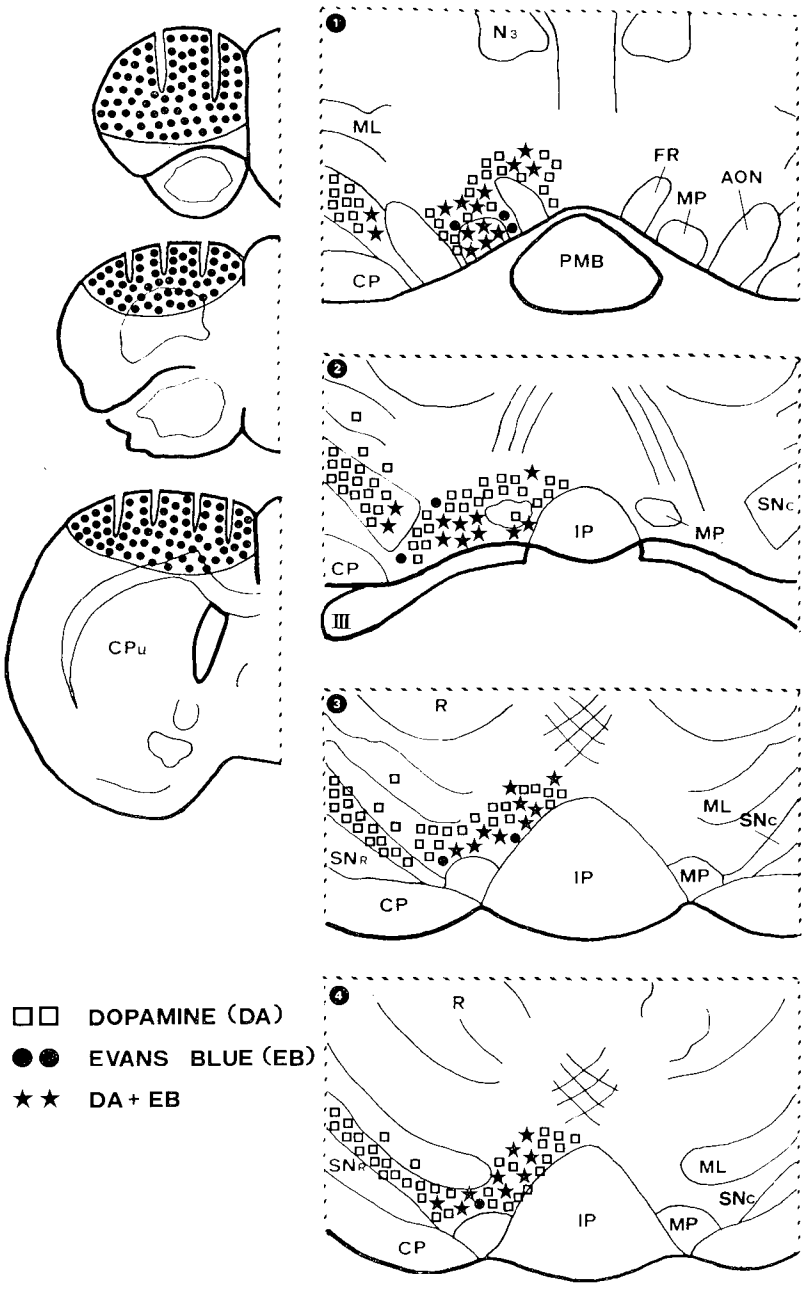

Fig. 1. Schematic representation of the extent of the EB injection area in the frontal cortex (left column) and of the distribution of catecholamine fluorescent and retrogradely labeled neurons in the ventromedial midbrain tegmentum (right column). For reasons of clarity DA cell bodies have been indicated only unilaterally. Each star and each open square in the VTA correspond approximately to two cells and each dot corresponds approximately to one cell. Numbers 1-4 indicate the rostrocaudal transverse sections. Abbreviations: AON, medial accessory optic nucleus; CP, cerebral peduncle; CPu, caudate-putamen; FR, fasciculus retroflexus; IP, interpeduncular nucleus; ML, medial lemniscus; MP, mammillary peduncle; N_3, nucleus of the third cranial nerve; PMB, posterior mammillary body; R, red nucleus; SN_C, substantia nigra pars compacta; SN_R, substantia nigra pars reticulata; III, third cranial nerve.

