SIMULTANEOUS VISUALIZATION OF AORTIC AND [³H]5-HYDROXYTRYPTAMINE-ACCUMULATING CELL BODIES IN THE NODOSE GANGLION OF THE CAT

By G. GAUDIN-CHAZAL, P. PORTALIER, J. J. PUIZILLOUT
AND D. VIGIER

From the Unité de Recherches Neurobiologiques, INSERM-U 6 and CNRS-GR.45, 280 Boulevard Sainte-Marguerite, 13009 Marseille, France

SUMMARY

1. Single- and double-tracer experiments were performed in cats to investigate the relationship between the aortic cells and the cell bodies accumulating 5-hydroxytryptamine (5-HT) in the nodose ganglion. In one series of experiments, horseradish peroxidase (HRP) was applied to the central end of the aortic nerve, anterogradely transported and accumulated in ganglionar perikarya. The distribution of HRP-positive neurones was reconstructed in serial sections through the nodose ganglion. In a second series of experiments, the distribution of [³H]5-HT-accumulating cell bodies was assessed following incubation of the nodose ganglion in [³H]5-HT. The third series of experiments combined the treatments of the preceding ones: anterograde transport of HRP in the aortic nerve followed by incubation of the nodose ganglion in [³H]5-HT.

2. The results from these experiments provide more information with regard to (i) the topographical relationship between the aortic and [³H]5-HT-accumulating cell bodies in the same ganglion and (ii) the distribution and number of double-labelled neurones, giving further indications about histochemical components of the aortic nerve.

3. The HRP experiments demonstrated that HRP-positive cells show a preferential pattern of topographical organization. They were mostly located in the medial border of the ganglion where the laryngeal and aortic nerves enter. On the other hand, [³H]5-HT-accumulating neurones were scattered throughout the ganglion.

4. In double-tracer experiments, three populations of labelled cell bodies were distinguished in the same nodose ganglion: (1) single HRP-cells; (2) single [³H]5-HT-accumulating cells and (3) double-labelled cells. The distribution of the latter population exhibited no preferential localization. Quantitative estimates indicated that double-labelled neurones constituted 65–85 % of the population of HRP-positive cell bodies.

5. These results show that most aortic neurones are able to take up exogenous serotonin and may be serotonergic neurones. They suggest that serotonin may be involved in physiological effects mediated via the aortic nerves.

<center>INTRODUCTION</center>

The nodose ganglion of the vagus nerve contains the cell bodies of afferent fibres innervating respiratory, cardiovascular and gastrointestinal systems. Approximately 80 % of the 30,000–50,000 fibres in the cervical vagus are sensory and 70 % of these are non-myelinated afferent fibres (Foley & Dubois, 1937; Jones, 1937; Mohiuddin, 1953; Agostoni, Chinnock, Daly De Burgh & Murray, 1957; Mei, Condamin & Boyer, 1980). Most of these fibres terminate in the caudal brain stem, in the nucleus of the solitary tract (Ramón y Cajal, 1909; Foley & Dubois, 1934; Ingram & Dawkins, 1945; Kerr, 1962; Cottle, 1964; Kalia & Mesulam, 1980).

The putative neurotransmitters used by these vagal afferents, and their functional significance, are unknown at present. Nevertheless, some data lead us to suspect that serotonin (5-hydroxytryptamine) plays a crucial role. The caudal part of the nucleus of the solitary tract has been observed to be richly supplied with 5-HT terminals (Fuxe, 1965; Fuxe, Hökfelt & Nilsson, 1965; Palkovits, Brownstein & Saavedra, 1974; Chan-Palay, 1977; Steinbusch, 1981). These 5-HT terminals may be connected with vagal afferent fibres. The first step was therefore to check the presence of 5-HT in the nodose ganglia by radioenzymatic assay (Gaudin-Chazal, Daszuta, Segu, Ternaux & Puizillout, 1978), and to visualize it by autoradiography (Gaudin-Chazal et al. 1978; Gaudin-Chazal, Segu, Seyfritz & Puizillout, 1981; Gaudin-Chazal, Seyfritz, Araneda, Vigier & Puizillout, 1982), histofluorescence (Cadilhac, Daszuta, Gaudin-Chazal, Portalier, Puizillout, Segu & Vigier, 1979) and immunohistochemistry (Puizillout, Gaudin-Chazal & Portalier, 1982).

