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THE USE OF HORSERADISH PEROXIDASE (HRP) IN THE STEROLOGICAL ANALYSIS OF MOTOR NUCLEI

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ABSTRACT

A quantitative analysis of the motor pools of the motor trigeminal nucleus in the rat was made. Localization of individual motor pools cannot be effected using standard histological techniques. Consequently, the muscles of mastication were separately injected with a 30% solution of HRP. The subsequent retrograde axonal transport of the enzyme, and its localization within the perikarya of efferent neurons permitted delineation of the motor pool subserving a particular muscle. In order to ensure that all cellular profiles in the pool were identifiable, the tissue sections were counterstained with Neutral Red. Mean diameters of neuronal somata were recorded, and frequency histograms constructed. All motor pools within the motor V nucleus were analysed; the results obtained from study of the mylohyoid motor pool are presented here.

INTRODUCTION

The aim of this investigation was to make a quantitative analysis of the individual motor pools of the motor nucleus of the trigeminal nerve in the rat. The partition of the nucleus into separate sub-nuclei subserving individual muscles cannot be effected using standard histological techniques since there are no definite anatomical boundaries between the pools. Consequently, a retrograde tracer technique was required, and the HRP method was selected.

Previous studies of localization within the motor

trigeminal nucleus have tended to be largely qualitative, and have involved the use of several tracer techniques. Szentagothai (1949) employed degeneration methods. He created lesions within the nucleus and then examined the masticatory muscles and trigeminal nerve muscular branches for evidence of degeneration of motor end-plates and nerve fibres respectively. This technique has obvious attendant disadvantages; the extent of the lesion is critical, as is the time course over which degeneration occurs. More recent studies are those of, for example, Mizuno et al (1975); Limwongse and De Santis (1977); Matsuda et al (1979) and Tal (1980). These authors have employed the HRP retrograde tracer technique.

To our knowledge, no-one has previously attempted the stereological analysis of individual motoneuron pools.

MATERIALS AND METHODS

Seven adult albino rats, of either sex, of weight 350-450 gm. were anaesthetised by intraperitoneal injection of sodium pentobarbitone (Sagatal).

In this paper, the example of the mylohyoid motor pool is presented. The mylohyoid muscle was exposed and injections of a 30% solution of HRP (Sigma. Type VI) in 0.9% normal saline were made into the muscle with a 5 µl Hamilton syringe. The volume of HRP solution introduced into the muscle varied from animal to animal since it is accepted that the muscle should change from a pinkish-red to a uniform brown colour. (Mizuno et al 1975).

After 48 hours the animals were sacrificed, under similar anaesthesia, by trans-cardial perfusion with 50-100 ml. of 0.9% normal saline, to ensure exsanguination followed by 500ml of fixative mixture. The fixative used was a standard solution composed of 4% Glutaraldehyde (TAAB Practical Grade) and 2% Paraformaldehyde in phosphate buffer pH 7.4 (Karnovsky, 1965).

The brainstem was removed and serial frozen transverse sections at 10µm thickness were cut through the motor trigeminal nucleus on a freezing microtome. The tissue sections were then processed for the blue reaction product of HRP neurohistochemistry, using Benzidine dihydrochloride (BDHC) as the chromogen, according to the method of Mesulam (1976). Subsequently the tissue sections were counterstained with 1% aqueous Neutral Red. This stain gives good colour contrast with the blue HRP reaction product.

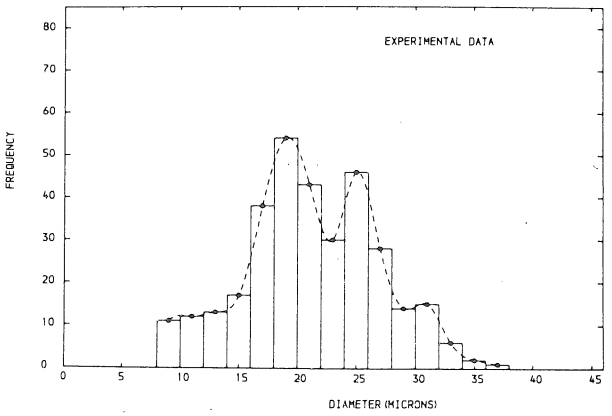

FIG. 1A. Mylohyoid. Experimental data.

