Light and electron microscopic immunocytochemical analysis of the serotonin innervation of the rat visual cortex

G. C. PAPADOPOULOS¹, J. G. PARNAVELAS² and R. M. BUIJS³

¹Laboratory of Anatomy, Veterinary School, University of Thessaloniki, Thessaloniki 54006, Greece

²Department of Anatomy and Embryology, University College London, Gower Street, London WC1E 6BT, UK

³Netherlands Institute for Brain Research, 1105 AZ Amsterdam, The Netherlands

Received 29 May 1987; revised 15 July 1987; accepted 27 July 1987

Summary

The serotonin afferents of the rat visual cortex were examined immunocytochemically at the light and electron microscopic levels. Immunoreactive fibres were typically thin, tortuous and varicose. Occasionally, some thicker fibres were found. The orientation of labelled axons varied according to laminar position, with fibres running parallel to the pial surface present mainly in layers I and VI, and radially oriented fibres prominent in layers II and III. Branches arising from horizontal or radially oriented fibres were seen to form irregularly shaped loops particularly in layers IV and V. The density of innervation and the prevailing axonal orientation in each cortical layer were similar in both coronal and parasagittal planes.

The ultrastructural features of serotonin-labelled axon terminals were examined in single and serial ultrathin sections. While in single sections the majority did not exhibit synaptic specializations, extensive serial section analysis showed that virtually all of these terminals were engaged in junctional complexes. Postsynaptic elements were spines and dendritic shafts, including pyramidal cell apical dendrites, with both symmetrical and asymmetrical membrane specializations. In axospinous synapses, the labelled terminals were usually adjacent to unstained axon terminals contacting the same postsynaptic element.

Introduction

The notion that the lower brainstem exerts a direct control upon the cerebral cortex was first suggested long ago (Moruzzi & Magoun, 1949). However, it was a significant time later that it was shown that a small number of brainstem neurons give rise to the monoamine fibres which were previously found to innervate the cerebral cortex (Carlsson *et al.*, 1962; Fuxe, 1965; Fuxe *et al.*, 1968a, b; Ungerstedt, 1971). Since then, numerous experimental approaches have been utilized to elucidate the morphological, neurochemical and physiological features of these afferent systems.

It is now widely believed that the cerebral cortex is innervated monosynaptically by three populations of brainstem neurons: (i) a group of noradrenaline-containing cells located in the locus coeruleus, (ii) a group of dopamine-containing neurons present in the ventral tegmental area-substantia nigra of the mesencephalon, and (iii) two groups of cells located in the mesencephalic nuclei, dorsal and medial

raphé, which contain serotonin (5-HT) (see Lindvall & Björklund, 1978, 1984; Moore & Bloom, 1978, 1979).

Compared to the catecholamine projection systems, the serotonergic system in the cerebral cortex was, until recently, less explored both anatomically and functionally. The serotonergic innervation of the cerebral cortex, studied by means of the formaldehyde-induced fluorescence method, was found to be sparse and restricted mainly in the molecular layer (Fuxe, 1965; Fuxe *et al.*, 1968b). However, biochemical studies (Gaudin-Chazal *et al.*, 1979; Reader *et al.*, 1979; Reader, 1981) and the use of more sensitive methods for localizing 5-HT-containing structures (Descarries *et al.*, 1975; Steinbusch, 1981) revealed the existence of a dense serotonergic innervation of the neocortex (Beaudet & Descarries, 1976; Morrison *et al.*, 1982; Takeuchi & Sano, 1983, 1984; Kosofsky *et al.*, 1984; Törk & Mulligan, 1984). In particular, the use of immunocytochemistry has revealed such a rich network of fibres that it has prompted some authors

to speculate that all cortical neurons are contacted directly by 5-HT-containing axons (Lidov *et al.*, 1980). The way in which these axons communicate with neuronal elements in the cerebral cortex has been the source of controversy. Using radioautography following topical application of tritiated 5-HT on the cortical surface, Descarries and his colleagues (Descarries *et al.*, 1975; Beaudet & Descarries, 1978; see also Beaudet & Descarries, 1984 for review) proposed that a very low proportion (< 5%) of the 5-HT terminals in the rat cerebral cortex exhibit junctional complexes. In contrast, Molliver *et al.* (1982) using immunocytochemistry and Parnavelas *et al.* (1985) using a similar approach to that of Descarries and co-workers, have reported that at least one-half of the labelled terminals form conventional synapses.

In the present study, we utilized an antibody to 5-HT to provide a detailed light and electron microscopic description of the 5-HT innervation of the rat visual cortex. The ultrastructural features of the axon terminals containing 5-HT were examined in a large number of series of serial ultrathin sections.