First, certain vagal afferents belonging to the aortic nerve are of particular interest. In addition to the main role played in cardiovascular control, we know that some baroreceptive fibres of this nerve may trigger sleep mechanisms (Dell, 1973; Puizillout & Foutz, 1977; Puizillout, 1980). We demonstrated recently that 5-HT injected into the circulation of the nodose ganglion in the cat has a hypnogenic effect (Sayadi, Gaudin-Chazal, Seyfritz & Puizillout, 1980). Moreover, the injection of 5-HT at the level of vagal afferent terminals can induce cortical synchronization (Key & Mehta, 1977). It would therefore appear that baroreceptive fibres, which have a hypnogenic role, may be serotonergic. To elucidate this issue, it is necessary to demonstrate that aortic cells are serotonergic. Consequently, we began by determining the exact location of the aortic cells within the nodose ganglia using the anterograde transport of horseradish peroxidase (HRP) described in the preliminary studies by Portalier & Vigier (1979) and Donoghue, Garcia, Jordan & Spyer (1982). Then, by combining a double-labelling with HRP and [³H]5-HT tracers, we compared this HRP labelling with that obtained by autoradiography following incubation of the nodose ganglion in [³H]5-HT.

<center>METHODS</center>

Experiments were performed on cats of both sexes. Animals were divided into three groups according to single- or double-tracer experiments.

Single-tracer studies

Group I: HRP anterograde transport. Twenty animals were used for single anterograde HRP transport in the aortic nerve. Under Nembutal anaesthesia (40 mg/kg, I.P.), the left or right aortic nerve was identified anatomically at its junction with the superior laryngeal nerve and 10 mm was dissected clear of the main trunk of the vagus nerve and the caudal part was cut. The central end of the cut nerve was placed in small watertight container filled with HRP solution (Sigma, 50 % in distilled water), and sutured to the muscles. After survival times of 23–72 hr the cats were deeply re-anaesthetized (Nembutal, 50 mg/kg, I.P.), and intracardially perfused with a solution containing 1 % paraformaldehyde and 1 % glutaraldehyde in 0·12 M-phosphate buffer pH 7·2–7·4. The nodose ganglion was dissected, post-fixed overnight in the fixative solution, and immersed in a solution of 30 % sucrose in phosphate buffer (0·1 M) until it sank. 40 μm thick serial sections were cut in a parasagittal plane on a vibratome. All the sections were incubated with P-phenylenediamine dihydrochloride and pyrocatechol (PPD–PC; Hanker, Yates, Metz & Rustioni, 1977) to react for HRP chemistry. Then the sections were counterstained with Cresyl Violet and mounted in DPX.

Group II: [³H]5-HT uptake. Six animals were used for [³H]5-HT uptake by nodose ganglion elements. Each animal was injected with pargyline (70 mg/kg, I.P.), a monoaminoxidase inhibitor, to prevent the metabolic breakdown of the 5-HT, and deeply anaesthetized with Nembutal (50 mg/kg, I.P.) 30 min before decapitation. The nodose ganglia (right and left) were rapidly dissected, removed and incubated for 30 min at 37 °C in oxygenated Tyrode solution containing 10^{-6} M-[³H]5-HT, (serotonin creatinine sulphate, generally labelled, specific activity 9–14 Ci/m-mole, Radiochemical Centre, Amersham) added to 10^{-5} M-noradrenaline hydrochloride (Sigma) to prevent uptake of 5-HT into other monoamine neurones. After a rinsing in Tyrode solution, the incubated ganglia were fixed with 4 % paraformaldehyde in 0·1 M-phosphate buffer, pH 7·4. Then the ganglia were dehydrated, embedded in paraffin and serially sectioned (6 μm thick). These sections deposited on glass slides were deparaffinized and then coated by dipping in Ilford K5 emulsion. The autoradiographs were developed 40 days later with Kodak D19, stained with Cresyl Violet and mounted in DPX.

Double-tracer studies

Group III: HRP transport combined with [³H]5-HT uptake. Five animals were used for this study. The first step was to place the left aortic nerve in a solution of HRP as described above in group I. Next, after a survival time of 72 hr, the animals received an injection of pargyline (70 mg/kg, I.P.), and were deeply anaesthetized (Nembutal, 50 mg/kg, I.P.) 30 min before decapitation. The right ganglion was taken as control. Both ganglia were rapidly dissected and immediately incubated for 30 min in a Tyrode solution containing [³H]5-HT (10^{-6} M) and noradrenaline (10^{-5} M), as described in group II.

