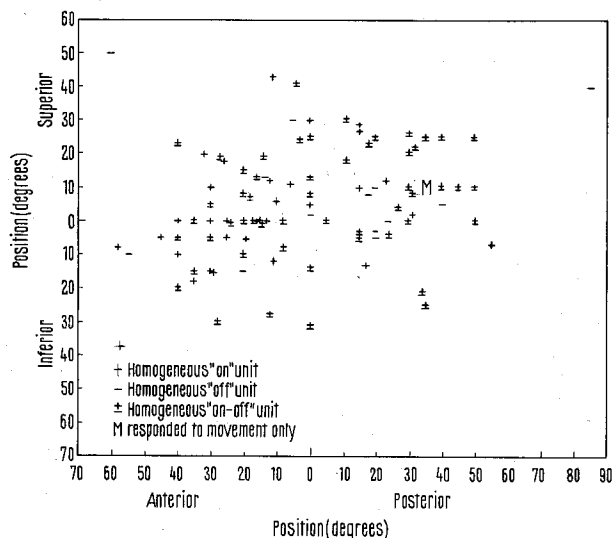


creased the number of spikes resulting per stimulus increased regularly to the field boundary as well, further supporting the absence of mutually inhibitory internal zones.

Table II shows the distributions of cells which could be stimulated by either retina or just one. The relative dominance, i.e. the eye requiring the minimum stimulation to give a response, is also shown. The contralateral predominance among the monocularly driven cells would be expected based on the high percentage of optic nerve fibers (about 80%) crossing at the optic chiasm. The grouping of cells of the same dominance seen successively within a single penetration suggests a vertical columnar organization similar, for example, to the columnar



Distribution in visual space of receptive fields of cells of the visual cortex of the marsupial *D. virginiana*.

Table II. Distributions of monocularly and binocularly responding cells from a marsupial (*D. virginiana*) visual cortex by receptive field geometry and dominance

	Monocular cells		Binocular cells		
	Right eye only <sup>a</sup>	Left eye only <sup>b</sup>	Right eye dominant <sup>a</sup>	Left eye dominant <sup>b</sup>	Equal
On	12	2	3	5	9
Off	11	2	—	1	1
On-Off	28	6	5	4	10
Unclassed	1				

<sup>a</sup> Contralateral eye. <sup>b</sup> Ipsilateral eye.

organization found for orientation of simple fields of cells of the cat cortex<sup>5</sup>. Summation between the 2 monocular field of a single cell, when stimulated simultaneously, could be seen as well.

Comparing the cortical visual response repertoire of this animal with that of his superior colliculus<sup>6</sup>, more differences than similarities are noted; e.g. (1) the presence of 3 basic geometrical field organizations at the cortex compared with 8 or more at the mesencephalic level; (2) the virtual absence of mutual inhibitory zones within a cortical receptive field; although similar homogeneous fields were found for some cells of the superior colliculus, many concentric, as well as asymmetric, antagonistic zones were found too; (3) the higher number of binocularly driven cells found at the cortical level, often with additive, i.e. right plus left eye summation characteristics; such binocular summation was less readily demonstrated at the mesencephalic level; (4) short-term habituation to repetitive stimulation was much more common at the collicular level<sup>7</sup>. In terms of similarity, however, cells at both the cortical and midbrain levels were seldom found selective to meridional or direction characteristics of the stimulus; cells at both levels, on the other hand, frequently demonstrated discrete ranges of velocity response and often had optimum velocities for maximum excitation.

This marsupial visual cortex then appears to offer in primitive, or at least early, form a wide variety of geometric and response properties found in more sophisticated stages of development among the higher placental mammals, suggesting this neo-cortex as an unique neurophysiological, anatomical model of inter-order, as well as synaptic level, development among the mammals<sup>8</sup>.