Materials and methods

Eight Sprague-Dawley and four Wistar albino rats (body weight 200–250 g) of both sexes were used in this study. All animals were perfused, under ether anaesthesia, through the heart first with a small quantity of saline and then with approximately 500 ml of a fixative solution containing 5% glutaraldehyde and 1% sodium metabisulphite in 0.05 M cacodylate buffer at pH 7.5. The brains were removed shortly after the perfusion and immersed in the same fixative for 1 h. Coronal and parasagittal sections through the visual and the frontoparietal cortex (areas 17, and 3, 4, 6 and 10, respectively) (Krieg, 1946) were cut at 50–60 µm using a Vibroslice (Campden Instruments) and collected in 0.05 M Tris buffer saline (TBS) containing 1% sodium metabisulphite, pH 7.5. They were then placed in sodium borohydride (5 mg ml⁻¹ TBS) for 10 min which reduces the double bonds formed by glutaraldehyde with the amine and tissue protein (McRae-Degueurce & Geffard, 1986). After several washes in TBS, sections were processed for immunocytochemical staining according to the unlabelled antibody peroxidase-antiperoxidase (PAP) technique (Sternberger, 1979). In this procedure, free-floating sections were incubated in 5-HT antiserum (dilution 1:1500 in TBS containing 1% sodium metabisulphite and 0.1% Triton X-100) first for 30 min at room temperature, then at 4°C for 18 h and then returned to room temperature for 30 min. Following a thorough wash with TBS they were transferred to goat anti-rabbit IgG (dilution 1:40 in TBS containing 0.1% Triton X-100) at room temperature for 1.5 h. Subsequently, they were washed in TBS for 1 h and placed in rabbit PAP (dilution 1:80 in TBS containing 0.1% Triton X-100) at room temperature for 3 h. After several rinses in TBS, sections were preincubated for 10 min in 3,3'-diaminobenzidine tetrahydrochloride (DAB) (7.5 mg in 14 ml TBS) and then transferred to a fresh solution of DAB containing 0.005% H₂O₂ for about 10 min. Following a rinse in TBS, sections to

be used for light microscopy were mounted on slides. A number of slides were counterstained with Cresyl Violet to enable the 5-HT innervation of individual cortical layers to be visualized. Sections destined for electron microscopy were placed in 0.1 M phosphate buffer and then into 1% OsO₄ for 30 min. After a brief rinse with cold sodium acetate buffer, sections were stained in 1% uranyl acetate for 30 min, dehydrated through an ethanol series and flat-embedded in Araldite. All sections were first examined with the light microscope and then representative areas of the cortex were selected for ultrathin sectioning. Ultrathin sections were cut serially, and series of between 10 and 15 consecutive sections were picked up on 200 mesh grids and slot grids. In order to obtain an estimate of the density of immunoreactive varicosities, sections through the thickness of the visual cortex were picked up on Robertson grids which have parallel bars, 110 µm apart, oriented in one direction. All grids were stained with uranyl acetate and lead citrate, each for 30 min, before examination under a Philips electron microscope.

Antiserum

The serotonin antiserum was produced in rabbits. The procedures for raising the antibody and all specificity tests have been previously described (Geffard *et al.*, 1985). In our studies, preincubation of the antiserum with 10⁻⁵ M purified synthetic 5-HT blocked all positive cortical staining. A similar result was obtained by omitting the anti-rabbit IgG from the immunocytochemical procedure.

Results

Light microscopy

Examination of coronal and parasagittal sections revealed an apparently highly arborizing plexus of 5-HT fibres in all cortical layers (Fig. 1). The great majority of these axons were fine and tortuous, bearing numerous and intensely stained varicosities. These varicosities were of variable size and were spaced irregularly along the fibres. In addition, some thick fibres with elongated large varicosities, resembling the A-fibres of Sano *et al.* (1982), were observed (Fig. 2). Very rarely, varicosities were at the end of short stalks which originated from a parent fibre (Fig. 3). It should be said that the types of 5-HT axons described in other parts of the brain on the basis of their thickness as well as the shape and size of their varicosities (Köhler *et al.*, 1981; Sano *et al.*, 1982) could not be recognized in the visual cortex. A description of the route of individual immunoreactive fibres, which on a few occasions were observed to terminate on cortical cell perikarya (Fig. 4), was hampered by the high density of innervation. However, the degree of arborization of 5-HT axons was not as extensive as previously suggested. Careful inspection, at relatively high magnification, showed that what at first appeared a highly arborizing plexus was actually created by segments of individual axons present at different levels of section thickness. Moreover, in

Fig. 1. Darkfield photomontage of a coronal strip through the rat visual cortex between pia and the subcortical white matter (WM) stained with an antibody against 5-HT. $\times 130$.

both coronal and parasagittal planes, three categories of 5-HT axons were recognized on the basis of the course they followed within the cortex: (i) fibres

running parallel to the pial surface, (ii) radially oriented fibres, and (iii) fibres emanating from horizontal or radially oriented axons, which formed irregularly shaped loops.

