STUDIORUM PROGRESSUS

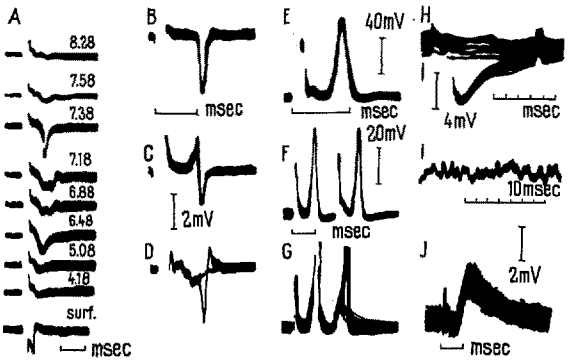
Intracellularly Recorded Antidromic Responses of Deiters' Neurones

The nucleus of Deiters is one of the most conspicuous structures in the brain stem, as it involves the well-known giant cells. Recently, knowledge of the cyto-architecture and fibre connections in this nucleus has greatly been advanced by BRODAL et al.[1,2]. On the other hand, though extracellular recording of unit spikes has been carried out by several workers[2], there are still many important gaps in our knowledge of electrical events occurring in the neurones of Deiters' nucleus. In the work to be reported, intracellular technique has been applied to these neurones which were identified by antidromic activation through the vestibulospinal tract.

Cats were anaesthetized with pentobarbitone sodium. In order to approach the nucleus of Deiters with microelectrodes, three different directions have been tried: (1) dorso-ventrally after complete removal of the cerebellum, (2) horizontally in the caudo-rostral direction after the posterior lobe vermis was lifted up so as to expose the floor of the fourth ventricle, and (3) ventro-dorsally through the medullary pyramid. The last procedure was most favourable because it caused little damage to the brain tissue, and therefore it was used throughout the later experiments. In this event, the cat was laid with its left flank down, the head being fixed upside down on a stereotaxic apparatus. After tracheal cannulation, the trachea and oesophagus were cut at the level of C_3 vertebra and their oral ends were lifted up. The base of the skull thus exposed was holed between the tympanic bullae and the ventral surface of the medullary pyramids was visualized for about 5 mm caudally from the edge of the pons. The C_3 vertebra was held rigidly by a metal clamp and its body was drilled through, the ventral surface of the C_3 spinal segment being exposed. The T_{13} to L_2 vertebrae were also laminectomized and the right side of the L_1 segment of the spinal cord was exposed. The L_3 vertebra was clamped rigidly with its left side down. The vestibulospinal tract was stimulated at both C_3 and L_1 levels on the right side by enamel wires placed on the ventral surface of the cord at about 2 mm lateral from the anterior median fissure. Strength of stimuli was adjusted by monitoring the spike potential on the surface of the pyramids which in part would involve the impulses up the vestibulospinal tract. Glass microelectrodes were filled with solution containing $3M$ KCl or $2M$ K citrate and those having electrical resistance of 15 to $20M\ \Omega$ were selected. They were inserted into the brain stem vertically with a lateral angle of 10 to 20 degrees at about 5 mm caudal from the edge of the pons and 2 mm lateral from the anterior fissure on the right side. The micromanipulator and the method of tracking through the brain tissue were similar to the methods employed for spinal motoneurones[3].

Figure A shows examples of the potential changes recorded with a microelectrode which, after once inserted to a depth of 10 mm from the surface of the pyramid with a lateral angle of 16°, was withdrawn in 100 μ steps, the cord being stimulated at the C_3 level homolaterally. It is seen that there occurs negative field potential at a depth of 4 to 8 mm, particularly prominently at 5.5 to 7.5 mm. When recorded along several tracks with different angles on the same transverse plane, it was revealed that this negative field potential was generated from a well-circumscribed area in the vestibular nuclei region. In the case of Figure A, for example, the area giving negativity of more than 500 μV in peak amplitude is enclosed by a circle with diameter of about 2 mm. This area was confirmed histologically to correspond exactly to the region of the lateral vestibular nucleus of Deiters. Ipsilateral stimulation at L_1 level produced negativity in the dorsocaudal portion of the area which was invaded by impulses when the cord was stimulated at the C_3 level. This is consistent with the somatotopical distribution in the nucleus of Deiters determined by a histological method[2].