carbonate-buffered (pH 5.5) solution of nialamide (Sigma; 100 mg/kg). The sections were coverslipped with mineral oil. They were studied with a Leitz Ploemopak fluorescence microscope using two different filter-mirror systems: system N2 (530–560 nm light excitation wavelength) was used to elicit the EB red fluorescence; system D (355–425 nm light excitation wavelength) was used to elicit the dopamine fluorescence, which appears as green granules in the neuronal cytoplasm and proximal dendrites. The material was studied screening each field with both systems. The distribution of the fluorescent cells was charted on serial drawings of the ventral midbrain tegmentum.

In all the animals many EB retrogradely labeled red fluorescent cells were found unilaterally in the VTA and in the most medial part of the substantia nigra pars compacta (SNc), while the green fluorescing dopamine-containing cells were observed throughout the SNc and the VTA bilaterally.

The results obtained in one representative case are shown in Fig. 1. The injection area was intensely red fluorescent and covered the rostral pole of the frontal cortex. Retrogradely EB-labeled cells were distributed throughout the rostrocaudal extent of the VTA and in the most medial part of the SNc. EB-labeled cells were mainly located ventromedially, but some were also scattered more dorsally in the VTA. The majority of EB retrogradely labeled cells also showed green granular catecholamine fluorescence, and thus fluoresced both red and green (Fig. 2). However some retrogradely labeled cells did not show any catecholamine fluorescence (Fig. 3); they were intermixed with the EB-labeled dopamine-containing cells.

Similar results were obtained in all the other cases. The number of the EB-labeled non-catecholamine-containing cells varied from 10% to 15% of the total amount of EB-labeled cells. Some of the EB-labeled cells showed an uneven catecholamine fluorescence. Observed at high magnification these neurons displayed a green fluorescence partially covering the cytoplasm or a thread-like rim bordering the cytoplasm and the nucleus with pale fluorescence in between. In this respect, the criterion was adopted that the EB-labeled cells were considered as dopamine-containing ones when catecholamine fluorescence could undoubtedly be detected in the cell body or in

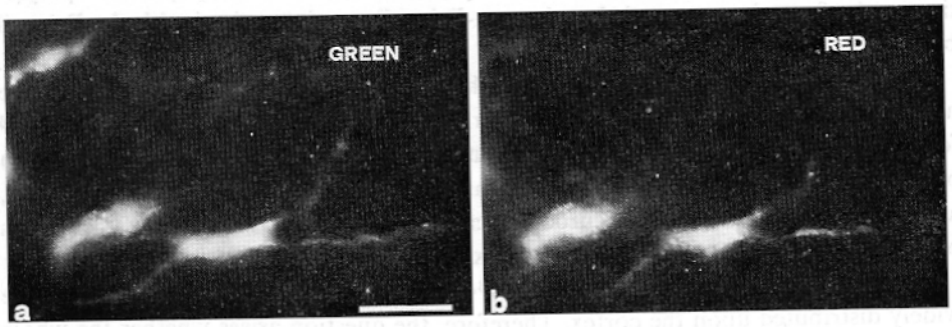

Fig. 2. Photomicrographs of dopamine-containing retrogradely labeled neurons in the VTA. a: catecholamine (green: fluorescent cells; excitation wavelength: 390 nm. b: same field illuminated with 550 nm light excitation wavelength reveals EB (red) fluorescence in the same cell bodies. Calibration bar: 20 μm.