Then the left and right ganglia were processed for both HRP histochemistry and [³H]5-HT autoradiography. The ganglia were fixed with 4 % paraformaldehyde in 0·1 M-phosphate buffer pH 7·4 and immersed in a solution of 30 % sucrose, as in group I. Serial sections 100 μm thick were cut with a vibratome. The sections were reacted first of all for HRP histochemistry according to the method of Hanker *et al.* (1977), and were subsequently processed for autoradiography. Then, the thick HRP-reactive sections were embedded in paraffin and serially sectioned (10 μm thick). Some cells may possibly contain both tracers: the brown granules of HRP reaction and the black reduced silver grains. So, we decided to number the sections and divide them into two groups, giving each group a different treatment, in order to see the reaction more clearly. The odd numbers were only deparaffinized and counterstained with Cresyl Violet and the even numbers were processed for autoradiography as described above in group II.

Distribution of labelled neurones

Computer graphic reconstructions of ganglia were used to observe the location of labelled cell bodies in a three-dimensional space. First, sections of the ganglia and labelled cell bodies in them were mapped precisely by means of an $X-Y$ plotter connected to the stage of an orthoplan microscope. When HRP alone was used, all the sections were mapped. When [³H]5-HT alone was used, sections were mapped at intervals of 42 μm. When both tracers were used, all the sections were mapped. Secondly, the drawings were entered into a Hewlett-Packard computer (model 9830 A) by means of a digitizer tablet. To obtain the three-dimensional computer reconstruction,

11-2

the data were then transferred to paper by an $X-Y$ plotter connected to, and controlled by the computer. Finally, the ganglion images were rotated through three-dimensional space to obtain the best visualization of the spatial relationship of labelled neurones.

<div align="center">RESULTS</div>

Single-tracer studies

HRP anterograde transport. After *in situ* incubation of the aortic nerve in a solution of HRP, HRP-positive cell bodies containing brown granules of reaction product in their cytoplasm were observed in the ipsilateral nodose ganglion. The satellite cells and cell bodies in the contralateral nodose ganglion were free of reaction product. The labelled cell bodies had the round or oval shape typical of the unipolar T type (Pl. 1 A). Their diameter varied between 30 and 50 μm. The three-dimensional reconstruction of a left nodose ganglion (Fig. 1 A) showed that the labelled cells were most numerous in the medial border of the ganglion. In this region they were concentrated near the entry of the laryngeal and aortic nerves. HRP-positive perikarya were also found in the rostral part of the dorsal portion of the nodose ganglion. Some other reactive cells were also distributed throughout the ganglion. The rostral and caudal extremities, where the fibres gather to constitute the two branches of the vagal nerve, were devoid of reactive cell bodies. The distribution of HRP-labelled cell bodies was similar in all cases and was approximately the same in both right and left ganglia. Great variations in the number of HRP labelled cells were observed ranging from one to several hundred.

Uptake of [^{3}H]5-*HT.* Following *in vitro* incubation of the nodose ganglion in [^{3}H]5-HT (10^{-6} M) with the addition of noradrenaline (10^{-5} M), a specific autoradiographic reaction was seen over some perikarya (Pl. 1 B), as previously described (Gaudin-Chazal *et al.* 1981). They were round or oval and their mean diameter was 30–40 μm. The three-dimensional reconstruction of a left nodose ganglion (Fig. 1 B) showed that the [^{3}H]5-HT-accumulating cell bodies were distributed throughout the ganglion, although they were more densely grouped in the rostral part. Labelled cell bodies were also found near the area where the laryngeal and aortic nerves enter the nodose ganglion, possibly indicating a double–labelling of some aortic cells.

Double-tracer studies

The HRP transport in the aortic nerve, combined with the incubation of the nodose ganglion in [^{3}H]5-HT, permitted a direct visualization of the topographic relationship between the aortic and [^{3}H]5-HT-accumulating cell bodies, and the identification of cells containing both HRP and [^{3}H]5-HT tracers. It was obvious from these experiments that four populations of cell bodies were distinguished in the nodose ganglion (Pl. 2): (1) unlabelled cells; (2) single HRP-positive cell bodies containing brown granules of reaction product (Pl. 2 A); (3) single [^{3}H]5-HT-accumulating cells labelled with black silver grains (Pl. 2 B); (4) double-reactive cell bodies labelled with both HRP and [^{3}H]5-HT tracers (Pl. 2 C).