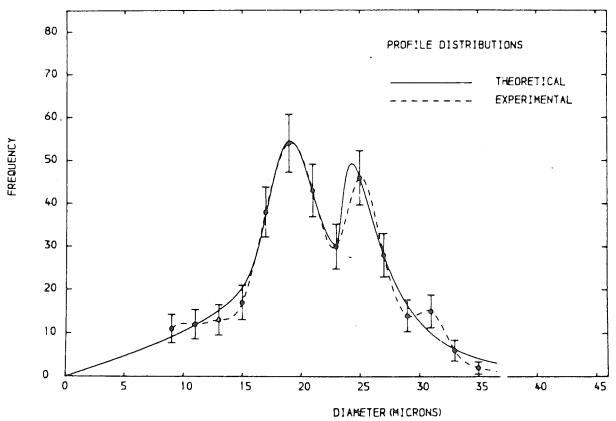

FIG. 1B. Mylohyoid. Profile distributions.

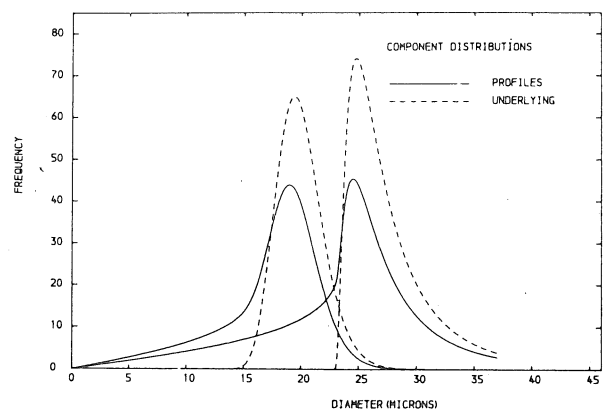

FIG. 1C. Mylohyoid. Component distributions.

The sections were examined on a Nikon Optiphot research microscope at a magnification of X400 for stereological analysis. Every ninth section was analysed. Fifty-three sections were examined in total. Starting from a random point within the labelled motor pool, two consecutive fields were examined using a square test system of side 200um (real units at x400) on a graticule located in one of the eyepieces. In the other eyepiece was a linear scale which was used to estimate the maximum and minimum diameters of each cell profile. The mean of the two measurements was recorded. 682 cell profiles were counted, and these were plotted on a frequency histogram using diameter increments of 2μm.

RESULTS

The results were analysed according to the methods detailed in Howard, Scales and Lynch (1980) and Scales and Howard (1982).

	Gamma	Alpha
Mean diameter μm	20.4	28.22
Standard deviation	1.7	5.21
N_v cells/10^6 μm^3	3.2	3.3

These results are shown graphically in Figure 1.

DISCUSSION

There is a relationship between the function of nerve cells and their size (Henneman et al. 1965). There also appears to be significance in the size distributions of individual populations of neurons (Howard 1981 a and b; Howard and Scales 1982). So far, the only successful stereological analyses of motoneuron pools have been performed on mixed populations of motoneurons serving several muscles. Because both population size distribution and individual neuron soma size appear to be so important in the normal functioning of the effector system, it is obviously desirable to examine pure populations. The technique outlined above, for the first time, allows this

aim to be achieved.

Once the vagaries of the HRP technique have been overcome, results can be obtained quickly and effectively. The error analysis in this experiment, which will be presented in a later, full report of this work, seems to indicate that the modelling of motoneuron populations with a double log normal model is better when used on pure populations than on mixed, which would support the statements made above.

In conclusion, it appears that HRP, a qualitative nerve cell tracing enzyme that is retrogradely transported in axons can be used successfully in stereological experiments when combined with a cell body counterstain. The HRP can be used to map areas within motor nuclei while the counterstained material is used for morphometric measurements.

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