

The electric overstimulation obviously depended on the conditions of the preparation under investigation; it should be discussed with respect to the single preparation only.

Discussion. The present investigations demonstrate that there is a static component of electrosensitivity in the Lorenzinian ampullae, which is positioned at least 3 min after a change in the current strength. This static component was in the same direction as the dynamic one over the whole range of temperatures investigated here (7 to 25°C); as a rule, a negative current applied to the orifice of the ampullary canal led to a rise of neural activity; a positive current yielded a fall. The amplitude of the static electrosensitivity, however, is very small compared to that of the dynamic component. The dynamic response in single afferent units to currents of sufficient strength could reach impulse rates of 160 sec⁻¹ (MURRAY⁴; in our own experiments computer evaluation of single spike intervals even gave values up to 260 sec⁻¹). Relative to steady state rates, the dynamic component reached values of up to several 1000%, depending on temperatures. In contrast, the static shift in steady discharge frequency due to current was in every case less than 100%.

AKOEV and ILYINSKY¹⁴ divided the ampulla of rays (*Raja clavata* and *Trygon pastinaca*) in phasic and tonic ones. In the dogfish, we found no basis for such a distinction. At 17°C, all fibres in a good condition showed a steady impulse rate greater than zero. Certainly the fibres were silent at extreme temperatures (< 7°C, > 25°C); in this case, as well as during the silent period following a quick drop in temperature, negative currents could induce neural activity.

It is obvious that under static conditions of temperatures and electric currents, no equivalent of both can be established. If such an equivalent existed, the static frequency versus temperature characteristic of the ampulla would have to be shifted along the temperature axis (abscissa in Fig. 3) by an additional constant current, whereas, in fact, the curves are shifted along the frequency axis (ordinate in Figure 3). In the case of an equivalent, for example, a depolarizing current would increase the discharge frequency at low temperatures and decrease it at high temperatures, which is in contradiction to our finding that depolarization led to a frequency increase over the whole temperature range.

Finally the question remains whether the static component of electroreception has any meaning for the fish. While this paper was being prepared, ANDRIANOV et al.¹⁵ described responses of central neurons of the electro-sensory system in skates to linearly rising magnetic fields (of more than 2 Gs/sec). This rate of change of magnetic field strength corresponds to that which a fish should feel when swimming in the earth's magnetic field

with a constant velocity of 50 cm/sec. However, the magnetic field was applied for 0.5 sec only, so that the dynamic responses to the induced electric fields were recorded. The static component of electroreception is two orders of magnitude smaller; therefore we should expect that the fish will record mostly quick changes of direction in the magnetic field¹⁶.

Summary. The effect of long-lasting electric currents on the Lorenzinian ampullae at constant temperatures between 7 and 25°C was investigated in the dogfish (*Scyliorhinus canicula*). Steady state neural impulse patterns in single afferent units were analyzed by plotting interval length histograms and computing mean values and standard deviations for currents between -100 and +100 nA. The mean values depended on temperature and on current strength; the relative standard deviations remained almost constant (ca. 20–30%). Negative currents, inserted at the orifice of the ampullary canal, led to higher, and positive currents to lower, steady impulse rates in the whole temperature range investigated here. This static component of electrosensitivity again disappeared at higher currents (of 50 nA and more; electric overstimulation). The maximum static response was two orders of magnitude less than the maximum dynamic component of electroreception. The electrosensitivity depended on temperature: the ampullae were most sensitive to electric currents between 13 and 19°C. The maximal neural activity at 19°C was not shifted to higher or lower temperatures by electric stimulation. A constant equivalent of electric and thermal stimulation throughout the tested temperature and current range could not be found.

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¹⁴ G. N. AKOEV and O. B. ILYINSKY, *Experientia* 29, 293 (1973).

¹⁵ G. N. ANDRIANOV, H. R. BROWN and O. B. ILYINSKY, *J. comp. Physiol.* 93, 287 (1974).

¹⁶ B. BROMM, H. HENSEL and K. NIER, *Pflügers Arch. ges. Physiol.* 347, R28 (1974).