Fig. 2 shows an example of the distribution of the three groups of labelled cells in a three-dimensional image of a single ganglion. The labelled cell bodies were scattered throughout the ganglion, but as first described in the single HRP
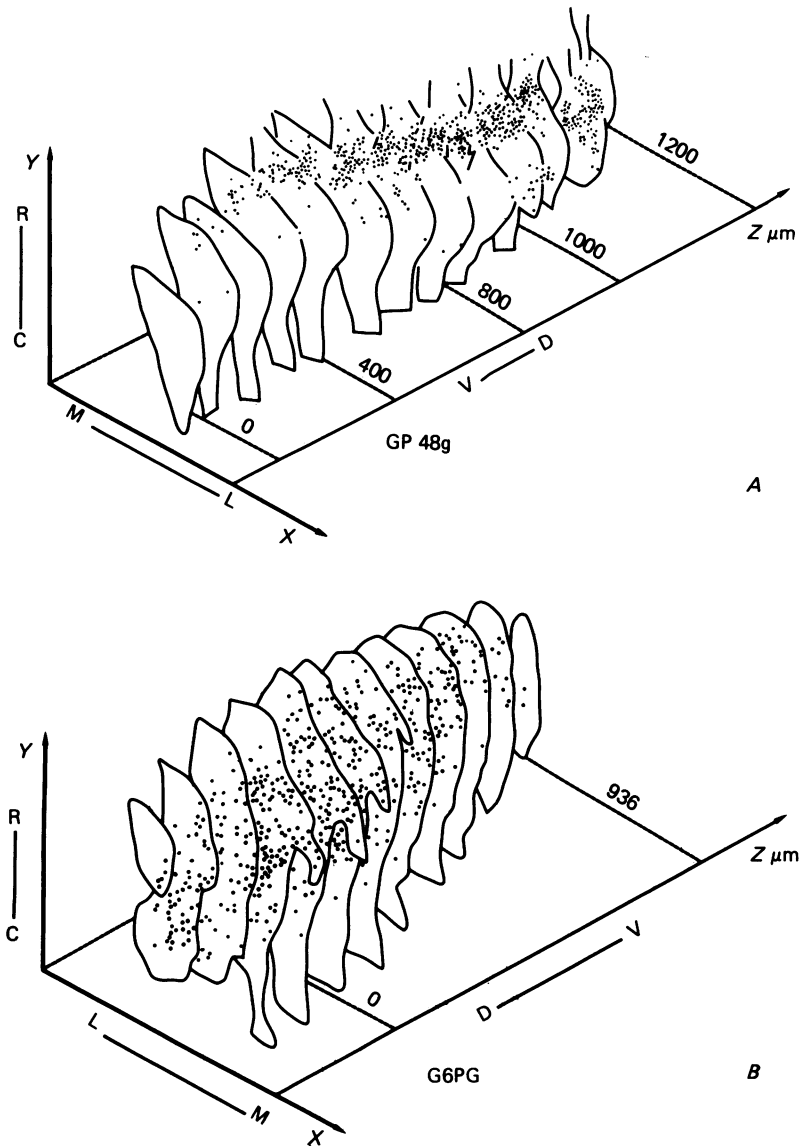
Fig. 1. Three-dimensional drawings of two left nodose ganglia. *A* is a ventral (V) to dorsal (D) view with the distribution of HRP-reactive cell bodies. These labelled cells were located in the medial border of the ganglion. *B* is a dorsal to ventral view showing the location of [³H]5-HT-accumulating cell bodies. These labelled cells were scattered throughout the ganglion. The values representing the cartesian co-ordinates system through which the computer images were rotated were: $X = -45$; $Y = -35.2$. M: medial; L: lateral; R: rostral; C: caudal.

