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THE CONCEPT OF 'NEUROMORPHOTAXIS' BASED ON A MINIMISATION PRINCIPLE. A CASE FOR THE CRITICAL ANALYSIS OF BIOLOGICAL VARIATION

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ABSTRACT

The size distributions of populations of neuronal somata, dendrite internode branch lengths and synapse areas are presented. These all show a positive skew and in some cases this is pronounced.

Arguments are put forward to explain this consistent finding. In general the tendency of biological systems to optimise their size on a minimising principle will be a contributory factor. In particular the excitable elements of the central nervous system possess the additional feature that size controls their physiological sensitivity and this is advanced as a major factor in the evolution of the experimental findings. The estimation of the majority of these measurements is possible only by the use of stereological techniques.

INTRODUCTION

The development and evolution of the nervous system has attracted the interest of neuroscientists over many decades. Ariens Kappers et al. (1960) summarised the principle of 'neurobiotaxis' which, it is proposed, governs the migration of groups of neurons in the direction of their main sensory input within the nervous system, throughout evolution.

The reporting of the size distributions of individual populations of neurons and their processes has in general been restricted to the first and, less commonly, the second moments. It is an exception to find published graphical evidence. Statistical procedures in general assume that the distributions are Gaussian, and

therefore, perfectly symmetrical.

In this paper we present two examples of neuron soma distribution from our own studies, and published corroborative evidence from several sources which indicate that the size distributions of structures associated with information processing are highly positively skewed. Work on the significance of asymmetric size distributions in general has been presented by Howard (1981a, 1981b). That discussion is extended in this paper and an hypothesis is advanced to explain our findings based firstly on the inherent conservation of biological systems and the concept of 'optimality' (Rashevsky 1960) and secondly on the fact that size and function are inseparably linked in the functions of neurons (Henneman et al. 1965). These findings appear to complement the theories of Kappers.

MATERIALS AND METHODS

The sizes of neuronal somata, dendrite internodal lengths and synapse areas were studied. In each case the size distributions were modelled according to Howard (1981b) and Scales and Howard (1982). The skewness of the model and the experimental data is presented for each example. Where the measurements have been made on the sections then the skewness has been calculated after the experimental data has been unfolded by the Cruz-Orive (1978) modification of the Schwartz - Soltikoff procedure. Graphical evidence is presented for each case. Where the raw data is from published work, sources are cited and very brief details of the methodology are given, there being full descriptions in the reference texts.

1. Neurons

Six adult albino rats were fixation perfused with 500 ml of 2% paraformaldehyde and 4% glutaraldehyde in phosphate buffer at pH 7.4. Blocks of tissue containing the medial reticular formation were removed and prepared routinely for electron microscopy by embedding in Epon. The cerebellar hemispheres were blocked routinely in paraffin for light microscopy.

a) Medial reticular formation. Gigantocellular nucleus. Transverse sections of 1µm were taken every 80µm and stained with toluidine blue. In the light microscope at x 450 the diameters of 1,339 reticular neurons were measured by taking the average of the maximum and minimum diameters. These were plotted as a density function.

b) Cerebellar granule cells.
Sections 3μm thick, perpendicular to the longitudinal axis of the folia of the paravermis were taken at 50μm intervals. The diameters of 603 of these highly spherical cells were measured.
c) Pigeon Retinal Ganglion Cells.
Hayes and Holden (1980) presented work in which they examined the central yellow field of five pigeon retinae which were examined in flat mount preparations. The areas of 542 cells were measured by tracing their outlines using a semi-automatic tracing device. The histogram ordinate is presented as the mean soma diameter.

2. Dendrites

Uyling's et al. (1978) presented frequency distributions of intermediate segment lengths of the basal dendrites of Golgi stained pyramidal cells from three areas of cortex in the rat. In the original, the histogram class widths were 1μm. In this paper the data have been aggregated into histogram bins of 4μm on only one set of data; that corresponding to the dorsal strip of cortex. In each case data for first, second and third order dendrites was given. The patterns found in this study were repeated in both the other areas of cortex studied.