**Zusammenfassung.** Etwa 40% von 100 Zellen vom visuellen Kortex des Beuteltieres *Didelphis virginiana* konnten entweder von der rechten oder linken Retina gereizt werden. In der Regel war eine Retina dominant. Areale Summation innerhalb und zwischen den monokulären Feldern war vorhanden, obschon gegenseitige Hemmung innerhalb eines rezeptiven Feldes nicht beobachtet wurde.

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<sup>5</sup> D. H. HUBEL and T. N. WIESEL, *J. Neurophysiol.* 28, 231 (1965).

<sup>6</sup> R. M. HILL, *Experientia* 24, 559 (1968).

<sup>7</sup> G. HORN and R. M. HILL, *Expl. Neurol.* 14, 199 (1966).

<sup>8</sup> This work was supported by U.S.P.H.S. Grants Nos. NB 06983 and NB 05416.

## Electron Microscopic Observations on the Innervation of the Intestinal Inner Muscle Layer

The inner muscle layer of mammalian small intestine is richly supplied with nerve fibres ('plexus interfasciculaires et plexus terminaux', CAJAL<sup>1</sup>). Ultrastructural observations on intramuscular nerve fibres have been provided by RICHARDSON<sup>2</sup> and TAXI<sup>3</sup>. It has recently been shown that these include adrenergic nerve fibres: fibres rich in adrenergic-type vesicles<sup>4</sup> have been detected by electron microscopy<sup>5</sup>; fluorescence microscopy (method of FALCK and HILLARP) has demonstrated the presence

of catecholamine containing fibres<sup>6</sup>. The present paper reports a more detailed analysis of the ultrastructural features of the intramuscular plexus.

For this purpose, samples of small intestine from 4–6-month-old albino rats (*Epimys norvegicus*, var. *albina*, Erxl.) were fixed in 4% glutaraldehyde in 0.1M phosphate buffer (pH 7.4) post-fixed in osmium, dehydrated in alcohol and embedded in Araldite (CIBA). Ultra-thin sections were stained with uranyl acetate and lead citrate,

and examined with a Siemens Elmiskop 1A electron microscope.

Intramuscular nerve fibres run in bundles ('innervation fasciculée'<sup>3</sup>), containing from a few to some dozens of individual fibres. Some bundles run near and parallel to blood vessels, commonly capillaries. Although they are thus vasa satellites, these bundles establish very close relationships with surrounding smooth muscle cells of the tunica muscularis. The fibres diameter ranges from 0.1 to 1.8  $\mu$ . Longitudinal sections reveal the alternation of dilated parts (varicosities, generally occupied by vesicles) and narrow parts, primarily occupied by microtubules. Three different types of varicosities or vesiculated nerve processes are observed: (1) one type has round vesicles of 400–700 Å, most of which show a very dense granule (Figures 1 and 2); (2) another type with round or oval vesicles of 1400–2000 Å, containing a material of medium electron density (Figure 1); (3) a third type shows densely packed agranular vesicles, mostly flattened<sup>7</sup>, with a major diameter of approximately 500 Å. In all 3 types of nerve processes, as also in intramural nerve cell bodies, granular vesicles of 800–1200 Å are occasionally observed<sup>9</sup>.

Full evidence to show that 3 distinct nerve fibre populations are involved is still lacking; large 3-dimensional reconstructions and observations under experimental conditions are in progress in our laboratory. Until now only one vesicle type has been observed in any given nerve process. Nerve processes with vesicles identical with those now described have been observed in the ganglia of the Auerbach's plexus<sup>11</sup>. As to the vesicles 800–1200 Å large, these do not seem, as such, specific of any nervous structure since in the small intestine they may be observed in perikaria and in several nerve processes, alone or accompanying any other kind of vesicles.

The vesiculated nerve processes usually occupy the surface of the nerve bundle and are situated only a few

hundred Å apart from smooth muscle cells; on other occasions, they may be found in the deep part of the bundle, surrounded by nerve fibres which, in that particular section, present only microtubules, or, although situated on the surface of the bundle, they may be covered with strips of Schwann or interstitial cells, or of fibroblasts, or with collagen fibres. Clusters of vesicles have sometimes been observed in close relationship with axolemma contacting glial cells.