experiments, the majority of the aortic perikarya were found mainly proximal to the aortic nerve entry. For a better understanding of the distribution of labelled cell bodies the reconstruction was subdivided into six portions. The histograms show the distribution of the three groups of labelled cell bodies in each portion. In portions I and VI no labelled cell bodies were visualized. The portions II and III
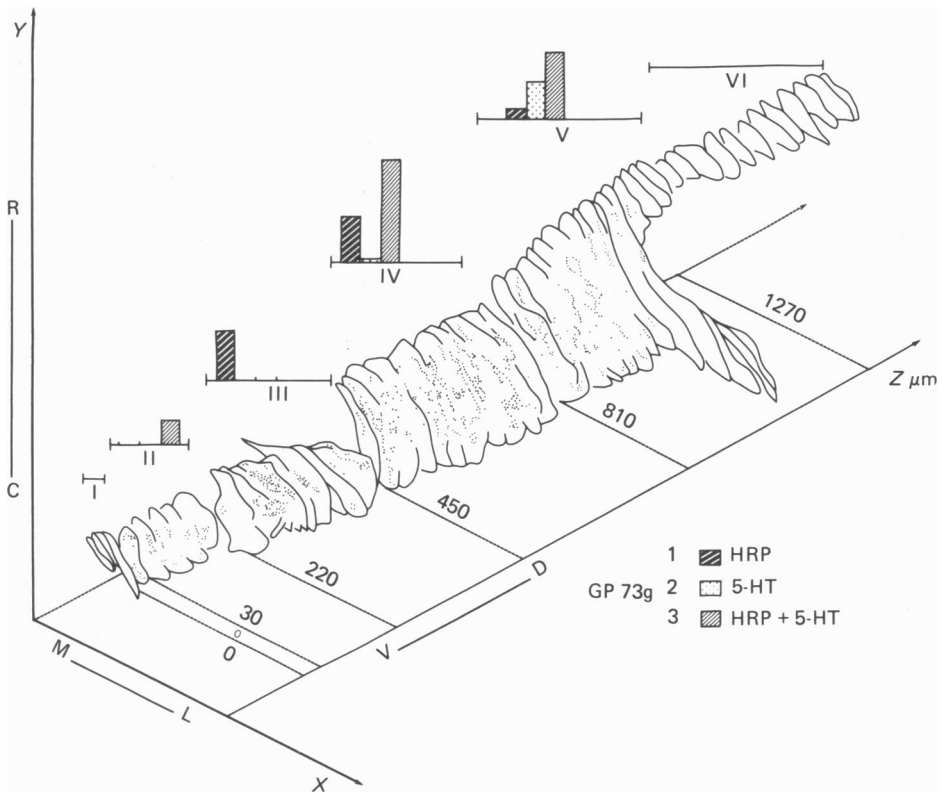

Fig. 2. A three-dimensional reconstruction of a left nodose ganglion showing the location of HRP, [³H]5-HT and double-labelled cell bodies. The values representing the cartesian co-ordinates system through which the computer images were rotated were: $X = -45$; $Y = -35.2$. The reconstruction was divided into six portions, numbered I–VI, and in each portion a histogram shows the distribution of the three populations of labelled cell bodies. The number of reactive cells in each portion was summarized in Table 1. D: dorsal; V: ventral; R: rostral; C: caudal; M: medial; L: lateral.

contained only one population of labelled cells, whereas in the portions IV and V all three groups of labelled cell bodies were present. The precise number of labelled cell bodies in each portion is given in Table 1. This quantitative study indicates that double-labelled perikarya are predominant. In the total population of HRP-positive cells counted, 65–80 % were double HRP–[³H]5-HT-accumulating cell bodies. Few [³H]5-HT-accumulating cells (11–19 %) presented a single autoradiographic reaction.

<center>DISCUSSION</center>

The present results collected from a series of single- and double-tracer studies represent the first step in a direct elucidation of neurotransmitters in the aortic nerve. Each single-tracer experiment (HRP transport and [³H]5-HT uptake) allowed us to determine the exact location, in the nodose ganglia of the cat, of two populations of neurones which were identified in different ways: the aortic cell bodies were characterized anatomically whereas the [³H]5-HT-accumulating cell bodies were identified biochemically. The double-tracer experiments provide a direct visualization of the topographic relationships between these two populations of cell bodies.

TABLE 1. Distribution of HRP, [³H]5-HT or double-labelled cell bodies in the six portions of the left nodose ganglion reconstructed in Fig. 2

Portion	HRP-positive cell bodies	[³H]5-HT-accumulating cell bodies	Double HRP–[³H]5-HT-accumulating cell bodies
I	0	0	0
II	1	0	134
III	249	0	0
IV	224	44	513
V	45	188	317
VI	0	0	0
Total	519	232	964

The majority of the labelled cell bodies were concentrated in portions IV and V. No labelled cells were found in portions I and VI. In the total population of HRP-positive cell bodies (1483), 65 % were double HRP–[³H]5-HT-accumulating cell bodies. Only 232 cells (19 %) were single [³H]5-HT-accumulating cell bodies.