In agreement with previous studies (Lidov *et al.*, 1980; Molliver *et al.*, 1982), the density of innervation and orientation of fibres within the visual cortex showed laminar variations in both coronal and parasagittal sections. Layer I contained the most dense plexus of axons labelled with 5-HT. This plexus, which was more prominent in the outer half of the layer, was formed mainly by diversely oriented axons running parallel to the pia and also by radial axons ascending from the lower layers. Thus, a horizontal grid-like network of 5-HT fibres penetrated by radially oriented immunoreactive axons was the characteristic feature of the molecular layer. An appreciable number of fibres was seen to terminate in the outermost surface of the cortex, merging with the pia. In the lower half of layer I, thick fibres were often observed running parallel to the pial surface for a long distance (Fig. 5). Layer II contained radially oriented axons which either penetrated into layer I or turned back to descend into layers III and IV. Labelled fibres in layer III were relatively straight, radially oriented axons which gave rise to very few collaterals. On rare occasions, one such axon was seen to oscillate between layers II through IV (Fig. 6). In contrast to the relatively long fibres visualized in layers I to III, fibres in layer IV and particularly in layer V were highly tortuous and, typically, only small segments of these fibres could be seen in individual sections (Fig. 7). Finally, a large number of tangential fibres running parallel to the subcortical white matter characterized layer VI (Fig. 8). Occasionally, individual 5-HT fibres arising in layer VI could be seen to traverse radially layers V to II. A small number of tangential labelled axons were also present within the white matter.

Electron microscopy

At the ultrastructural level, 5-HT-positive profiles appeared as unmyelinated intervaricose axon segments or as axonal enlargements filled with synaptic vesicles (= varicosities). In our quantitative analysis, the identity of individual profiles was confirmed in an adequate number of serial sections. However, a certain percentage (approximately 20%) of immunoreactive profiles could not be identified with certainty because of poor ultrastructural preservation or because of the intensity of the immunoreactive product. In a randomly photographed sample, varicosities had a diameter of $0.5\text{--}0.8\mu\text{m}$, whereas intervaricose segments were considerably thinner ($<0.4\mu\text{m}$). Significantly larger varicosities were rarely observed. The contents of intervaricose segments were often obscured by the immunoreactive product,

but occasionally mitochondria and microtubules could be seen. Labelled varicosities were observed either as separate profiles or along fine intervaricose segments, and in single sections they appeared round, elongate or irregular in shape. Often one or two mitochondria and a plethora of vesicles could be identified. Counts in strips, 110 μm wide, through the entire thickness of the visual cortex showed an average density of 30 to 35 immunoreactive varicosities.

Examination of single ultrathin sections showed that, although a number of labelled varicosities made typical synaptic contacts with various postsynaptic targets, the majority (approximately 80%) did not exhibit synaptic specializations. In an attempt to obtain an accurate estimate of the proportion of varicosities involved in junctional complexes, we examined 600 varicosities in 25 uninterrupted series of 12 to 15 sections through the visual cortex (Fig. 9a-i). Surprisingly, 550 out of the 600 profiles (90.2%) were found to form conventional synapses. A similar proportion was obtained in five series of serial sections through the frontoparietal cortex. Typically, the synaptic contacts were of both symmetrical and asymmetrical varieties, and the postsynaptic elements were dendritic spines, pyramidal cell apical dendrites or small to medium-sized dendritic shafts (Fig. 9j). In axospinous synapses, the terminals labelled with 5-HT were typically adjacent to unstained axon terminals contacting the same postsynaptic element (Fig. 9k).

Discussion

It has been known for some time that the cerebral cortex receives a widespread innervation by 5-HT-containing afferents originating in the dorsal and medial raphe nuclei (see Lindvall & Björklund, 1984 for review). Knowledge of the organization of these afferents and their relationship with cortical elements remains vague and controversial. In this study, these

aspects of the 5-HT system were examined in the rat visual cortex.

