varied from nerve-like threads to complex plexuses in which individual fibres could be seen (Figures 3 and 4). In the 3 areas from each of the experimental sections at least 4 labelled structures were found, typical photometer readings being as shown in the Table. No such structures were found in lung tissue from the contralateral side or the untreated control hen.

Conclusion. We conclude that this technique can be used to demonstrate the peripheral distribution of vagal afferent nerve fibres which have their cell bodies in the nodose ganglion and this work is now in progress.

Résumé. Par marquage du ganglion nodosum avec la ^{3}H-leucine nous avons étudié la repartition des afférences vagales au niveau de la partie inférieure du système respiratoire du poulet par des techniques d'autoradiographie.

A. J. BOWER, V. MOLONY and C. M. BROWN

Department of Anatomy and Department of Veterinary Anatomy, University of Liverpool, Brownlow Hill, P.O. Box 147, Liverpool L69 3BX (England),
12 November 1974.

Sampling of the Rodent Dorsal Lateral Geniculate Nucleus for Electron Microscopy

In our laboratory we have been concerned with the electron microscopy of retinal axon termination and distribution of the neurons of the dorsal lateral geniculate nucleus (DLGN) in the rat. In order that correlation could be made with other anatomical and physiological data it was necessary to develop an accurate sampling technique to insure precise localization within the DLGN of information obtained in ultrastructural studies.

The brains of laboratory rats were perfused through the aorta with a mixture of 4% paraformaldehyde and 0.5% glutaraldehyde with 4% sucrose in 0.1 *M* phosphate buffer (pH 7.3). The perfusate was used at room temperature. Sufficient pressure for gravity perfusion was achieved by maintaining the perfusate reservoir at a height of 3.5 feet (78.5 mm Hg) above the animals. The perfusions continued for a total of 20 min.

Immediately after perfusion each animal was decapitated by first severing the soft tissue structures around the neck region. This was followed by a laminectomy to expose the upper cervical cord. The cervical spinal cord was transected with a sharp razor blade. This step is performed to avoid stretching artifact of the brainstem. The calvarium was removed with a small pair of 'rongeurs'.

After removing the brain from the cranial cavity, the brain was sliced sagittaly. Each half of the brain was trimmed so that the entire desired nuclear region was contained in one tissue block. The tissue block was immersed for two hours in cold 4% glutaraldehyde in the same buffer as the perfusate. The block was transferred to a rinse solution of 0.1 *M* phosphate buffer and 4% sucrose. The block of brain tissue was further trimmed under a dissecting microscope to the smallest dimensions that still contained the entire nuclear group of choice. With the aid of a Smith-Farquhar tissue sectioner this tissue block was cut into serial slices 250 µm thick. Integrity of the slice series was maintained by the agar media used to support the block. The slices were placed serially in partitioned plastic cases that contained the rinse solution. Sections were rinsed for an additional 10 min. Prior to post-fixation each slice was transferred to a small petri dish that contained a solution of 0.5% osmium-tetroxide and 4% sucrose in 0.1 *M* phosphate buffer. After 1 to 2 min in this solution the surface of the slice acquired a light brown color. Each slice was next transferred to a pool of phosphate-buffered sucrose on a clear plastic disk on the transilluminating stage of a dissecting microscope (Figure 1). The osmicated myelinated