Superposed on the field potentials, there often appeared unit extracellular spike potentials which were large negative (Figure B), positive-negative (Figure C) or sometimes negative-positive (Figure D) spikes. Amplitude of these spikes was usually within several millivolts, but that of the positive-negative spikes sometimes amounted to 30 to 40 mV. When the microelectrode penetrated the cell membrane, there appeared suddenly a resting potential



A, electric field potentials occurring along a track through the nucleus of Deiters during its antidromic activation. The ventral surface of the C_3 spinal segment was stimulated homolaterally with condensor-discharge shocks (time constant, 50 μsec). Figures on each trace indicate the depth of the microelectrode measured from the ventral surface of the pyramid. Time constant of the amplifier was 0.2 sec. Voltage scale of 2 mV is shown in C. Negativity is represented by downward deflections. The bottom trace (surf.) is the potential change recorded by a ball-tipped electrode from the ventral surface of the pyramid and illustrated with negativity upwards. B, C, D, unit spikes recorded extracellularly in Deiters' nucleus during antidromic activation from C_3 level. Time constant of amplifier was 0.02 sec. The same voltage scale of 2 mV and time scale of msec apply to all of B, C and D. E, spike potential of a Deiters' neurone antidromically induced from C_3 level and recorded intracellularly, d.c. recording. F, G, responses of another Deiters' neurone for double antidromic stimuli at C_3 level, d.c. recording. H, afterpotential following a spike antidromically induced in the same cell as in F and G, d.c. recording. I, synaptic noise occurring spontaneously in Deiters' neurone, d.c. recording. J, excitatory postsynaptic potential produced in a Deiters' neurone by stimulating the ipsilateral vestibular nerve which was prepared for stimulation by opening the bulla and removing the first turn of cochlea[3]. Voltage scale of 2 mV applies to both I and J. In B–J, negativity is shown downwards. In all records of the Figure except I, ten to forty traces were superimposed at a sweep rate of 3 to 10 c/s.

[1] G. L. RASSMUSSEN and W. F. WINDLE, *Neural Mechanisms of the Auditory and Vestibular Systems* (C. C. Thomas, Springfield, Ill. 1960).

[2] A. BRODAL, O. POMPEIANO, and F. WALBERG, *The Vestibular Nuclei and their Connections* (C. C. Thomas, Springfield, Ill. 1962).

[3] J. C. ECCLES, P. FATT, S. LANDGREN, and G. J. WINSBURY, J. Physiol. *125*, 590 (1954).

of 50 to 70 mV and stimulation of the C_3 segment elicited with a short latency (0.5 to 1.9 msec) action potentials which amounted to 80 mV in amplitude and showed an inflection on their rising slope (Figure E). That these spikes were induced by antidromic invasion was indicated by the fact that, when the stimulus strength was reduced, or when conditioned by another stimulus with a short interval, the spike disappeared abruptly without leaving any prepotential. When two stimuli were given at appropriate intervals, the inflection of the succeeding spike became prominent (Figure F), or the spike was fractioned into two component potentials (Figure G). On analogy to the antidromically evoked spike in cat spinal motoneurones[4], this inflection would be assumed to occur due to the step conduction from the initial segment to the cell soma. The spike potential was followed by an after-hyperpolarization of several millivolts with a duration of 20 to 50 msec (Figure H). This relatively short duration of the after-hyperpolarization in comparison with that of cat spinal motoneurones[5] would be relevant to the fact that the impaled cells tended to fire repetitively at a rate up to 400 c/s, presumably due to depolarization caused by penetration. In general, the cells in Deiters' nucleus were much more susceptible to injury due to microelectrode penetration than spinal motoneurones of the same animal species. In addition to such configurations of the action potential as the inflection and after-hyperpolarization, presence of irregular fluctuation of the membrane potential of a few millivolts (Figure I), probably caused by spontaneous synaptic activity, may be taken as evidence that the nerve cells but not the axons were penetrated.

When a Deiters' cell was invaded antidromically from both C_3 and L_1 levels, the conduction velocity along its axon could be calculated from the distance between these two segmental levels and the difference of latencies for antidromic invasion therefrom. This ranged from 25 to 119 m/sec. If HURSH's[6] relation between the fibre calibre and the conduction velocity holds for fibres in the vestibulospinal tract, its calibre spectrum should cover a wide range from 4 to 20 μ. Under the assumption that larger cells issue thicker axons, the above results would be consistent with the histological finding that the cells in a Deiters' nucleus are of various sizes, including the giant cells, which practically all send axons down to the spinal cord[2].

In Figure H strength of the antidromic stimulus was set just at threshold value for the axon of the impaled cell, there being failure of stimulation at about half the trials. With this procedure of threshold-straddling no evidence has been obtained of the recurrent inhibition such as that through the Renshaw cell pathway for spinal motoneurones[7]. Excitatory and inhibitory postsynaptic potentials could be induced in the cells of Deiters by stimulating various sources of their afferent connections, i.e. vestibular nerve, cerebellum and spinal cord. For example, Figure J illustrates that stimulation of the ipsilateral vestibular nerve elicited an excitatory postsynaptic potential with a latency as short as 0.6 msec. This potential is presumed to be induced monosynaptically through the primary vestibular afferent which makes direct contact with the cells of Deiters. These postsynaptic potentials are now being studied in relation with the somatotopical distribution of individual cells in the nucleus and the conduction velocities of their axons.

Résumé. Chez le chat anesthésié au Nembutal, les réponses évoquées dans les neurones de Deiters par les stimulations antidromiques ont été étudiées au moyen de microélectrodes intracellulaires.

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