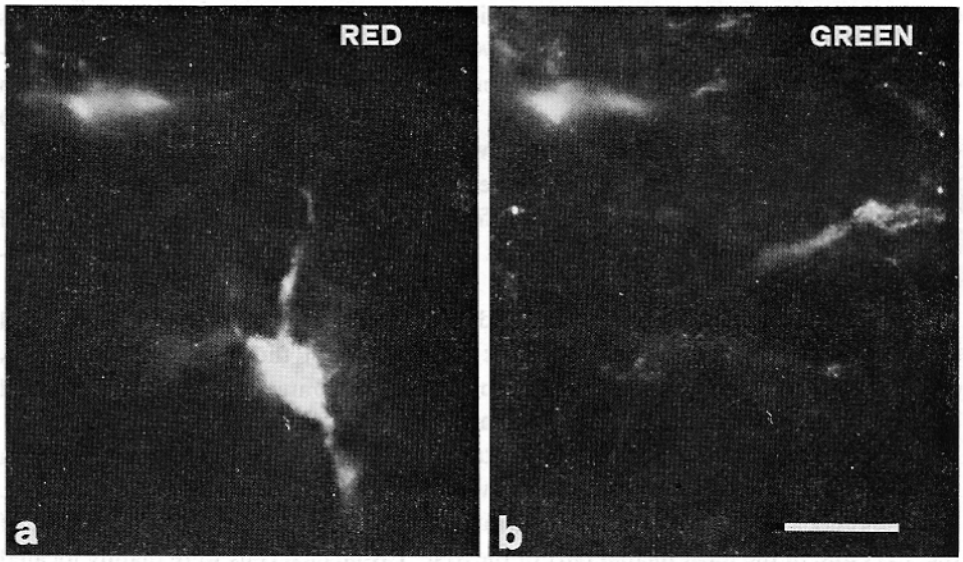

Fig. 3. Photomicrographs of EB retrogradely labeled non-DA neuron in the VTA. a: EB (red) fluorescent retrogradely labeled cell; excitation wavelength: 550 nm. b: the cell does not show any catecholamine fluorescence under a 390 nm light excitation wavelength. Calibration bar: 20 μm.

part of it. Although the possibility of false negative results cannot be ruled out completely, it must be stated that dubious findings were very rare and were seen in the cases in which the catecholamine fluorescence was not optimal, in agreement with the results observed by van der Kooy and Wise[17].

The distribution of the VTA cells projecting to the frontal cortex, as shown in the present study, is in agreement with previous retrograde tracing studies in the rat[2,6]. Moreover, the present results indicate that the projections from the VTA to the frontal cortex take origin both from DA and non-DA cell bodies. These findings confirm previous electrophysiological data[14]. In addition, the present study evidences that non-DA neurons represent a minor part (10–15%) of the VTA neural population projecting to the frontal cortex and that the non-DA cells are intermixed with the DA cell bodies.

Recent studies[14,18,19] reported that the VTA efferents to the septum and to the nucleus accumbens in part take origin from non-DA cell bodies. Van der Kooy et al.[16] found that a minor number (about 5%) of non-DA cells of the ventral mesencephalon project to the striatum and that they are mainly located in the VTA. Taken together, all these data suggest that the neurochemical organization of the VTA cells as well as of their efferent pathways is more complex than previously supposed.

Retrograde tracing studies in the cat[3,12] evidenced that the VTA projections are widely distributed upon the cortex. Therefore, the question arises whether the whole VTA-neocortical system takes origin both from DA and non-DA cell bodies. It has recently been shown[15] that the VTA projections to the visual cortex originate from DA cell bodies; however, this latter study[15] does not favour or exclude the existence of

non-DA cell bodies projecting to the visual cortex. DA as well as some non-DA cells of the ventral midbrain tegmentum contain the peptide cholecystokinin (CCK)[9]; CCK terminals are present in the rat neocortex[8]. Future anatomo-chemical studies will probably clarify whether CCK or other possible neurotransmitters are involved in the mesencephalo-cortical pathway.

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