Previous studies have reported that the 5-HT system in the brain comprises a heterogeneous population of fibres based on the length and diameter of the intervaricose segments, and the incidence, shape and size of the varicosities (Köhler *et al.*, 1981; Sano *et al.*, 1982; Takeuchi & Sano, 1983). In our material, the serotonergic system appeared as a relatively homogeneous network of fine and tortuous axons with small varicosities. However, some thick fibres with large, spherical or elongated varicosities were also observed. These thick fibres, which closely resembled the 5-HT-containing tract fibres of Sano *et al.* (1982), have also been described in other cortical areas of the rat (Lidov *et al.*, 1980) and in the monkey neocortex (Morrison *et al.*, 1982; Takeuchi & Sano, 1983). The thick tract fibres of Sano *et al.* (1982) were reported to give rise to numerous branches which form a terminal plexus of fine fibres. In our system, thick axons in the lower half of layer I could be traced for long distances (sometimes up to a few hundred micrometres) without giving rise to a significant number of branches. These thick fibres appeared separate from the dense plexus of thin axons concentrated in the outer portion of the layer. These observations suggest that two axonal 5-HT systems exist, exhibiting different arborization patterns. It is pertinent to note that Kosofsky & Molliver (1987) have recently reported that cortical serotonergic afferents with large varicosities arise from cells in the median raphe nucleus, while those with small varicosities originate in the dorsal raphe. Thus, similar to the suggestions made for other aminergic systems in the cortex (see Lindvall & Björklund, 1984; Berger & Verney, 1984), distinct 5-HT cortical systems appear to exist. It would be interesting to compare the physiological properties and the cellular targets of these systems.

The uniform density of 5-HT afferents through all neocortical layers, and their similar distribution

Figs 2-8. Darkfield (Figs 5, 7, 8) and brightfield (Figs 2-4, 6) photomicrographs of fibres labelled with 5-HT. Pia is towards the top.

Fig. 2. Obliquely oriented fibres bearing varicosities of various sizes as they course through layer III. $\times 600$.

Fig. 3. Varicosities at the end of short stalks (arrows) originating from a common axon. The section was counterstained with Cresyl Violet. $\times 1280$.

Fig. 4. A tortuous fibre appearing to terminate on a cortical cell body (arrow). The section was counterstained with Cresyl Violet. $\times 880$.

Fig. 5. A thick fibre (arrows) in layer I coursing parallel to the pia. $\times 500$.

Fig. 6. Radial axon 'oscillating' in layer III before leaving the plane of section. $\times 500$.

Fig. 7. A network of short, tortuous axonal branches characterize layer V. At times these elements form irregular loops (arrows). $\times 200$.

Fig. 8. Tangentially oriented fibres in layer VI. $\times 200$.

throughout most neocortical regions of the rat, originally gave the impression that the 5-HT system in the mammalian cerebral cortex is a diffuse transmitter system. However, subsequent studies in primates (Brown *et al.*, 1979; Goldman-Rakic & Brown, 1982; Takeuchi & Sano, 1983; Morrison & Foote, 1986) showed regional variations in terms of the density and laminar distribution of the 5-HT fibres. These studies suggested a greater degree of organization in more highly developed cortices as well as a close relationship between individual cortical fields and their patterns of innervation. However, interspecies similarities appear to exist with regard to the orientation of 5-HT fibres in individual layers. The basic orientation pattern, which resembles that of the noradrenergic system (Papadopoulos *et al.*, 1988) shows that tangential fibres predominate in layers I and VI, radial axons in layers II and III and short curved axon segments in layers IV and V. The subtle laminar variations of the 5-HT fibre orientation that has been reported in some cortical fields of primates (Takeuchi & Sano, 1983; Morrison & Foote, 1986) may represent variations from the basic pattern due to features unique to certain cortical areas.

Early studies on the mode of action of the monoamine systems have proposed that these systems act non-synaptically in a variety of brain sites (Hökfelt, 1968; Ajika & Hökfelt, 1973; Richards *et al.*, 1973; Tennyson *et al.*, 1974; Chan-Palay, 1975, 1976; Descarries *et al.*, 1975, 1977). More recent studies have reported conflicting findings concerning the incidence of specialized aminergic synapses in the CNS (Wilson *et al.*, 1977; Koda & Bloom, 1977; Arluisson *et al.*, 1978; Cuello & Iversen, 1978; Groves, 1980; Groves & Wilson, 1980; Itakura *et al.*, 1981; Olschowka *et al.*, 1981; Molliver *et al.*, 1982; Ruda *et al.*, 1982; Takeuchi & Sano, 1984). Concerning the 5-HT system in various brain regions, this incidence has been reported to range from 2 to 84% (Descarries *et al.*, 1975; Chan-Palay, 1978a,b; Ruda *et al.*, 1982; Pickel *et al.*, 1984; Takeuchi & Sano, 1984; Parnavelas *et al.*, 1985; Soghomonian *et al.*, 1986). While the differential incidence of typical synaptic contacts may be explained by the different modes of action of the 5-HT system in various parts of the brain (Beaudet & Descarries, 1978), studies which have focused on the cerebral cortex have produced equally conflicting results (Descarries *et al.*, 1975; Molliver *et al.*, 1982; Takeuchi & Sano, 1984; Parnavelas *et al.*, 1985).

