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Monoaminergic fibers form conventional synapses in the cerebral cortex

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The morphological characteristics of the monoaminergic axon-terminals in the mammalian cerebral cortex have been the subject of controversy in recent years. Systematic analysis of serial ultrathin sections, immunocytochemically stained with antibodies against noradrenaline and serotonin, has shown that nearly all stained terminals form synapses characterized by specialized junctional appositions. These results suggest that, contrary to the widely held view, monoamines in the cortex are released at specialized junctions.

The ultrastructural features of the monoaminergic terminals in the cerebral cortex have attracted great interest during the past 20 years. It has been thought that knowledge of the fine features of these terminals could provide clues concerning the mode of action of the monoamines. However, it has been difficult to visualize monoaminergic terminals in the cortex. Various methods have been utilized in the past resulting in questionable findings [1, 5, 9, 15]. Electron microscope autoradiography following topical application of labelled noradrenaline (NA) and serotonin (5-HT) has been the most extensively applied method, particularly by Beaudet and Descarries [1]. They have reported, and it is now widely believed, that only a small proportion (less than 5%) of monoaminergic terminals in the rat neocortex are engaged in typical synaptic contacts, with the majority lacking morphologically defined synaptic specializations (for reviews see refs. 1 and 2). The implication of these reports is that monoamines are released non-synaptically in the cortex influencing not only adjacent neuronal elements but also more distant neurons and perhaps for prolonged periods [6]. However, contradictory evidence has been slowly accumulating [11, 13, 18, 19]. Here we report results of a systematic analysis of serial ultrathin sections of rat cerebral cortex, immunocytochemically stained with recently developed antibodies against NA and 5-HT, which show that at least 90% of labelled terminals form

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synapses characterized by specialized junctional appositions. We suggest that, similar to other conventional neurotransmitters, monoamines in the cortex are released at specialized junctions to act on specific receptors of adjacent neuronal elements.

Six Sprague-Dawley and two Wistar albino rats (approximately 250 g), anesthetized with ether, were perfused transcardially first with 0.9% NaCl and then with 500 ml of fixative containing 5% glutaraldehyde and 1% sodium metabisulfite in 0.05 M cacodylate buffer, pH 7.5. The brains were subsequently removed and placed in the same fixative for 1 h. Coronal sections through the visual cortex (area 17) [10] were cut at 50 μ m using a Vibroslice (Campden Instruments) and collected in 0.05 M Tris-buffered saline (TBS) containing 1% sodium metabisulfite, pH 7.5. In addition, sections through the frontoparietal cortex (corresponding to areas 3, 4, 6 and 10) [10] were cut from the brains of 4 animals. Those destined for NA immunocytochemistry were placed in sodium borohydride (5 mg/ml 0.05 M TBS) for 10 min. After several washes with TBS, sections were processed with the peroxidase-antiperoxidase immunocytochemical method using antibodies against 5-HT and NA in dilutions of 1:1500 and 1:2500 respectively. At the end of the procedure sections were transferred into 1% OsO₄ for 30 min, block-stained with 1% aqueous uranyl acetate, dehydrated through an ethanol series and flat-embedded in Araldite. Procedures for raising the anti-sera and all specificity tests have been described elsewhere [7]. It should be mentioned that the antiserum against NA cross-reacts slightly with dopamine, but as it is known that dopamine amounts to only 6% of the monoamine content in the visual cortex [21], this cross-reaction does not affect our results. In addition, preabsorption of the NA and 5-HT antisera with 10⁻⁵ M synthetic NA and 5-HT respectively blocked all positive staining. Ultrathin sections were cut and picked up on 200 mesh grids, slot grids or Robertson grids which have parallel bars, 110 μ m apart, oriented in only one direction. The latter grids were used to obtain an estimate of labelled varicosities throughout the thickness of the cortex.