3. Synapses

a) Cat cerebellar cortex granular layer terminals.
Vrensen et al. (1982) studied the size distribution of these terminals 'en face' in the electron microscope on material stained with ethanolic phosphotungstic acid (EPTA). This method is described in detail in Vrensen et al. (1980)
b) Cat cerebellar molecular layer climbing fibre active zone cord length.
Vrensen et al. (1982) measured this parameter in the electron microscope on Osmium stained synapses associated with autoradiographically labelled boutons, the injection site being the medial accessory olive. The unfolding procedures made the assumption of disc shape which was confirmed in a separate EPTA study.

The significance of skewness of all data has been assessed by the Kolmogoroff-Smirnov test, as in Table 1. The single negative skewness value was assumed to be spurious and due to a poor non-parametric unfolding. This is

probably due to the fact that there are relatively few bins in that histogram and that the skewness result therefore was unduly sensitive to an uneven unfolding.

In all cases the data are presented as frequency density histograms. The error bars at each of the data points indicate the absolute expected error of either the experimental data or, where unfolding has been performed, of the unfolded non-parametric data, assuming a Poisson distribution.

The disparity between the cubic spline interpolation between the data points of the raw data and the model may be explained by the fact that the former minimises curvature between points while the latter does not. The important observation is that they both pass near or through the data points.

RESULTS

TABLE 1

	BIOLOGICAL MODEL	SAMPLE SIZE	LOG NORMAL MODEL		RAW DATA OR NON-PARAMETRIC UNFOLDED DATA	
			COEFF. OF SKEWNESS	SKEWNESS P-VALUE	COEFF. OF SKEWNESS	SKEWNESS P-VALUE
NEURONES	MEDIAL RETICULAR FORMATION NEURONES	1,339	3.599	1.000	1.874	1.000
	SPINO-CEREBELLAR GRANULE CELLS	603	2.788	1.000	-1.471	1.000
	PIDGEON RETINA GANGION CELLS	524	0.245	0.000	0.939	0.997
DENDRITES	1ST ORDER PYRAMIDAL CELL BASAL DENDRITE LENGTH	468	2.146	1.000	1.404	1.000
	2ND ORDER etc.	448	1.810	1.000	1.284	1.000
	3RD ORDER etc.	239	2.006	1.000	0.849	1.000
SYNAPSES	MOSSY FIBRE PRE-SYNAPTIC GRID AREAS	1,307	0.342	0.552	0.810	1.000
	ACTIVE ZONE LENGTHS MOLECULAR LAYER	615	0.712	0.998	0.601	0.977

FIGURES

Figures 1, 2 and 3 correspond to the three headings neurons, dendrites and synapses found under the heading Materials and Methods. Where profile distributions have been obtained from measurements made on thin sections then both the underlying distribution and profile distrib--ution are provided.

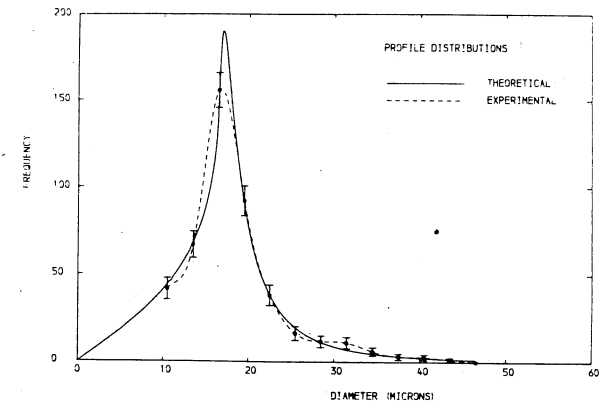

Fig. 1a Rat medial reticular formation gigantocellular nucleus neuron profile distribution.