Immunocytochemistry, using a specific antibody to 5-HT, combined with extensive serial section analysis provides an approach free of some of the problems inherent in the methods previously applied in the cerebral cortex (i.e. steep diffusion gradient after topical application of [^{3} H]5-HT and lack of extensive serial section analysis). The present light and electron microscopic observations further support the notion of highly organized innervation of the cerebral cortex by serotonergic afferents. These afferents, which are part of the 'réseau régulateur' (Glowinski, 1984), appear to be specifically organized with respect to their targets with which they communicate at spatially restricted sites (see also Morrison *et al.*, 1982). Thus, the postulated global influence of 5-HT upon cortical function is apparently selective, and the modulation of a large range of brain functions is accomplished in a way which preserves the 'orderly function in complex neuronal circuits' (Molliver *et al.*, 1982).

An observation which should not go unnoticed is the close association of 5-HT varicosities with other unstained terminals forming synapses upon the same target, especially dendritic spines. This finding suggests that the analogous arrangement of 5-HT afferent terminals reported in the oculomotor nucleus (Soghomonian *et al.*, 1986) may not be a peculiar element of this nucleus but rather a feature of the 5-HT projection system. The physiological significance of these closely linked afferents remains elusive. However, it could be hypothesized that the associated terminals and their common target represent the anatomical substrate for the sustained influence that the 5-HT system may exert on modality specific signals. Evidence is now increasing for an extensive interaction between 5-HT and other putative neurotransmitters at the level of pre- and postsynaptic sites (see Reader & Jasper, 1984). Recent morphological evidence for a competitive interaction between noradrenergic and 5-HT cortical systems (Blue & Molliver, 1987) emphasizes the complexity of the mode of 5-HT action(s).

Acknowledgements

We wish to thank Dr M. Geffard for the 5-HT antiserum, Eva Franke for the help with the electron microscopy, and Anna Tsipinia and Chris McCourt for secretarial help. The work was supported by The Wellcome Trust.

Fig. 9. (a-i) A 5-HT-labelled varicosity shown in serial sections. It forms an asymmetrical synaptic contact with a dendritic spine in five sections (d-h). (j) A 5-HT-labelled varicosity containing numerous vesicles and a mitochondrion seems to form an asymmetrical synapse with a dendritic shaft. (k) A 5-HT-immunoreactive varicosity forming an asymmetrical synapse with a dendritic spine (sp) which also receives a synaptic contact from an unlabelled axon terminal. $\times 41\,000$.

