Numerical Capacities of Cerebellar Cell and Fiber Systems

Contemporary brain research still treats unit element quantities of cell and fibre systems as quasi infinite. At the turn of the century a figure of 14.2×10^6 was suggested for the number of Purkinje cells in the cerebellar cortex of man[1]. A more recent estimate[2] for this cell type is 15×10^6, the number of granular cells was estimated to range from 10^{10}–10^{11}. The 2 available estimates for the number of Purkinje cells agree so well as to reinforce each other. The figure of 10^{10} is routinely quoted as comprising the total neuron capacity of all levels of the human nervous system.

The major source for neurites entering the cerebellum are the pontine nuclei. They extend into 1125 sections of 25 μ thickness in a brain-stem of a 56-year-old male. For the same subject estimates were carried out earlier, to determine fibre numbers in the cerebral commissures[3] and cell numbers in the inferior olivary nuclear complex[4]; details of cell and fibre-counting methods are outlined in these two papers.

In one single section at the midpontine level, all nerve cells within the pontine grey were counted out field by field. The figure of 23,653 cells thus obtained was used for the testing of the reliability of sampling procedures. By the count in every 25th slide of nucleoli in 100 randomly chosen fields of 10,000 μ each, 23.085×10^6 neurons were estimated within the pontine grey. Of this total one half may be expected to send its axons via the middle cerebellar peduncle of one side into the cerebellum. Due to axon branching known to occur in the superior cerebellar peduncle, the actual figure may be higher. If this eventuality, or that of a proportion of these cells having short axons that synapse within the pons itself are neglected, 23.085×10^6 neurites project onto the granular cells in the form of mossy fibres[5]. There is an overall dispersion ratio of 1:434 to 1:4347, depending on the high or the low estimate for the granular cells, from pontine level to cerebellar cortex. In the cortico-pontine to ponto-cerebellar system, the pontine nuclei provide the intermediate synaptic link. Together with the cortico-bulbar and the cortico-spinal fibres, the neurites of the cortico-pontile system constitute the 2 cerebral peduncles. Nerve fibres within the peduncles have a wavy course at the level before entering the pons. This pattern requires special consideration when counting axon numbers in an ocular grid, as many fibres in groups are cut at oblique angles. It was found, however, that compared to a square area wherein fibres are cut at right angle, in an area of the same size with obliquely cut fibres, there is a reduction by a factor equal to the sine of the cutting angle (Figure). In a reverse application of this relationship, the difference of numbers of fibres counted on 2 adjacent sides of the grid square may be used for the calculation of the sine and thus the degree of the cutting angle. For the programming of expected future attempts at automated counting, this relation may prove useful.

In the section at the level before the fibres of the cerebral peduncle merge into the pons, 36 strata of differing fibre composition were delineated and for each stratum the proportions were established of surface areas with fibres cut at right angle, and at varying degrees of obliquity and also of non-fibre-carrying parts of the cross-section. This estimation procedure rendered a figure of 20.763×10^6 neurites for the right side peduncle. Applying the same criteria, an estimate was also made of 1.127×10^6 axons in the pyramid, at the level where it leaves the pons. Thus about 19.6×10^6 axons are left to terminate in some form within the pontine region.

An as yet undeterminable proportion of these, but fewer than estimated for the pyramidal system, are expected to belong to the cortico-bulbar tract. If this latter proportion is neglected, there is a 1:1.7 convergence ratio from the cortico-pontine to the ponto-cerebellar system. There is in the ferret a proportion of 2.23:1 of fibres entering the pons to those leaving via the pyramid[6] in man this proportion rises to 23:1.

The second largest source of fibres entering the cerebellum are the inferior olivary nuclei. Among 4 male subjects ranging in age from the new-born to the above-

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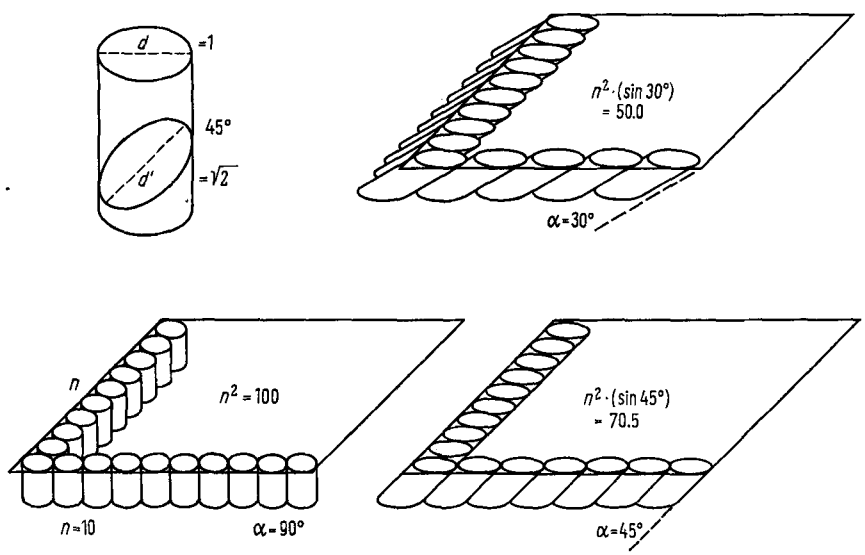

