Initial Axon Collaterals from Nerve Cells in the Lateral Cervical Nucleus

The degeneration of terminal boutons in the lateral cervical nucleus (NCL) has been studied in the electron microscope after axonal transection[1]. Although the lesions were made in the dorsal part of the lateral funiculus immediately below the nucleus and thus should cut off all known afferent fibres to it, there was a surprisingly small population of structurally altered boutons. A similar finding has been made in the cuneate nucleus in which only 18% of the boutons degenerated after lesions immediately below the nucleus[2].

To investigate if there are other afferent fibre systems to the NCL than that which was known previously[3], studies have been undertaken on normal as well as on experimental material with the aid of the Golgi and the Nauta (1957) techniques, respectively. This is a preliminary note on the observation of initial axons collaterals from NCL-neurones in Golgi preparations.

Impregnation according to the rapid Golgi technique[4] was carried out in 62 cats ranging in age from new-born to adult. Three of them, all 7 days old, were perfused with osmium tetroxide and potassium permanganate according to a method recently published[5].

NCL-axons were found to give off collaterals at a distance of 70–130 μ from the cell body. The collaterals were very thin, leaving at a straight angle from the axons. So far only 1 collateral per axon has been observed. Most of the collaterals branched richly and terminated within the NCL with small terminal boutons or with what seemed to be free endings.

NCL has been studied with the Golgi technique before[6] but initial axon collaterals have not been described. In this investigation, axon collaterals have been demonstrated in the perfused material only. Whether this means that perfusion is definitely superior to immersion fixation is not yet possible to decide from this material, especially when the hazardous outcome of the Golgi impregnation is considered.

The presence of initial axon collaterals fits well with what is known about the functional organization of the NCL. Neurophysiological studies have shown recurrent inhibition in the NCL after antidromic as well as orthodromic stimulation[7]. Whether the initial collaterals represent the whole number of unchanged boutons in the electron microscopical material[1] cannot be decided yet. The neurophysiological findings[7] indicate the presence of internuncial neurones within the NCL. This would mean that at least a third group of boutons should be present in the nucleus beside those from the fibres ascending in the dorsal part of the lateral funiculus and the recurrent initial axon collaterals.

Zusammenfassung. Nach Perfusion mit Osmiumtetroxyd und Kaliumbichromat wurde das Rückenmark 7 Tage alter Katzen nach der raschen Methode von Golgi imprägniert. Es wurden initiale Axonkollateralen von Nervenzellen in Nucleus cervicalis lateralis beschrieben, was mit physiologischen Befunden übereinstimmt.

J. Westman

Department of Human Anatomy, University of Uppsala (Sweden), 16th February 1967.

1 J. Westman and G. Grant, Acta Soc. Med. upsal. *70*, 259 (1965).
2 F. Walberg, Expl Brain Res. *2*, 107 (1966).
3 A. Brodal and B. Rexed, J. comp. Neurol. *98*, 179 (1953).
4 O. Strong, J. comp. Neurol. *6*, 101 (1896).
5 K. Morest and R. Morest, Am. J. Anat. *118*, 811 (1966).
6 S. R. Y. Cajal, *Histologie du système nerveux de l'homme et des vertébrés* (Consejo superior de investigaciones cientificas, Madrid 1952), vol. I, p. 986; T. A. Leontovich and G. P. Zhukova, J. comp. Neurol. *121*, 347 (1963); H. Ha and C.-N. Liu, Expl Neurol. *8*, 318 (1963).
7 G. Gordon and M. G. M. Jukes, J. Physiol., Lond. *169*, 28 (1963); D. F. Horrobin, Q. Jl exp. Physiol. *51*, 351 (1966).

The Toxic Action of *Bacillus thuringiensis* 'Exotoxin' on *Drosophila* Reared in Yeast-Containing and Yeast-Free Media

A prepurified preparation of the so-called 'exotoxin' of *Bacillus thuringiensis* was prepared by differential precipitation with ethanol after the method of Benz[1]. Its toxic activity was bioassayed with larvae of *Drosophila* on two basically different rearing media.

Medium A was a corn-yeast-agar medium (water 750 ml, agar 9 g, sugar 50 g, corn semolina 100 g, dry yeast 20 g), while medium B was the synthetic medium C of Sang[2]. Our third medium C was medium B with 2% of dry yeast added. After the media were cooked 0.66 ml of a 20% solution of nipagin in ethanol was added per 100 ml of medium and mixed thoroughly. Polystyrene beakers with a capacity of 200 ml were used for all tests. 0.5 ml of a 2.2% solution of streptomycin, and 2 ml of different dilutions of 'exotoxin' or water were pipetted into each beaker, and, in the case of the media B and C an additional 0.5 ml of a vitamin mixture. 30 ml of warm (not hot) medium were added per beaker and well mixed with the liquid before the medium solidified. Not all brands of casein can be used for the preparation of synthetic *Drosophila* medium. We tested vitamin-free caseins manufactured by Nutritional Biochemical Co., Merck, and Fluka. Only the first preparation was good (maximal control mortality 6%), while the other two brands were toxic for *Drosophila* larvae (control mortalities of 45 and 100% respectively).

Drosophila eggs were collected from culture bottles, placed on fine meshed gauze and washed several times with water of 25 °C in order to remove yeast and other impurities. The washed eggs were then kept on wet filter paper in a Petri dish. Fifty freshly hatched larvae were placed on the medium of each beaker. The beakers were closed with perforated polystyrene lids and kept at 24–25 °C and 80% relative humidity. Flies that eclosed were recorded, and the results were corrected by Abbot's formula.

The Figure presents results of such tests. They indicate a marked difference between the mode of action of the

1 G. Benz, Experientia *22*, 81 (1966).
2 J. H. Sang, J. exp. Biol. *33*, 45 (1956).