Examination of the sections with the light microscope showed a widespread distribution of the NA and 5-HT fiber systems in both cortical areas. The density and distribution of the immunoreactive fibers resembled descriptions of previous histochemical and immunocytochemical studies (see ref. 12). NA and 5-HT fibers were mostly thin and varicose and branched profusely, revealing a dense network throughout the cortex.

Examination of ultrathin sections showed that NA- and 5-HT-immunoreactive profiles may be divided on the basis of size and the presence of synaptic vesicles into intervaricose segments and varicosities. An estimate of the densities of labelled varicosities was obtained by counting these profiles in 110 μ m wide strips of visual cortex between the pia and the subcortical white matter. A comparison between 5-HT- and NA-labelled varicosities showed the former to be more numerous (5-HT, 30-35; NA, 25-30) but generally smaller (5-HT, 0.5-0.8 μ m; NA, 0.5-1.0 μ m) in diameter. In an attempt to quantify the varicosities involved in synaptic contacts, we excluded a certain percentage (approximately 20%) of labelled profiles if the reaction product was too heavy or if the ultrastructural preservation was not adequate to permit identification. Examination of single ultrathin sections showed that only a small proportion

Fig. 1. (a-c) NA-labelled varicosity shown in 3 of 4 consecutive sections in which it was observed to form an asymmetrical synapse (arrows) with a dendritic spine. (d-f) 5-HT-labelled varicosities forming asymmetrical synapses with dendritic profiles (arrows). $\times 28,500$.

(approximately 20%) of varicosities formed conventional synapses with postsynaptic targets. However, systematic analysis of all labelled profiles in 50 uninterrupted series of 12–15 serial sections of visual cortex revealed that virtually all labelled varicosities in all layers formed synaptic contacts with other neuronal structures (Fig. 1). In this analysis, 550/600 (90.2%) of labelled 5-HT and 530/600 (88.3%) of stained NA varicosities formed synapses with postsynaptic profiles. A similar estimate was obtained in 10 series of serial sections through the frontoparietal cortex. Typically, a varicosity was observed to form a synaptic contact in 2–4 consecutive sections in each set of serial sections (Fig. 1a–c). The varicosities not found to be engaged in synaptic contacts may belong to a small number which were not cut in their entirety or those which were cut in an unfavorable plane. Both symmetric and asymmetric types of membrane specializations were observed for both 5-HT- and NA-stained varicosities, and the postsynaptic targets were mainly spines and dendritic shafts. However, on a few occasions axosomatic contacts with pyramidal and non-pyramidal neurons were also noted.

The present findings clearly suggest that the monoaminergic system exerts its influence through conventional synaptic junctions in the cerebral cortex. Molliver and colleagues [15, 18], using antibodies against dopamine- β -hydroxylase, the

enzyme that converts dopamine to NA, and 5-HT were the first to provide immunocytochemical evidence suggesting that the monoamine fiber system forms classical synaptic junctions in the central nervous system including the neocortex. Our findings are also in concurrence with other ultrastructural observations in a variety of sites in the central nervous system [3, 4, 8, 20], but in sharp contrast to the view that the monoamine fibers rarely form synaptic contacts but instead release transmitter in the extracellular space to influence not only adjacent structures but also more distant neuronal elements. Beaudet and Descarries have been the chief proponents in this view [1, 2]. Their negative evidence is based on observations of varicosities labelled autoradiographically by application of tritiated NA or 5-HT onto the cortical surface. It may be that their estimate of labelled varicosities was influenced by the diffuse reaction which is usually observed around the site of the topical application. Another reason for the discrepancy between their uptake studies and the immunocytochemical results presented here is that they did not utilize extensive serial section analysis which we found to be essential for a precise description of the fine features of the monoamine fibers. Our results suggest that the monoaminergic system shows the type of specificity displayed by other afferent systems in the cortex. This is consonant with the notion emerging from more recent studies [14, 16, 17] of monoamine innervation of the mammalian cortex that the two projections from the brainstem are highly specific in their mode of termination, have separate postsynaptic targets and play different roles in the processing of information in the cortex.

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