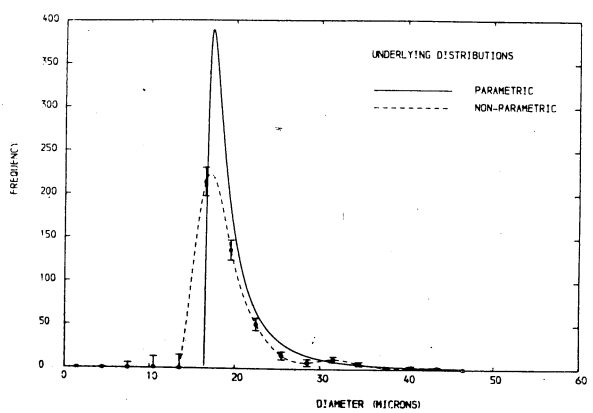

Fig. 1b Rat medial reticular formation gigantocellular nucleus neuron underlying distribution.

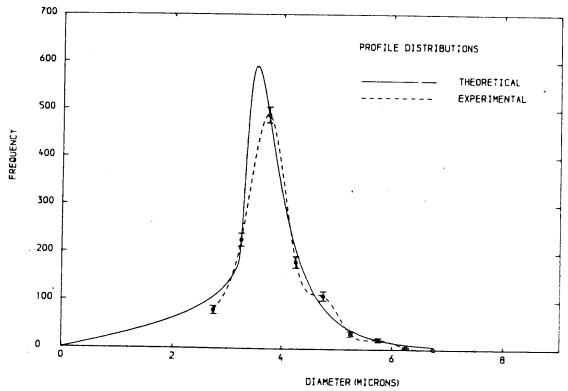

Fig. 1c Rat spino-cerebellar granule cells profile distribution.

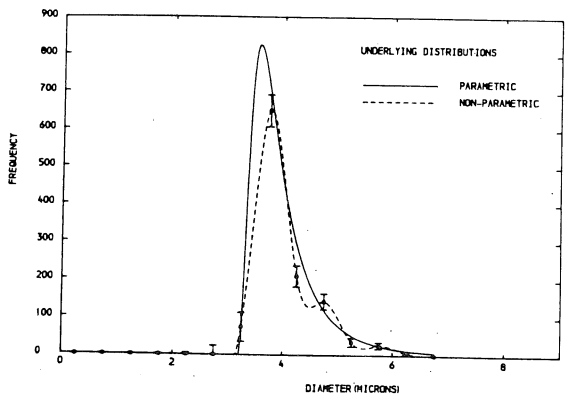

Fig. 1d Rat spino-cerebellar granule cells underlying distribution.

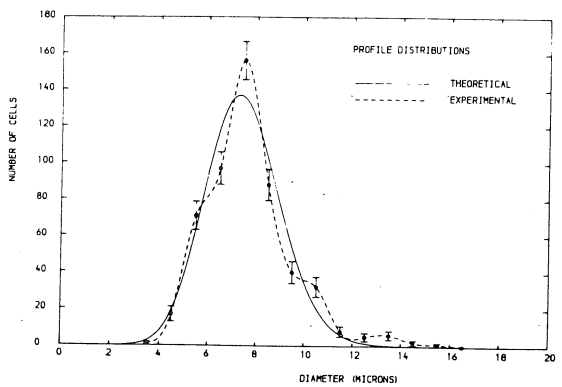

Fig. 1e Pigeon retinal ganglion cells.

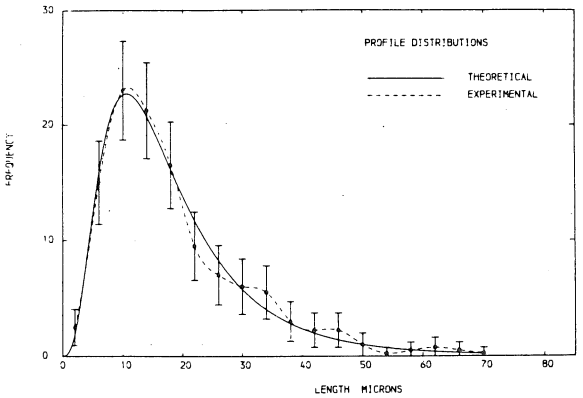

Fig.2a Rat pyramidal cell basal dendrite length. 1st order.