The distribution of HRP-labelled cells confirms preliminary results (Portalier & Vigier, 1979) and is consistent with earlier studies on different mammals (Kalia & Welles, 1980; Donoghue *et al.* 1982). In the cat, such a distribution has been shown by Mei (1970) by recording unitary activity of electrophysiologically identified neurones. In the rabbit, an early anatomical study localized the cells of the aortic nerve, using the degeneration method (Molhant, 1913). In this species, recent experiments have reported the distribution of the aortic cells following anterograde transport of HRP, and by electrophysiological approach (Garcia, Jordan & Spyer, 1979; Donoghue *et al.* 1982).

The distribution of [³H]5-HT-accumulating cells was different from that of aortic cell bodies. The former labelled cell bodies were not concentrated in a particular area of the ganglion although some were observed in the region where the majority of aortic cells were to be found. The size of the [³H]5-HT-accumulating cell bodies was comparable to that of the aortic cell bodies. They were significantly smaller than unreactive cells. So there was a reasonable chance that some aortic cell bodies might be [³H]5-HT-accumulating cells too. This was proved by double-labelling experiments. Quantitative estimates carried out in two cases indicated that the double-labelled cells constituted 65–83 % of the population of aortic cell bodies. The total population of aortic cells (single and double-labelled cells) was seen to be higher than that

estimated in single HRP experiments (Portalier & Vigier, 1979), possibly due to the method of counting. It was, however, lower than the number of aortic cells reported by Donoghue *et al.* (1982). This discrepancy may be explained by the superior sensitivity of the tetramethylbenzidine (TMB) technique as demonstrated by the latter. It should also be noted that all the [^{3}H]5-HT-accumulating cells did not belong to the aortic population.

The specificity of [^{3}H]5-HT uptake by some ganglion cell bodies being well demonstrated (Gaudin-Chazal *et al.* 1981; Segu, Gaudin-Chazal, Seyfritz & Puizillout, 1981), the present studies give, for the first time, a clear indication of the existence of [^{3}H]5-HT-accumulating aortic cells. The presence of endogenous 5-HT has been recently described in the nodose ganglion of the cat (Puizillout *et al.* 1982). Further experiments are necessary to ascertain whether these cells and those able to take up exogenous tracer are the same ones. However we know that in the central nervous system, monoaminergic neurones are reported to take up and retain their own transmitter (Descarries, Beaudet & Watkins, 1975; Calas & Segu, 1976; Bosler & Calas, 1982). If peripheral monoaminergic neurones have the same property, these 5-HT-accumulating neurones may be regarded as true serotonergic neurones. Then, the majority of aortic cell bodies able to take up and retain [^{3}H]5-HT may also be serotonergic cell bodies.

At the moment the physiological importance of serotonin in this population of neurones is not wholly clear. Apart from its role in cardiovascular regulation (Wolf, Kuhn & Lovenberg, 1981), the aortic pathway may be involved in sleep mechanisms, as demonstrated by electrophysiological studies. It is possible to activate the large baroreceptive fibres of the aortic nerve alone and selectively, and so induce a complete and caricatural sleep cycle characterized by a progression of all stages of sleep (Dell & Padel, 1965; Dell, 1973; Puizillout & Foutz, 1977; Puizillout, 1980). It could be suggested that the serotonergic aortic neurones are the population of neurones involved in the vago-aortic stimulation. In addition, the findings of our group (Sayadi *et al.* 1980), who reported the hypnogenic effect of serotonin following its peripheral injection in the nodose ganglion circulation, provide further support for this hypothesis. Further experiments are necessary to prove the exact role of these serotonergic aortic neurones in sleep mechanisms.

REFERENCES

AGOSTONI, E., CHINNOCK, J. E., DALY DE BURGH, M. & MURRAY, J. G. (1957). Functional and histological studies of the vagus nerve and its branches to the heart, lungs and abdominal viscera in the cat. *J. Physiol.* **135**, 182–205.

BOSLER, O. & CALAS, A. (1982). Radioautographic investigation of monoaminergic neurons. An evaluation. *Brain Res. Bull.* (in the Press).