There are no special differentiations in smooth muscle cell membrane at the nerve process site, though membrane differentiations were observed by THAEMERT<sup>12</sup> on the same material. Small vesicular in-pocketings of the cell

<sup>1</sup> S. R. CAJAL, *Histologie du Système nerveux de l'Homme et des Vertébrés* (Maloine, Paris 1909).

<sup>2</sup> K. G. RICHARDSON, *Am. J. Anat.* 103, 99 (1958).

<sup>3</sup> J. TAXI, *Annls. Sci. nat., Zool.* 7, 413 (1965).

<sup>4</sup> K. G. RICHARDSON, *Nature* 210, 756 (1966).

<sup>5</sup> G. GABELLA, *J. Microscopie* 6, 863 (1967).

<sup>6</sup> G. GABELLA and M. COSTA, *G. Accad. Med. Torino* 130, 198 (1967).

<sup>7</sup> Criticism of F. WALBERG<sup>8</sup> about these so-called elongated or flattened vesicles as a result of aldehyde fixation cannot be discussed here, as our specimens were always fixed in glutaraldehyde. The fact that technical artifacts may be distinguished does not serve to deny that a clearly distinct type of fibre is involved.

<sup>8</sup> F. WALBERG, *Acta anat.* 65, 224 (1966).

<sup>9</sup> These may be considered equivalent to GRILLO and PALAY<sup>10</sup> type I vesicles, while the first type of granular vesicles above described correspond to GRILLO and PALAY type II vesicles.

<sup>10</sup> M. A. GRILLO and S. L. PALAY, *Vth Int. Congress Electron Microscopy* (Academic Press, New York 1962), p. U1.

<sup>11</sup> G. GABELLA and G. PAGLIARDI, *C. r. Ass. Anat.*, 53e Réunion, Tours, avril 1968, p. 884.

<sup>12</sup> J. C. THAEMERT, *J. Cell Biol.* 16, 361 (1963).



Fig. 1. Intramuscular nerve bundle in the inner muscle layer of the rat small intestine. (a) Nerve fibre with small granular vesicles (catecholamine-storage vesicles); (b) nerve fibre with large granular vesicles (more than 1500 Å large) and an elongated mitochondrion; (m) smooth muscle cell.  $\times 46,000$ .

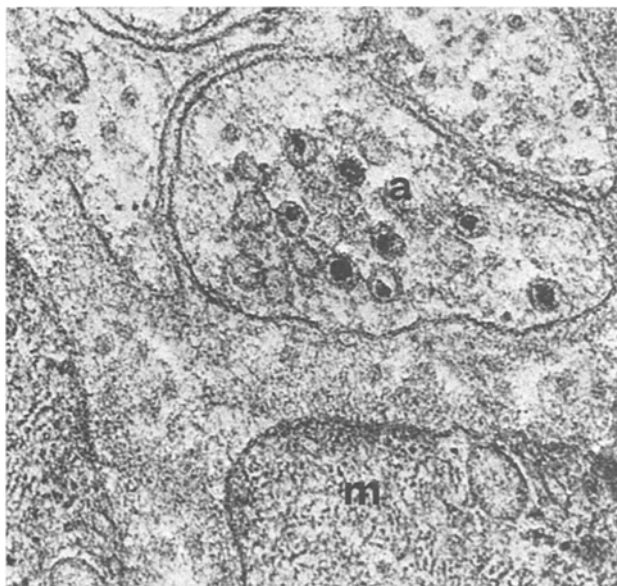


Fig. 2. Intramuscular nerve bundle in the inner muscle layer of the rat small intestine, showing a nerve process (a) with small granular vesicles (catecholamine-storage vesicles); (m) smooth muscle cell.  $\times 83,000$ .

membrane (micropinocytosis vesicles) can be observed in this segment of a smooth muscle cell, as well as on any other part of its surface.