References

- AJIKI, K. & HÖKFELT, T. (1973) Ultrastructural identification of catecholamine neurones in the hypothalamic periventricular-arcuate nucleus-median eminence complex with special reference to quantitative aspects. *Brain Research* **57**, 97-117.
- ARLUISON, M., AGID, Y. & JAVOY, E. (1978) Dopaminergic nerve endings in the neostriatum of the rat. I. Identification by intracerebral injections of 5-hydroxydopamine. *Neuroscience* **3**, 657-73.
- BEAUDET, A. & DESCARRIES, L. (1976) Quantitative data on serotonin nerve terminals in adult rat neocortex. *Brain Research* **111**, 301-9.
- BEAUDET, A. & DESCARRIES, L. (1978). The monoamine innervation of rat cerebral cortex: synaptic and nonsynaptic axon terminals. *Neuroscience* **3**, 851-60.
- BEAUDET, A. & DESCARRIES, L. (1984) Fine structure of monoamine axon terminals in cerebral cortex. In *Monoamine Innervation of Cerebral Cortex* (edited by DESCARRIES, L., READER, T. R. & JASPER, H. H.), pp. 77-93. New York: Alan R. Liss.
- BERGER, B. & VERNEY, C. (1984) Development of the catecholamine innervation in rat neocortex: morphological features. In *Monoamine Innervation of Cerebral Cortex* (edited by DESCARRIES, L., READER, T. R. & JASPER, H. H.), pp. 95-121. New York: Alan R. Liss.
- BLUE, M. & MOLLIVER, M. E. (1987) 6-Hydroxydopamine induces serotonergic axon sprouting in cerebral cortex of newborn rat. *Developmental Brain Research* **32**, 255-70.
- BROWN, R. M., CRANE, A. M. & GOLDMAN, P. S. (1979) Regional distribution of monoamines in the cerebral cortex and subcortical structures of the rhesus monkey: Concentrations and in vivo synthesis rates. *Brain Research* **168**, 133-50.
- CARLSSON, A., FALCK, B. & HILLARP, N.-A. (1962) Cellular localization of brain monoamines. *Acta physiologica scandinavica* **56**, Suppl. 196, 1-28.
- CHAN-PALAY, V. (1975) Fine structure of labelled axons in the cerebellar cortex and nuclei of rodents and primates after intraventricular infusions with tritiated serotonin. *Anatomy and Embryology* **148**, 235-65.
- CHAN-PALAY, V. (1976) Serotonin axons in the supra- and subependymal plexuses and in the leptomeninges; their roles in local alterations of cerebrospinal fluid and vasomotor activity. *Brain Research* **102**, 103-30.
- CHAN-PALAY, V. (1978a) The paratrigeminal nucleus. II. Identification and interrelations of catecholamine axons, indoleamine axons, and Substance P immunoreactive cells in the neuropil. *Journal of Neurocytology* **7**, 419-42.
- CHAN-PALAY, V. (1978b) Morphological correlates for transmitter synthesis, transport, release, uptake and catabolism: a study of serotonin neurons in the nucleus paragigantocellularis lateralis. In *Amino Acids as Chemical Transmitters* (edited by FONNUM, F.), pp. 1-30. New York: Plenum Press.
- CUELLO, A. C. & IVERSEN, L. L. (1978) Interactions of dopamine with other neurotransmitters in the rat substantia nigra: a possible functional role of dendritic dopamine. In *Interactions Between Putative Neurotransmitters in the Brain* (edited by GARATTINI, S., PUJOL, J. F. & SAMANIN, R.), pp. 127-49. New York: Raven Press.
- DESCARRIES, L., BEAUDET, A. & WATKINS, K. C. (1975) Serotonin nerve terminals in adult rat neocortex. *Brain Research* **100**, 563-88.
- DESCARRIES, L., WATKINS, K. C. & LAPIERRE, Y. (1977) Noradrenergic axon terminals in the cerebral cortex of rat: III. Topometric ultrastructural analysis. *Brain Research* **133**, 197-222.
- FUXE, K. (1965) Evidence for the existence of monoamine neurons in the central nervous system. IV. The distribution of monoamine terminals in the central nervous system. *Acta physiologica scandinavica* **64**, Suppl. 247, 39-85.
- FUXE, K., HAMBERGER, B. & HÖKFELT, T. (1968a) Distribution of noradrenaline nerve terminals in cortical areas of the rat. *Brain Research* **8**, 125-31.
- FUXE, K., HÖKFELT, T. & UNGERSTEDT, U. (1968b) Localization of indolealkylamines in CNS. In *Advances in Pharmacology*, part A, Vol. 6 (edited by GARATTINI, S. & SHORE, P. A.), pp. 235-51. New York: Academic Press.
- GAUDIN-CHAZAL, G., DASZUTA, A., FAUDON, M. & TERNAUX, J. P. (1979) 5-HT concentration in cat's brain. *Brain Research* **160**, 281-93.
- GEFFARD, M., DULLUC, J. & ROCK, A.-M. (1985) Antisera against the indolealkylamines: tryptophan, 5-hydroxytryptophan, 5-hydroxytryptamine, 5-methoxytryptophan, and 5-methoxytryptamine tested by an enzyme-linked immunosorbent assay method. *Journal of Neurochemistry* **44**, 1221-8.
- GLOWINSKI, J. (1984) Functional properties: introductory remarks. In *Monoamine Innervation of Cerebral Cortex* (edited by DESCARRIES, L., READER, T. R. & JASPER, H. H.), pp. 229-31. New York: Alan R. Liss.
- GOLDMAN-RAKIC, P. S. & BROWN, R. M. (1982) Postnatal development of monoamine content and synthesis in the cerebral cortex of rhesus monkeys. *Developmental Brain Research* **4**, 339-49.
- GROVES, P. M. (1980) Synaptic endings and their postsynaptic targets in neostriatum: synaptic specializations revealed from analysis of serial sections. *Proceedings of the National Academy of Sciences USA* **77**, 6926-9.
- GROVES, P. M. & WILSON, C. J. (1980) Monoaminergic presynaptic axons and dendrites in rat locus coeruleus seen in reconstructions of serial sections. *Journal of Comparative Neurology* **193**, 853-62.
- HÖKFELT, T. (1968) *In vitro* studies on central and peripheral monoamine neurons at the ultrastructural level. *Zeitschrift für Zellforschung und mikroskopische Anatomie* **91**, 1-74.
- ITAKURA, T., KASAMATSU, T. & PETTIGREW, J. D. (1981) Norepinephrine-containing terminals in kitten visual cortex: laminar distribution and ultrastructure. *Neuroscience* **6**, 159-75.
- KODA, L. Y. & BLOOM, F. E. (1977) A light and electron microscopic study of noradrenergic terminals in the rat dentate gyrus. *Brain Research* **120**, 327-35.
- KÖHLER, C., CHAN-PALAY, V. & STEINBUSCH, H. (1981) The distribution and orientation of serotonin fibers in the entorhinal and other retrohippocampal areas. An