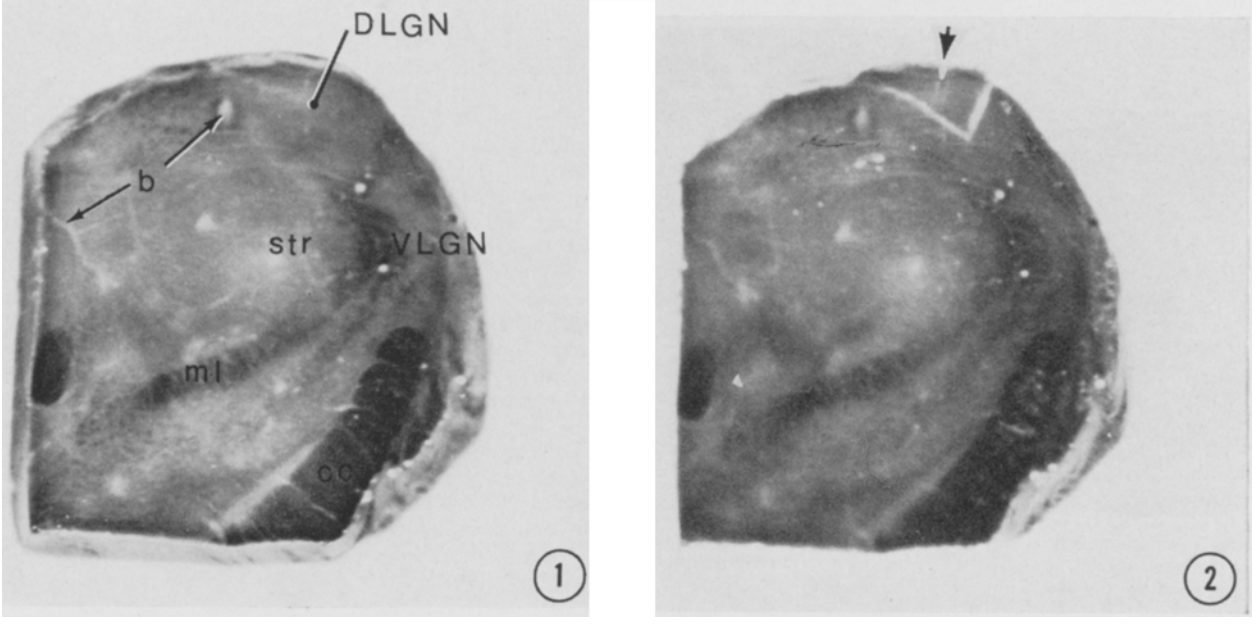

Fig. 1 and 2. Transverse slices through the diencephalon of the rat. The slice has been lightly osmicated. Note the prominent landmarks of myelinated fibre tracts and blood vessels. An arrow in Figure 2 indicates sample taken for electron microscopy. Dorsal and ventral lateral geniculate nuclei (DLGN, VLGN); superior thalamic radiation (str); crus cerebri (cc); blood vessels (b). ×12.

fibre bundles provided the necessary orientation so that, in these studies, wedge-shaped tissue samples of the DLGN could be taken (Figure 2). The wedge-shaped samples were placed in individual glass vials and further processed by post-fixation for 2 h in 1% osmium-tetroxide and 4% sucrose in 0.1 *M* phosphate buffer. The samples were rapidly dehydrated through a graded series of ethyl alcohols and propylene oxide then infiltrated in Epon. All wedges were carefully oriented as they were embedded in flat molds that contained Epon so that transverse semi-thin and thin sections could be cut.

Semi-thin sections of the entire block face, 1 to 3 µm thick, were cut with glass knives on a Sorvall Porter-Blum MT2 ultra-microtome. These sections were stained with Mallory's azure II-methylene blue[1] and examined with the light microscope. From the study of the semi-thin sections an appropriate area coud be chosen for subsequent thin sectioning and study.

This sampling technique provided for the precise localization within the DLGN of the thin sections used in electron microscopy. The localization and orientation of a DLGN tissue sample is illustrated in Figures 1 and 2. The curved side of the wedge-shaped sample (arrow in Figure 2) permitted the immediate identification of the optic tract surface. This, along with orientation drawings completed at the time of initial osmication, enabled one to correctly embed the wedge-shaped sample in a flat mold. By this method the thin sections could always be obtained in the desired plane of section. To date the samples were all embedded so that transverse sections were made through the nucleus from rostral to caudal. Since the thickness of each sample was known, it was possible to determine the rostral to caudal location of each of the semi-thin and thin sections within the DLGN.