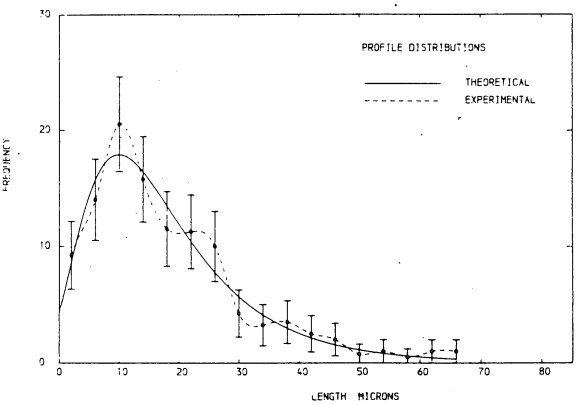

Fig.2b Rat pyramidal cell basal dendrite length. 2nd order.

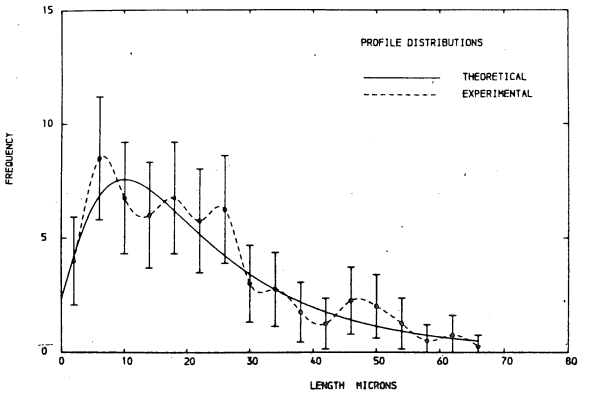

Fig.2c Rat pyramidal cell basal dendrite length. 3rd order.

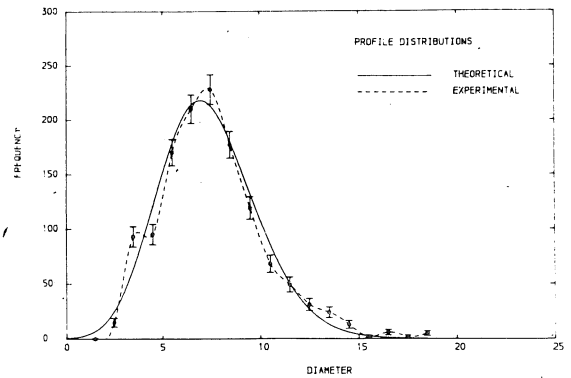

Fig.3a Cat cerebellar cortex granular layer terminals.

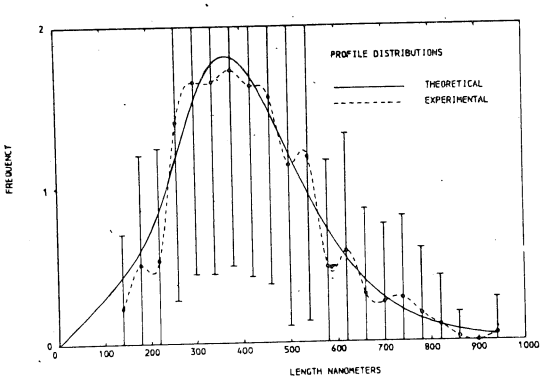

Fig.3b Cat climbing fibre active zone profile distribution.

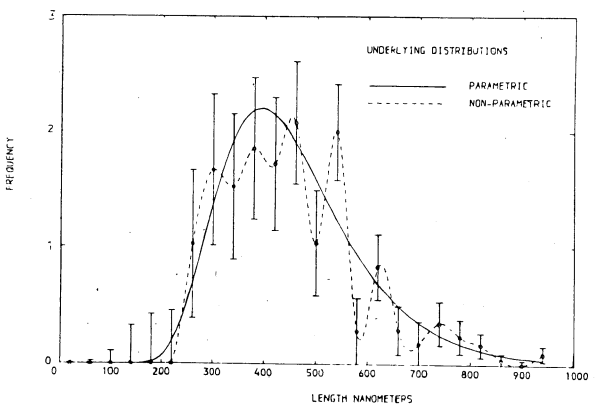

Fig.3c Cat climbing fibre active zone underlying distribution.