- immunohistochemical study with anti-serotonin antibodies in the rat's brain. *Anatomy and Embryology* **161**, 237–64.
- KOSOFKY, B. E. & MOLLIVER, M. E. (1987) The serotonergic innervation of cerebral cortex: different classes of axon terminals arise from dorsal and median raphe nuclei. *Synapse* **1**, 153–68.
- KOSOFKY, B. E., MOLLIVER, M. E., MORRISON, J. H. & FOOTE, S. L. (1984) The serotonin and norepinephrine innervation of primary visual cortex in cynomolgus monkey (*Macaca fascicularis*). *Journal of Comparative Neurology* **230**, 168–78.
- KRIEG, W. G. S. (1946) Connections of the cerebral cortex. I. The albino rat. A. Topography of the cortical areas. *Journal of Comparative Neurology* **84**, 221–76.
- LIDOV, H. G. W., GRZANNA, R. & MOLLIVER, M. E. (1980) The serotonin innervation of the cerebral cortex in the rat – an immunohistochemical analysis. *Neuroscience* **5**, 207–27.
- LINDVALL, O. & BJÖRKLUND, A. (1978) Organization of catecholamine neurons in the rat central nervous system. In *Handbook of Psychopharmacology*, Vol. 9 (edited by IVERSEN, L. L., IVERSEN, S. D. & SNYDER, S. H.), pp. 139–231. New York: Plenum Press.
- LINDVALL, O. & BJÖRKLUND, A. (1984) General organization of cortical monoamine systems. In *Monoamine Innervation of Cerebral Cortex* (edited by DESCARRIES, L., READER, T. R. & JASPER, H. H.), pp. 9–40. New York: Alan R. Liss.
- MCRAE-DEGUEURCE, A. & GEFFARD, M. (1986) One perfusion mixture for immunocytochemical detection of noradrenaline, dopamine, serotonin and acetylcholine in the same rat brain. *Brain Research* **376**, 217–19.
- MOLLIVER, M. E., GRZANNA, R., LIDOV, H. G. W., MORRISON, J. H. & OLSCHOWKA, J. A. (1982) Monoamine systems in the cerebral cortex. In *Cytochemical Methods in Neuroanatomy* (edited by CHAN-PALAY, V. & PALAY, S. L.), pp. 255–77. New York: Alan R. Liss.
- MOORE, R. Y. & BLOOM, F. E. (1978) Central catecholamine neuron systems: anatomy and physiology of the dopamine systems. *Annual Review of Neurosciences* **1**, 129–69.
- MOORE, R. Y. & BLOOM, F. E. (1979) Central catecholamine neuron systems: anatomy and physiology of the norepinephrine and epinephrine systems. *Annual Review of Neurosciences* **2**, 113–68.
- MORRISON, J. H. & FOOTE, S. L. (1986) Noradrenergic and serotonergic innervation of cortical, thalamic, and tectal visual structures in old and new world monkeys. *Journal of Comparative Neurology* **243**, 117–38.
- MORRISON, J. H., FOOTE, S. L., MOLLIVER, M. E., BLOOM, F. E. & LIDOV, H. G. W. (1982) Noradrenergic and serotonergic fibers innervate complementary layers in monkey primary visual cortex: an immunohistochemical study. *Proceedings of the National Academy of Sciences USA* **79**, 2401–5.
- MORUZZI, G. & MAGOUN, H. W. (1949) Brain stem reticular formation and activation of the EEG. *Electroencephalography and Clinical Neurophysiology* **1**, 455–73.
- OLSCHOWKA, J. A., MOLLIVER, M. E., GRZANNA, R., RICE, F. L. & COYLE, J. T. (1981) Ultrastructural demonstration of noradrenergic synapses in the rat central nervous system by dopamine- β -hydroxylase immunocytochemistry. *Journal of Histochemistry and Cytochemistry* **29**, 271–80.
- PAPADOPOULOS, G. C., PARNAVELAS, J. G. & BUIJS, R. M. (1988) Light and electron microscopic immunocytochemical analysis of the noradrenaline innervation of the rat visual cortex. *Neuroscience* (in press).