Relationship of cutting angle to reduction of fibre number accommodated in a square.

mentioned 56-year-old subject, cell frequencies for the main olivary nucleus are 447,785 ± 14,375 neurons[4]. The 2 small accessory olivary nuclei added, slightly more than 0.5×10^6 axons project to one cerebellar hemisphere. If these neurites project to the granular cells in form of mossy fibres[5] the divergence ratio may range from 1:10,000 to 1:100,000; if according to another view[7] they synapse in the form of climbing fibres with the Purkinje cells directly, the ratio is 1:15.

Cerebellar outflow is mainly concentrated in the superior peduncle and with few exceptions originates from the cells of the cerebellar nuclei. Counts and estimates carried out on sections of cerebelli of 3 male subjects, rendered for all 4 nuclei of one hemisphere a figure of 311,404 ± 3835 neurons. For the individual nuclei cell frequencies are 284,009 neurons in the dentate nucleus, 16,153 in the nucleus globosus, 10,381 in the emboliforme nucleus and 5210 in the nucleus fastigii. Details of these findings are to be published elsewhere.

At the level where the superior cerebellar peduncle enters the midbrain, an estimate for its constituent number of axons rendered a figure of 782,310 neurites in the right side peduncle. Thus there is a ratio of 1:2.5 of cell numbers of cerebellar nuclei, to that of axons in the main cerebellar efferent fibre tract. The discrepancy may either be due to branching of axons or the presence of incoming fibres. For the occurrence of branchings conclusive evidence was provided by CAJAL[8]. The existence of substantial incoming rubro-cerebellar fibres in the superior cerebellar peduncle was pointed out recently[9].

A proportion of cerebellar nuclear cells are known to project via the fastigio-bulbar bundle directly to the vestibular nuclei. The number of neurons in the vestibular nuclei of man, as recently established[10], is in the order of 245,000 cells. The low figure for the number of neurons found in the fastigial nucleus make it doubtful whether all projections attributed to it, can be served from this limited source[11].

Zusammenfassung. Mit Bezug auf Probleme der Kybernetik wurde die Informationsübertragungskapazität der afferenten und efferenten Zell- und Fasersysteme des menschlichen Kleinhirns numerisch definiert.

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Cytochrome Oxidase Activity in Different Hypothalamic Areas. Influence of Castration[1]

It has previously been shown that sexual activity modifies the oxidative metabolism of hypothalamus. Cyclic changes in the oxygen uptake[2] and in the succinic-dehydrogenase activity[3] were observed in female rats. Experiments performed in castrated male rats showed a decrease in the hypothalamic capacity to oxidize several substrates of the Krebs cycle[4].

In order to determine if the respiratory chain participates in the modifications of the oxidative metabolism of hypothalamus after gonadectomy, the cytochrome oxidase activity in different hypothalamic areas has been studied in normal and castrated rats.

Material and methods. Adult white male rats, fed on the standard diet of the Institute of Physiology, and weighing 150–180 g, were used. Light and temperature were controlled and kept constant (25 °C; 12 h light and 12 h darkness). Gonadectomy was performed 25–30 days before sacrifice. The animals were decapitated and the entire hypothalamus removed. The sample was divided into 3 portions, anterior hypothalamus, middle hypothalamus and posterior hypothalamus, as described previously[5].

The samples were gently blotted on filter paper, weighed on a torsion balance and rapidly transferred to micro-Warburg vessels. Each determination was made on pooled tissue from 2 rats.

Cytochrome oxidase was determined according to the manometric method of SCHNEIDER and POTTER[6] adapted as follows: 0.2 ml of saturated OHNa solution were added to the central well of the micro-Warburg vessel; the main vessel contained the hypothalamic tissue with 1.0 ml of Krebs-Ringer phosphate buffer and the lateral vessel cytochrome C 0.0009 m*M* and ascorbic acid neutralized to pH 7.4, 0.030 m*M* (total volume 1.5; pH 7.4). The vessels were gassed for 5 min with 100% O_2; tipping was performed after allowing 10 min for equilibration; the observation period lasted 60 min. Cytochrome oxidase activity was expressed as µl of O_2/mg wet tissue·h. The results were analyzed for variance following SNEDECOR[7] and the statistical significance was determined according to TUKEY'S[8] method. The minimal significant difference of the means was 0.340 in the anterior hypothalamus and 0.300 in the posterior hypothalamus.

Results. The cytochrome oxidase activity of the anterior, middle and posterior hypothalamus is presented in the Table. The analysis of variance showed that there are significant differences between the experimental groups in the anterior ($p < 0.01$) and posterior ($p < 0.05$)

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