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¹⁸ The experiments were performed at the Biologische Anstalt Helgoland. We wish to thank Prof. Dr. O. KINNE for his hospitality and support and Mr. J.-K. HOLTMANN for his help during the adaption procedure. For the digital analysis of spike frequency on the IBM 1130 we wish to thank Dipl. Ing. A. TAGMAT, Bochum.

PRO EXPERIMENTIS

A Section Stretching Apparatus for Ultracryotomy¹

Several procedures for stretching and handling semithin and ultrathin frozen sections have been described²⁻⁷. None of these methods, however, seems to be satisfactory, especially with respect to section stretching. Therefore a section stretching apparatus will be described, the main feature of which is that it is inherently adapted to the cutting edge of the glass knife. According to this principle,

¹ Patent pending.

² T. KOLLER, *J. Cell Biol.* 27, 441 (1965).

³ S. A. HODSON and J. MARSHALL, *J. Physiol., Lond.* 207, 63P (1969).

⁴ S. A. HODSON and J. MARSHALL, *J. Microsc., Lond.* 89, 373 (1969).

⁵ S. A. HODSON and J. MARSHALL, *J. Microsc., Lond.* 91, 105 (1970).

⁶ W. BERNHARD and A. VIRON, *J. Cell Biol.* 49, 731 (1971).

⁷ A. K. CHRISTENSEN, *J. Cell. Biol.* 51, 772 (1971).

is obtained. (At the end of this procedure, screw No. 2 also serves to hold the section stretcher in place, as mentioned above). This adjustment by means of screw No. 2 is made possible by the fact that the axis of the holder (No. 4) is arranged in such a way that triangle B (the section stretcher) is positioned slightly to the right prior to fastening. Throughout the initial procedure, triangle B is guided by the feather (No. 5). Technical details of the apparatus are given in Figure 3.

Semithin and ultrathin sections with and without the synthetic coating described by BOLL et al.⁸ have been produced with this section stretching apparatus.

Advantages of the apparatus described are that it provides an ideally fitting section stretcher, allowing for good observation of the cutting process, and that even sections can be obtained without the use of a floating

medium. The removal of the frozen sections, which usually adhere to the glass knife (A in Figures 1 and 2), can follow without the use of liquids by any of the known methods^{2,3,6,7,9-11}. In any case, it is advantageous that the sections are protected from drying until the opening of the section stretcher at the desired moment. Our experience has been, furthermore, that static electricity causes less disturbance with the use of this method.

Zusammenfassung. Es wird ein Schnittstreck-Gerät für die Ultramikrotomie, besonders für die trockene Gewinnung und Abnahme von semidünnen und ultradünnen Gefrierschnitten beschrieben. Das Prinzip des Gerätes besteht darin, von den bei der Herstellung (Brechen) von Glasmessern aus Vierecken erhaltenen beiden komplementären Dreiecken je eines als Messer, das zweite als Schnittstreckgerät zu verwenden.

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(German Federal Republic, BRD), 29 July 1974.*

- ⁸ H. U. BOLL, G. REB and R. TAUGNER, *Experientia* 30, 1103 (1974).
⁹ A. K. CHRISTENSEN, *Proc. 7th Int. Congr. Electron Microscopy*, Grenoble (1970), Vol. 1, p. 503.
¹⁰ W. F. GEYMER, *8th Int. Congr. on Electron Microscopy*, Canberra (1974), Vol. 2, p. 60.
¹¹ K. T. TOKUYASU, *J. Cell Biol.* 57, 551 (1973).

An Autoradiographic Method for the Demonstration of Vagal Afferent Nerve Fibres in the Lower Respiratory Tract of the Chicken (*Gallus gallus domesticus*)

The innervation of the lungs and extrapulmonary airways of the chicken have been extensively studied¹⁻⁴. In these and other investigations of the innervation of viscera, one of the most difficult problems has been to distinguish between afferent and efferent fibres. We believe that we have overcome this problem by utilizing axonally transported ³H-leucine. Autoradiographic techniques have previously been used to study nerve pathways within the CNS⁵, central connections of dorsal root ganglia⁶, the rate of axonal transport⁷, and the optic pathways of several species⁸⁻¹⁰. The present study was undertaken to determine the feasibility of using this method to demonstrate the distribution of afferent nerve fibres to the lower respiratory tract of the chicken.

