Morphometric Characterization of Central Molluscan Neurons

The central neurons of the pulmonate gastropod *Cryptomphallus aspersa*, mainly the giant ones, have been proved to be a useful model for both neurophysiological[1] and morphological[2] purposes. In this paper, three problems will be discussed in relation to central neurons of *C. aspersa*: their size, their basophilia and the interrelationship among the types, classically described as a product of poliploidy.

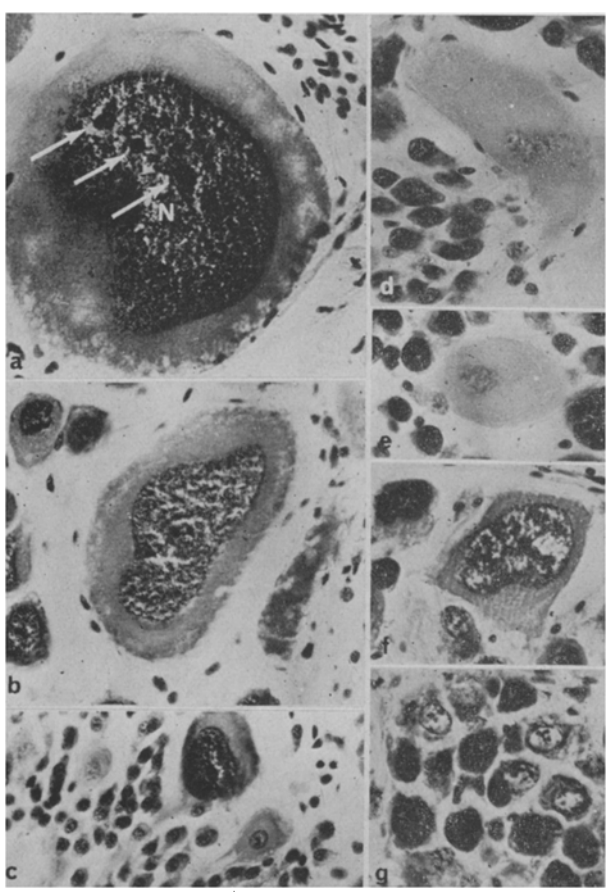
The abdominal ganglionic mass of 6 adult specimens, each of them of about 7 g, were used. The whole nervous periesophagic ring was excized, pinned on a cork sheet and plunged into Carnoy fixative[3] for 2 h at room temperature. They were then dehydrated, cleared in terpineol and embedded in Paraplast. Frontal serial sections were cut at 8 µm and stained with Gallocyanin-chrome alum according to EINARSON[4, 5] at pH 3.4.

For each cell type 50 measurements were taken[6]. The cells to be measured were selected by a random method and, since serial sections were used, the necessary care not to measure the same cell twice was taken. The maximal and minimal diameters were determined with an ocular micrometer. After the calculation of the statistically descriptive parameters (mean, standard deviation, standard error, total correlation coefficient, etc.), an analysis of variance for all the cell types was performed.

Figures a), b) and c) show neurons of the types conventionally named here A, B and C, whereas the Table I shows the mean and standard deviation of maximal and minimal diameters of these cell types. Glial cells (Figure g) are also included in Table I as they were studied in comparison to the neuron populations. Table I shows a distribution of data in 3 populations with widely separated diameter magnitudes. The total correlation coefficients for both diameters, tested by a *t*-test, were significant at the 10% level.

The significance of the difference between diameters of the 3 neuron types was tested by an analysis of variance, of which the results are shown in Table II. All the ratio of variances obtained were significative for the 0.1% level, and this fact provides a good statistical basis for the hypothesis of the difference between the mean maximal diameters of these cells. The sizes of *C. aspersa* giant neurons are comprized in the range between 30 µm (*Ferrissia shimekii*[7]) and 800 µm (*Tritonia diomedia*[8]), the latter being the biggest neurons found in molluscs. However, in no case was statistical treatment mentioned.

In the 3 neuron types, the basophilia does occur as a finely particulated material which fills all the cytoplasm. This is in agreement with classical description[9], whereas other authors have reported irregular patches similar to



a) Type A (giant) neuron, showing in the nucleus (N) 3 distinct nucleoli (arrows) and few unstained vacuoles in the cytoplasm (×432). b) Type B neuron (×432). c) Type C neuron (×432). d) and e) 'Chromatolitic' images (×440). f) Atypical neuron showing clumped chromatin (×440). g) Glial cells with dark and clear nuclei (×1930).

Table I.

