MONOAMINE NEURON SYSTEMS IN THE NORMAL AND SCHIZOPHRENIC HUMAN BRAIN: FLUORESCENCE HISTOCHEMISTRY OF FETAL, NEUROSURGICAL AND POST MORTEM MATERIAL*

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THREE sources of material are presently being used to study the distribution of monoamine neuron systems in the human brain using Falck–Hillarp fluorescence histochemistry (FALCK *et al.*, 1962). The possibility of using fetal brain material from abortions was shown by OLSON and UNGERSTEDT (1970) and has recently resulted in detailed mapping studies (NOBIN and BJÖRKLUND, 1973, OLSON *et al.*, 1973a). Neurosurgery material has demonstrated the presence of catecholamine (CA) nerve terminals in the cerebral and cerebellar cortices (NYSTRÖM *et al.*, 1972). Using *post mortem* material, the presence of CA terminals in selected areas was shown by CONSTANTINIDIS *et al.* (1969) and by DE LA TORRE (1972). Our recent studies have shown that the *post mortem* time limit for histochemical analysis given by DE LA TORRE, 45 min, can be extended by several hours, especially when using *in vitro* incubations in amine solutions. Thus aspects on the distribution of CA cell bodies and of CA and 5-hydroxytryptamine (5-HT) nerve terminal areas have been described (OLSON *et al.*, 1973b).

While fetal material is excellent for mapping studies, due to the well-known increased histochemical detectability of non-terminal monoamine axons during development, the possibility of using *post mortem* brain material no doubt provides the most important source of material in order to gain insights into the possible involvement of the monoamine neurons in various diseases. The need for fluorescence histochemistry of the schizophrenic brain has been directly expressed by PLUM (1972). It provides a possibility to test the hypothesis of Stein and Wise (see STEIN, WISE and BERGER, 1972) of a destruction of noradrenergic nerves by endogenous 6-OH-dopamine (6-OH-DA) formation in schizophrenia. In the following we will briefly describe methodology and results mainly of the *post mortem* analyses.

METHODOLOGY

For distribution of analysed cases of schizophrenia, see Table 1. The histochemistry of two of the cases (F9 + F15) have been briefly described elsewhere, together with our control material (see OLSON *et al.*, 1973). The procedure followed was a so called "partial autopsy". Following death, as diagnosed by an independent physician, and after permission of the relatives, dissection was commenced as soon as possible. This procedure has been approved upon by the staff of the Ulleråker Hospital.

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We use smears (OLSON and UNGERSTEDT, 1970b) and freeze-dried material (OLSON and UNGERSTEDT, 1970a). Fluorescence microscopy of the adult human brain is complicated by the heavy accumulations of lipofuscin in neurons and glia having a strong yellow autofluorescence. The specific paraformaldehyde-induced green and yellow neuronal fluorescence is, however, easily recognized in the microscope due to color, morphology, diffusion and fading characteristics, especially using a narrow band excitation filter (TAL405) and a Zeiss 47 barrier filter. *In vitro* incubations of thin slices of tissue with α-methyl-NA (10^{-6} or 10^{-5} M) sometimes following preincubation in 6-OH-DA (10^{-4} M) or DMI (10^{-4} M) were carried out according to HAMBERGER (1967). Nerve densities and fluorescence intensities were estimated semiquantitatively. For the reliability of such estimations as correlated to biochemistry see OLSON *et al.* (1968), OLSON and MALMFORS (1970), JONSSON (1971) and LIDBRINK and JONSSON (1971).

RESULTS AND DISCUSSION

We conclude from our three sources of material (about 40 fetal brains, 75 neurosurgery operations and 20 *post mortem* brains) that the basic cytoarchitecture of the monoamine neurons present in the rat (see FUXE *et al.*, 1973) is present also in man. Thus, there are descending bulbospinal noradrenaline (NA) and 5-HT systems, as well as ascending NA and 5-HT systems reaching almost all areas of the brain, having their cell bodies in the lower brain stem. Furthermore, the predicted nigro-neostriatal dopamine (DA) system with cell bodies in the substantia nigra area and a dense arrangement of terminals in the nuc. caudatus and putamen has been visualized.

Endogenous CA fluorescence located to varicose nerve terminals was a constant finding in subcortical areas of all the *post mortem* brains, the largest *post mortem* time interval being 7·3h. Like in the rat, there was a mixture of thin fibers with small varicosities and thick fibers with large, strongly fluorescent varicosities. In addition, scattered fibers showed extremely large "varicosities" with an intense yellow-green fluorescence not seen in the rodent brain. Especially dense innervation patterns were found in hypothalamic areas, e.g. periventricularly, in the tuber cinereum and in nuc. supraopticus, but moderate numbers of CA nerve terminals were found throughout the central nervous system from the filum terminale, and the spinal cord, through the lower brain stem, the hypothalamic areas, amygdala and in the olfactory bulb, to give some examples.