DISCUSSION

The work of four separate groups of neurobiologists has been presented. The consistent pattern of positive skewing to the size distributions of neurons, dendrites and synapses invites comment. These recurring findings were incidental and unpredicted in the four laboratories, with the exception of the cerebellar granule cells. This makes it unlikely that the significant positive skewing was an artefact.

In this paper we put forward an hypothesis concerned with the control of the sizes of individual neurons within a population. This does not apply solely to the sizes of the somata of a population, but also those other morphological features involved in information processing, i.e. the branching patterns of dendrites and the size of the apposition zones of synapses.

Biological systems are inherently conservative. (Rashevsky 1960). Nature does not appear to build a 'safety factor' into any particular design, at least in the animal kingdom. Living tissue requires energy merely to maintain its normal anatomico-physiological form. This is particularly so in the case of the central nervous system (CNS), which has a very high metabolic rate. Therefore, it is reasonable to hypothesise that there will be a selection pressure to optimise the size of any particular species' CNS to a working minimum, thus avoiding the energy 'penalty' that would be incurred by maintaining redundant tissue. This should apply to all the components of the CNS.

There is a second and possibly more important influence at work in the evolution of the CNS. Henneman et al. (1965) showed that the size of a neuronal soma affected its physiological sensitivity. In a population the smaller the cell, other things being equal, the more sensitive to depolarization it becomes. Therefore, it is possible to envisage a process whereby, as populations of neurons evolve they will, by a process of natural selection, change in size. This will in general, though not necessarily invariably, be a decrease. It seems reasonable to assume that the overall effect of such a decrease would be an increase in 'sensitivity' to incoming stimuli. That would be reflected in the overall behaviour of the species and it is on this that natural selection may act. It would be unreasonable to hypothesise such a selection pressure acting solely on the somata of nerve cells. It is more than probable that

this would also affect the other receptive elements of the neuron, the dendrites. Furthermore, the inputs could be similarly selected for and it is possible to see how a complete heuristic of neuron populations could evolve optimum size distributions of all their elements to provide maximum refinement of function. Indeed, as we study the evolution of the nervous system we observe a general increase in complexity of behaviour, with animals being able to discriminate progressively smaller changes in their environment and to produce more refined responses to those stimuli. Brodal (1969) has noted that "In the phylogenetic scale the relative proportion of large cells decrease". This is evident in comparative anatomy of the medial reticular formation and corpus striatum.

The effect that these pressures would have on the size distributions of the neuronal characteristics under discussion, marked positive skewing, has been discussed at length by Howard (1981a, 1981b).

The finding that the skewness of the internodal length distribution of dendrites increases disto - proximally is interesting. Rall (1964) has provided a convincing model that the effect of a single synapse on the resting membrane potential of a neuron, other things being equal, increases as it is moved proximally. It is possible that the 'hardwired' part of the neuronal circuitry impinges upon the soma and proximal dendrites, while the 'softwired' neuronal circuitry terminates on more distal dendrites, the latter being able to respond in a plastic manner to the environment. This would certainly be supported by Uyling's data. It may be interpreted as showing that there is an increasing design constraint on the lower limit of dendritic internodal length, disto - proximally.

The data from the synaptic studies are less concrete. There may be mixtures of terminals both within and between populations of axons and there is the problem of 'crossfire' in the interpretation of autoradiographs. However, the findings are consistent with the pattern observed in the other structures under consideration. This lends credance to the idea mootedby Vrensen et al. (1982) that a pure population of neurons will give rise to a population of synapses with a specific size distribution. We predict that these will almost invariably be positively skewed distributions.

In conclusion, we have observed a pattern in the shape of distributions of various structures within the

CNS, associated with information processing. We predict that this will prove to be a general rule which can be interpreted (Howard, 1981b) to indicate that the environment acts as a negative feedback on the CNS through the evolutionary ploy of 'size optimality'. Populations of neurons and their processes progressively evolve smaller size distributions, thereby increasing the sensitivity of the heuristic system. We propose that this process be called 'neuromorphotaxis'. It can be imagined to work in conjunction with Kapper's neurobiotaxis' in a complementary way, as evolution progresses.

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