Materials and methods. The nodose ganglia of 2 adult hens each weighing 1.5 kg were treated with ³H-leucine. A slow i.v. infusion of a solution of 400 mg/ml of urethane in avian Ringers solution was given until a constant plane of anaesthesia was obtained, at a dose of about 1.5 g/kg body weight. This gave deep anaesthesia throughout the duration of the experiment (12-18 h). The right nodose ganglion which lies in the thoracic inlet was exposed and carefully dissected free, the bird maintained by artificial respiration. A small sheet of dental wax was placed beneath the ganglion to form a shallow bath and the ganglion was bathed for 30 min in a 1% solution of hyaluronidase (Koch-Light Labs; Cat. No. 3137 t) in normal saline. The ganglion was then surrounded by small pieces of gelatin foam soaked in ³H-leucine solution (Radiochemical Centre, Amersham, Bucks; Cat. No. TRK 70) which were maintained in position by wrapping the dental wax carefully around the ganglion. A specific activity of 5 mCi/ml was obtained by freeze-drying the ³H-leucine and then redissolving in the appropriate volume of normal saline. It was assumed that the rate of rapid transport was about 400 mm/day, therefore the 10 or more h which the bird remained alive should have been sufficient to permit the rapid transport of the amino acid to the receptor endings. The hens were finally killed by intracardiac perfusion with 10% formalin. The nodose ganglion, the vagus just distal to the ganglion, the

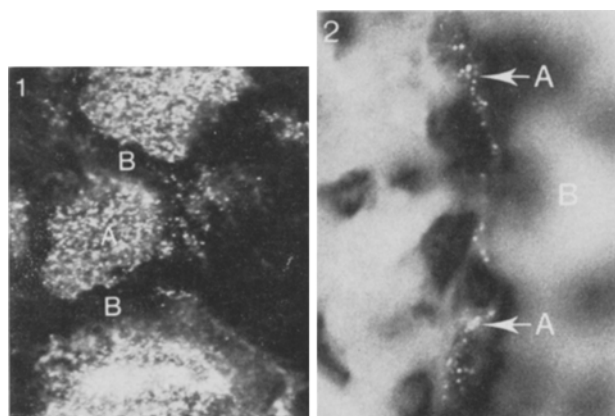


Fig. 1. Nodose ganglion from the treated side. The cell bodies (A) have taken up the ³H-leucine but there is little activity present between the cells (B). $\times 550$.

Fig. 2. Syrinx. A labelled nerve (A) can be seen lying beneath the epithelium (B) of the air sac wall. The epithelium has shrunk more than the other tissue and is out of focus. $\times 550$.

- ¹ R. D. COOK and A. S. KING, *J. Anat.* 106, 273 (1970).
² J. McLELLAND, *Acta anat.* 85, 418 (1973).
³ T. BENNETT, *Z. Zellforsch.* 114, 117 (1971).
⁴ H. P. GROTH, *Z. Zellforsch.* 127, 87 (1972).
⁵ W. M. COWAN, D. I. GOTTLIEB, A. E. HENDRICKSON, J. L. PRICE and T. A. WOOLSEY, *Brain Res.* 37, 21 (1972).
⁶ R. J. LASEK, *Brain Res.* 7, 360 (1968).
⁷ R. J. LASEK, *Int. Rev. Neurobiol.* 13, 289 (1970).
⁸ W. J. CROSSLAND, W. M. COWAN and J. P. KELLY, *Brain Res.* 56, 77 (1973).
⁹ J. S. ELAM and B. W. AGRANOFF, *J. Neurochem.* 18, 375 (1971).
¹⁰ J. O. KARLSON and J. SJÖSTRAND, *Brain Res.* 11, 431 (1968).