- PARNAVELAS, J. G., MOISES, H. C. & SPECIALE, S. G. (1985) The monoaminergic innervation of the rat visual cortex. *Proceedings of the Royal Society of London, Series B* **223**, 319–29.
- PICKEL, V. M., JOH, T. H., CHAN, J. & BEAUDET, A. (1984) Serotonergic terminals: ultrastructure and synaptic interaction with catecholamine-containing neurons in the medial nuclei of the solitary tracts. *Journal of Comparative Neurology* **225**, 291–301.
- READER, T. A. (1981) Distribution of catecholamines and serotonin in the rat cerebral cortex: absolute levels and relative proportions. *Journal of Neural Transmission* **50**, 13–27.
- READER, T. A. & JASPER, H. H. (1984) Interactions between monoamines and other transmitters in cerebral cortex. In *Monoamine Innervation of Cerebral Cortex* (edited by DESCARRIES, L., READER, T. R. & JASPER, H. H.), pp. 195–225. New York: Alan R. Liss.
- READER, T. A., MAASE, P. & DE CHAMPLAIN, J. (1979) The intracortical distribution of norepinephrine, dopamine and serotonin in the cerebral cortex of the cat. *Brain Research* **177**, 499–513.
- RICHARDS, J. G., LOPEZ, H. P. & TRANZER, J. P. (1973) Indolealkylamine nerve terminals in cerebral ventricles: identifications by electron microscopy and fluorescence histochemistry. *Brain Research* **57**, 277–88.
- RUDA, M. A., COFFIELD, J. & STEINBUSH, H. W. M. (1982) Immunocytochemical analysis of serotonergic axons in laminae I and II of the lumbar spinal cord of the cat. *Journal of Neuroscience* **2**, 1660–71.
- SANO, Y., TAKEUCHI, Y., KIMURA, H., GOTO, M., KAWATA, M., KOJIMA, M., MATSUURA, T., UEDA, S. & YAMADA, H. (1982) Immunohistochemical studies on the processes of serotonin neurons and their ramification in the central nervous system – with regard to the possibility of the existence of Golgi's rete nervosa diffusa. *Archivum Histologicum Japonicum* **45**, 305–16.
- SOGHOMONIAN, J.-J., DESCARRIES, L. & LANOIR, J. (1986) Monoamine innervation of the oculomotor nucleus in the rat. A radioautographic study. *Neuroscience* **17**, 1147–57.
- STEINBUSCH, H. W. M. (1981) Distribution of serotonin-immunoreactivity in the central nervous system of the rat – cell bodies and terminals. *Neuroscience* **6**, 557–618.
- STERNBERGER, L. A. (1979) *Immunocytochemistry*. New York: John Wiley.
- TAKEUCHI, Y. & SANO, Y. (1983) Immunohistochemical demonstration of serotonin nerve fibers in the neocortex of the monkey (*Macaca fuscata*). *Anatomy and Embryology* **166**, 155–68.
- TAKEUCHI, Y. & SANO, Y. (1984) Serotonin nerve fibers in the primary visual cortex of the monkey. Quantitative and immunoelectronmicroscopical analysis. *Anatomy and Embryology* **169**, 1–8.
- TENNYSON, V. M., HEIKKILA, R., MYTILINEOU, C.,

- COTE, L. & COHEN, G. (1974) 5-Hydroxydopamine 'tagged' neuronal boutons in rabbit neostriatum: inter-relationship between vesicles and axonal membrane. *Brain Research* **82**, 341-8.
- TÖRK, I. & MULLIGAN, K. A. (1984) Dense serotonergic innervation of select cortical neurons in cat neocortex. *Society for Neuroscience Abstracts* **10**, 63.
- UNGERSTEDT, U. (1971) Stereotaxic mapping of the monoamine pathways in the rat brain. *Acta physiologica scandinavica*, Suppl. **367**, 1-48.
- WILSON, C. J., GROVES, P. M. & FIFKOVA, E. (1977) Monoaminergic synapses, including dendro-dendritic synapses in the rat substantia nigra. *Experimental Brain Research* **30**, 161-74.