CADILHAC, J., DASZUTA, A., GAUDIN-CHAZAL, G., PORTALIER, P., PUIZILLOUT, J. J., SEGU, L. & VIGIER, D. (1979). The nodose ganglia of the cat. I. Localization and ultrastructural studies of aortic cell bodies. II. Identification of serotonergic cells. *Neurosci. Lett.* **3**, Suppl. S 231.

CALAS, A. & SEGU, L. (1976). Radioautographic localization and identification of monoaminergic neurones in the central nervous system. *J. Microsc. & Biol. cell.* **27**, 249–252.

CHAN-PALAY, V. (1977). Indoleamine neurons and their process in the normal rat brain and in

chronic diet-induced thiamine deficiency demonstrated by uptake of [³H]serotonin. *J. comp. Neurol.* **176**, 467–494.

COTTLE, M. K. (1964). Degeneration studies of primary afferents of IX-th and X-th cranial nerves in the cat. *J. comp. Neurol.* **122**, 329–343.

DELL, P. (1973). Afférences baroceptives, phases de synchronisation corticale et sommeil. *Archs ital. Biol.* **111**, 553–563.

DELL, P. & PADEL, Y. (1965). Rapid falling asleep provoked by selective stimulation of vagal afferents in the cat. *Electroenceph. clin. Neurophysiol.* **18**, 725.

DESCARRIES, L., BEAUDET, A. & WATKINS, K. C. (1975). Serotonin nerve terminals in adult rat neocortex. *Brain Res.* **100**, 563–588.

DONOGHUE, S., GARCIA, M., JORDAN, D. & SPYER, K. M. (1982). Identification and brain stem projections of aortic baroreceptor afferents neurones in nodose ganglia of cats and rabbits. *J. Physiol.* **322**, 337–352.

FOLEY, J. O. & DUBOIS, F. S. (1934). An experimental study of the rootlets of the vagus nerve in the cat. *J. comp. Neurol.* **60**, 137–159.

FOLEY, J. O. & DUBOIS, F. S. (1937). Quantitative studies of the vagus nerve in the cat. The ratio of sensory to motor fibres. *J. comp. Neurol.* **67**, 49–67.

FUXE, K. (1965). Evidence for the existence of monoamine neurons in the central nervous system. IV. Distribution of monoamine nerve terminals in the central nervous system. *Acta physiol. scand.* **64**, suppl. 247, 39–85.

FUXE, K., HOKFELT, T. & NILSSON, O. (1965). A fluorescence and electron-microscopic study on certain brain regions rich in monoamine terminals. *Am. J. Anat.* **117**, 33–46.

GARCIA, M., JORDAN, D. & SPYER, K. M. (1979). Identification of aortic nerve cell bodies in the nodose ganglion of the rabbit and their central projections. *J. Physiol.* **290**, 23–24*P*.

GAUDIN-CHAZAL, G., DASZUTA, A., SEGU, L., TERNAUX, J. P. & PUIZILLOUT, J. J. (1978). Serotonin containing neurones in the nodose ganglia of the cat. *Waking and Sleeping* **2**, 149–151.

GAUDIN-CHAZAL, G., SEGU, L., SEYFRITZ, N. & PUIZILLOUT, J. J. (1981). Visualization of serotonin neurones in the nodose ganglia of the cat. An autoradiographic study. *Neuroscience* **6**, 1127–1137.

GAUDIN-CHAZAL, G., SEYFRITZ, N., ARANEDA, S., VIGIER, D. & PUIZILLOUT, J. J. (1982). Selective retrograde transport of ³H-serotonin in vagal afferents. *Brain Res. Bull.* **8**, 503–509.

HANKER, J. S., YATES, P. E., METZ, C. B. & RUSTIONI, A. (1977). A new specific, sensitive, and non-carcinogenic reagent for the demonstration of horseradish peroxidase. *Histochem. J.* **9**, 789–792.

INGRAM, W. R. & DAWKINS, E. A. (1945). The intramedullary course of afferent fibres of the vagus nerve in the cat. *J. comp. Neurol.* **82**, 157–168.

JONES, R. L. (1937). Cell fibre ratios in the vagus nerve. *J. comp. Neurol.* **67**, 469–482.

KALIA, M. & MESULAM, M. M. (1980). Brain stem projections of sensory and motor components of the vagus complex in the cat. I. The cervical vagus and nodose ganglion. *J. comp. Neurol.* **193**, 435–465.