Summing up, the inner muscle layer of rat small intestine displays numerous nerve bundles; a distinction between true intramuscular and perivascular nerve

fibres is not possible since each bundle type is intimately related to smooth muscle cells of the tunica muscularis. Three types of nerve processes are recognized as to their vesicular content and transition forms were not observed; this would seem to indicate that there are 3 types of nerve fibres; at present, identification extends only to the nerve fibres with small dense-core vesicles (catecholamine-storage vesicles), which are to be considered postganglionic orthosympathetic fibres of extrinsic origin. Varicosities seem to bear no constant relationship to any definite structure, nor to structures situated within a definite distance. Transmitters released by these fibres are expected to act on plasma membranes situated only 200 Å away or on membranes lying at much greater distances. Nerve processes with the same kind of vesicles are observed inside the ganglia of Auerbach's plexus<sup>13</sup>.

*Riassunto.* Nella tonaca muscolare interna dell'intestino tenue di ratto si osservano numerosi fasci di fibre nervose; una distinzione tra fibre intramuscolari vere e proprie e fibre perivascolari non è possibile in quanto ogni ordine di fasci ha rapporti intimi con cellule muscolari lisce proprie della tonaca muscolare. L'osservazione di tre tipi di espansioni a vescicole distinte, senza forme di passaggio, fa ritenere verosimile l'esistenza di tre tipi differenti di fibre nervose.

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## Some Characteristics of the 'Auditory Neurophonic'<sup>1</sup>

The cochlear microphonic (CM) is a well-known electrophysiological phenomenon which is a close replicate of the original auditory stimulus. However, the CM is not a neural phenomenon; it has no latency and is present after death, sometimes for hours. A second phenomenon, which has been studied less extensively although originally reported many years ago<sup>2</sup>, also resembles the stimulus. Unlike the CM, it is of neural origin and is not restricted to the cochlea. We have termed this phenomenon the 'auditory neurophonic' (AN), a phrase we will attempt to justify below.

The AN has been investigated in some detail recently by BOUDREAU and TSUCHITANI<sup>3-5</sup>, after having been virtually ignored for several years. These investigators extended observations of the AN, originally recorded in the VIIIth nerve<sup>2</sup>, to the trapezoid body and superior olivary complex. It is mildly surprising that they did not emphasize the neural substrate of the phenomenon. We chanced upon the AN and at the time, unaware of Boudreau's work, we assumed that it was either stimulus artifact or the CM recorded by volume conduction. Control experiments to verify these assumptions revealed instead that the waveforms resembling the stimulus were indeed of neural origin. We report briefly these observations.

*Materials and methods.* The subjects were 6 adult cats, anesthetized with sodium pentobarbital and fixed in a conventional stereotaxic instrument. They were maintained at normal body temperature in an acoustic chamber. Auditory stimuli, consisting of tone bursts, were presented from PDR 10 earphones through hollow ear bars. Stimulus intensity was always between 80 and 90 db re: 0.0002 dyne/cm<sup>2</sup>. Bipolar electrodes consisting of 0.010 inch stainless steel wire, insulated except for 0.5 mm at their tips and affixed side by side with their tips offset vertically by 1 mm, were used to explore the auditory system from the level of the trapezoid body to the medial geniculate nucleus. Histological controls verified intended placements. Bioelectric activity was amplified by conventional differential amplifiers and displayed on a multi-

<sup>1</sup> This study was supported by research grant No. 11250 from the National Institute of Mental Health to N.M.W.

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<sup>3</sup> C. TSUCHITANI and U. C. BOUDREAU, *J. Neurophysiol.* 27, 814 (1964).

<sup>4</sup> J. C. BOUDREAU, *Nature* 208, 1237 (1965).

<sup>5</sup> J. C. BOUDREAU, *J. Acoust. Soc. Am.* 34, 779 (1965).