The neuropil of the DLGN is packed between the myelinated axon bundles. Careful examination of such semi-thin sections enabled us to identify pockets of neuropil (dotted line in Figure 3) in the DLGN. In these neuropil pockets the reduced number of myelinated structures was noticeable. Such areas were usually surrounded by several neuronal perikarya. These areas of special interest were isolated into pyramid form by the 'mesa-pyramid' technique[2]. Thin sections of these neuropil pockets usually demonstrated many areas of complex synaptic contacts.

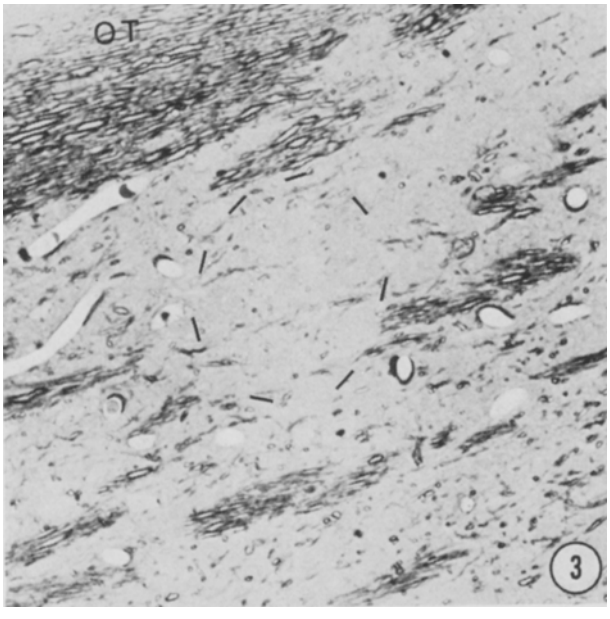

Fig. 3. Transverse 1 µm section through DLGN. Dashed line indicates pocket of neuropil. Note optic tract (OT) on surface of sample. ×600.

Zusammenfassung. Um eingehend elektronenmikroskopisch den Verlauf, die Endigungen und die Verteilung der Axone von der Retina des Auges im Nucleus geniculatus dorsalis lateralis untersuchen zu können, wurde die hier beschriebene sichere Methode für Übersicht und Lokalisierung ausgearbeitet. Mit dieser Methode können nun genau lokalisierte Stellen im Nucleus geniculatus dorsalis lateralis aufgesucht und anschliessend mit dem Elektronenmikroskop untersucht werden.

R. M. Kriebel[3]

Department of Anatomy, P. O. Box 906, Medical College of Virginia, Richmond (Virginia 23298, USA), 13 November 1974.

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Separation of Homovanillic Acid and Vanillylmandelic Acid with Ion-Exchange Resin Column

In the study on the roles of dopamine (DA) and norepinephrine (NE) in brain functions, it is essential to examine the metabolic changes of these amines. Although various methods for the simultaneous estimation of catecholamines and their metabolites in the brain have been presented, they leave much to be improved with regard to the separation, the recovery rate and the practical simplicity of procedures. Above all, the separation of homovanillic acid (HVA) and vanillylmandelic acid (VMA) has been considered most difficult. There are two studies on the separation method of HVA and VMA by column chromatography. Taylor and Laverty[1] had separated these acids of the brain by elution of a Dowex-1-X2 anion exchange resin column with increasing concentrations of HCl. Under our experimental conditions, however, some overlap of the acids into adjacent fractions remained with their method. Messiha et al.[2] reported separation of urinary HVA and VMA with basic alumina column.

We have successfully separated HVA from VMA using the method here given. Ion-exchange resin employed was Dowex-1-X2 anion exchange resin, 200–400 mesh (chloride form) (Dow Chemical Co.). The brain extract of Wistar rats was obtained by homogenizing the brain, except for the olfactory bulb, pineal gland and cerebellum, in 5–6 volumes of ice-cold 0.4 *N* perchloric acid. After

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