KALIA, M. & WELLES, R. V. (1980). Brain stem projections of the aortic nerve in the cat: a study using tetramethyl benzidine as the substrate for horseradish peroxidase. *Brain Res.* **188**, 23–32.

KERR, F. W. L. (1962). Facial, vagal and glossopharyngeal nerves in the cat. Afferent connections. *Archs Neurol., Chicago* **6**, 264–281.

KEY, B. J. & MEHTA, V. H. (1977). Changes in electrocortical activity induced by the perfusion of 5-hydroxytryptamine into the nucleus of the solitary tract. *Neuropharmacology* **16**, 99–106.

MEI, N. (1970). Disposition anatomique et propriétés électrophysiologiques des neurones sensitifs vagaux chez le chat. *Exp. Brain Res.* **11**, 465–479.

MEI, N., CONDAMIN, M. & BOYER, A. (1980). The composition of the vagus nerve. *Cell & Tissue Res.* **209**, 423–431.

MOHIUDDIN, A. (1953). Vagal preganglionic fibres to the alimentary canal, *J. comp. Neurol.* **99**, 289–318.

MOLHANT, M. (1913). Le nerf vague. Etude anatomique et expérimentale. III. Les ganglions périphériques du vague. *Névraxe* **15**, 521–579.

PALKOVITS, M., BROWNSTEIN, M. & SAAVEDRA, J. M. (1974). Serotonin content of the brain stem nuclei in the rat. *Brain Res.* **80**, 237–249.

PORTALIER, P. & VIGIER, D. (1979). Localization of aortic cells in the nodose ganglion by HRP retrograde transport in the cat. *Neurosci. Lett.* **11**, 7–11.

PUIZILLOUT, J. J. (1980). Caricatural sleep reflexely induced in the cat by vegetative afferents. In *Sleep 1978* (4th European Congress on Sleep Research, Tirgu-Mures, 1978), ed. KOELLA, W. P. & LEVIN, P., pp. 4–16. Basel: S. Karger.

PUIZILLOUT, J. J. & FOUTZ, A. S. (1977). Characteristics of the experimental reflex sleep induced by vago-aortic nerve stimulation. *Electroenceph. clin. Neurophysiol.* **42**, 552–563.

PUIZILLOUT, J. J., GAUDIN-CHAZAL, G. & PORTALIER, P. (1982). Neurones with serotonin-like immunoreactivity in the nodose ganglia of the cat. *Neurosci. Lett.* (in the Press).

RAMON Y CAJAL, S. R. (1909). *Histologie du Système Nerveux de l'Homme et des Vertébrés.* vol. I. Paris: Maloine.

SAYADI, A., GAUDIN-CHAZAL, G., SEYFRITZ, N. & PUIZILLOUT, J. J. (1980). Effet hypnogène de la sérotonine par mise en jeu de la voie vago-aortique afférente. *C. r. hebd. Séanc. Acad. Sci. Paris* **291**, 569–572.

SEGU, L., GAUDIN-CHAZAL, G., SEYFRITZ, N. & PUIZILLOUT, J. J. (1981). A serotoninergic system in the nodose ganglia of the cat: radioautographic studies. *J. Physiol., Paris* **77**, 187–189.

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.

WOLF, W. A., KUHN, D. M. & LOVENBERG, W. (1981). Blood pressure responses to local application of serotonergic agents in the nucleus tractus solitarii. *Eur. J. Pharmacol.* **69**, 291–299.

EXPLANATION OF PLATES

PLATE 1

Labelled cell bodies in the nodose ganglion of the cat following single-tracer studies. *A*, an HRP-aortic cell body containing brown granules of reaction product observed following anterograde transport of HRP in the aortic nerve. *B*, three [^{3}H]5-HT-accumulating cell bodies containing silver grains observed after incubation of the ganglion in [^{3}H]5-HT (10^{-6} M) plus an excess of noradrenaline (10^{-5} M). Bar = 30 μm.

PLATE 2

Labelled cell bodies in the same nodose ganglion of the cat following double-tracer study. Three populations of labelled cell bodies were observed: A, single HRP-positive cell bodies; B, single [^{3}H]5-HT-accumulating cells, and C, double-reactive cells labelled with both HRP and [^{3}H]5-HT tracers. Bar = 30 μm.