The pineal glands were yellow fluorescent, the habenulae richly provided by CA and 5-HT fibers.

The nuc. caudatus and putamen were always diffusely green fluorescent. Following incubation in α-m-NA an extremely dense innervation by fine CA varicosities were disclosed in these areas, identical in appearance to the DA innervation of the neostriatum as seen in rats.

Untreated cerebral and cerebellar cortex showed no or very few CA nerve terminals *post mortem*. A marked concentration dependent uptake of α-m-NA could always be demonstrated. Following incubation in α-m-NA the typical (as compared to rats) thin varicose fibers were found in all layers of the cerebral cortex and in the molecular layer of the cerebellar cortex. This uptake was inhibited by 6-OH-DA and by DMI. 5-HT nerve terminals were likewise found in the cerebral cortex.

TABLE. 1. DISTRIBUTION OF ANALYZED CASES.
(All four patients were repeatedly diagnosed by different psychiatrists as having typical schizophrenia with a relatively early onset and a long duration.)

Case	Age	Sex	Duration of disease (years)	Analyzed material	Post mortem time (min)
F9	85	female	45	post mortem brain	100
F15	63	female	38	post mortem brain	65
F19	69	male	45	post mortem brain	70
Op.*	53	female	32	cortex cerebri resected at tumour neurosurgery	—

* This patient had also undergone a frontal lobotomy.

In case "op." (Table 1), where fresh cerebral cortex from a schizophrenic was obtained at neurosurgery, both CA and 5-HT nerve terminals showed endogenous fluorescence. Work in progress shows that these terminals are able to take up and accumulate labelled NA and 5-HT, respectively, and to release their labelled amines upon field stimulation *in vitro* (FARNEBO, NYSTRÖM, OLSON and SEIGER, in preparation).

Green fluorescent cell bodies, simultaneously heavily loaded by neuromelanin were found in the locus coeruleus and substantia nigra.

A sympathetic adrenergic innervation of larger intracranial blood vessels was constantly found.

In view of the Stein and Wise theory (cited above) it is important to note that we found *no differences between the schizophrenic and non-schizophrenic brains* (Fig. 1).

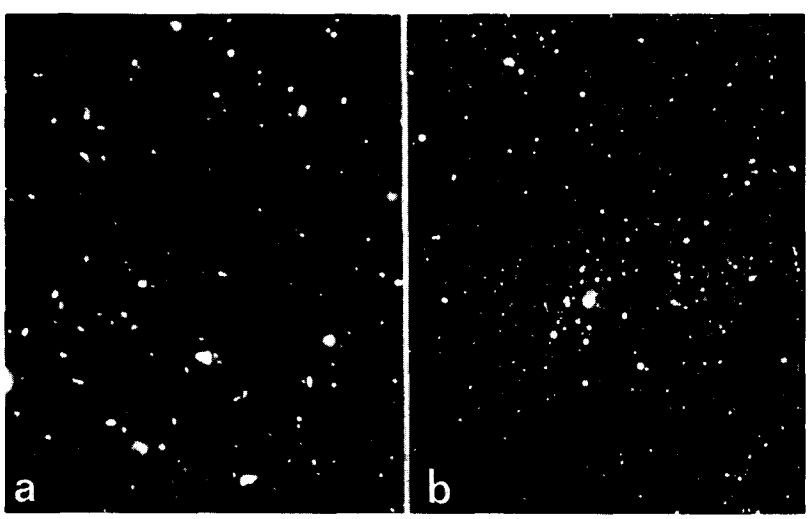

FIG. 1.—Fluorescence micrographs of smears from a schizophrenic brain (case F19). (a) A moderate number of green fluorescent varicosities is seen in the cerebral cortex after incubation in α-m-NA (10^{-5} M) × 300. (b) A high number of varicosities showing endogenous CA fluorescence seen in the hypothalamus close to the ventricular surface. Very small, sharply outlined dots represent unspecific lipofuscin fluorescence in both pictures × 120.

Thus, although our data are only preliminary, not fully quantitative, and does not discriminate between DA and NA, they clearly demonstrate an abundant presence of NA-like nerve terminals in all investigated areas of the schizophrenic brain. The schizophrenic brain likewise seem to contain an amount of DA and 5-HT nerve terminals similar to that of the non-schizophrenic and to have a well developed sympathetic adrenergic blood vessel innervation.

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