Cell type	Diameter	N	$\bar{x}$	Standard deviation
A	maximal	25	94,2	21,0
	minimal	25	67,4	17,4
B	maximal	25	40,4	7,9
	minimal	25	33,1	8,7
C	maximal	25	22,1	3,8
	minimal	25	18,2	3,2
Glial	maximal	25	6,30	1,09
	minimal	25	5,03	0,85

Table II.

Comparison	N	F
A–B–C-Glial	100	280
A–B	50	143
B–C	50	109
C-Glial	50	40
A–C	50	284
A-Glial	50	436
B-Glial	50	454

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Table III.

Comparison	D.F.	t	P
A–B	48	0,053	>95%
B–C	48	0,100	>90%
A–C	48	0,021	>98%

those of the vertebrates Nissl bodies, such as in *Helix pomatia*[10] and in *Lymnaea stagnalis*[11]. In the light of current knowledge, the intensity and arrangement of the basophilia reflect more a physiological stage than a constant and conspicuous feature. Moreover, there is evidence that basophilia seems to have variations in correlation with the phasic periodical activity of adductor muscles in *Anodonta cygnea*[12]. By cytophotometric analysis, it was also found that concentration of cytoplasmic RNA is equivalent to the total nucleic acids (DNA + RNA) within the nucleus[12]. Typical images of 'chromatolysis' were found at random in the ganglia studied, as is shown in Figures d) and e) while others show atypical distribution of the chromatin (Figure f). Both 'anomalies' did not exceed 2% of the neurons.

Finally, another point to be stressed is the hypothesis, advanced by many authors[13–15], related to the poliploid nature of molluscan neurons. As is well known, the degree of poliploidy causes nuclear (and total) enlargement of the cell, and a ratio 1:2:4 among the types can be expected[7]. Nuclear sizes, as well as maximal and minimal cell diameters, were studied related to this ratio, but since these data show a significative correlation, only the statistical treatment for the maximal cell diameter will be given here. This relationship was tested making a t-test of the null hypothesis of the difference between the mean of a given diameter and the mean of the other times the assumed coefficient ($\bar{x}_1 - n\bar{x}_{1+k} = 0$). For this purpose the pooled variance was the same of the analysis of variance. All values obtained (see Table III) show that the null hypothesis cannot be disproved, in consequence it is possible to assume safely that these differences are not significant, and the 1:2:4 ratio can be statistically proved. Assuming a generalized elipsoidal shape for these neurons, their volumes were estimated in about 3.2×10^4 μm^3 for the type C, 2×10^5 μm^3 for the type B and 2×10^6 μm^3 for the type A. These data gave a ratio among the volumes of the types of 1:6.3:63.

The presence of at least 2 different cell populations was reported in *Aplysia californica* after the measurement of the DNA content in central giant neurons, by a fluorescent method[16]. The enormous amount of DNA found per nucleus suggest that a part or all the genome replicates many times[16]. The occurrence of poliploidy in mammalian central neurons has also been reported[17]. However, no definite conclusions on the possibility of a functional significative increment of the DNA in such neurons appear well established[18].

Resumen. Se realiza en la masa ganglionar ventral del molusco *Cryptomphallus aspersa* (Gasteropoda, Pulmonata) la caracterización morfologico-estadística de las neuronas centrales y células gliales. Se establece la existencia de tres tipos neuronales, las características de su basofilia y la relación entre los tipos, lo que se discute en relación a los hallazgos obtenidos en otras especies por diversos autores.

C.A. SANCHIS and R.J. CASTRO

Instituto de Anatomía General y Embriología, Facultad de Medicina, and Departamento de Ciencias Biológicas, Facultad de Farmacia y Bioquímica, Buenos Aires (Argentina), 27 July 1971.

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[18] Acknowledgments. The authors are deeply indebted to Prof. E. DE ROBERTIS for the valuable advice given during the preparation of the manuscript, and to Mr. R. CALCAGNO for his skillful assistance. Supported by a grant from the Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina.

Sialic Acid in Human Cancer

The relative proportion of the two main sialic acids, N-acetyl-neuraminic acid (NANA) and N-glycolyl-neuraminic acid (NGNA), varies considerably in different mammalian species; but man seems to be unique in that only NANA has been found[1]. In human cancer, quantitative changes in sialic acid have been reported. Analyses of several types of human cancer tissues revealed that the area of malignancy contained almost twice as much sialic acid as the normal areas of the same tissues[2].

Qualitative changes in sialic acids have been found in experimental cancer. Reports have indicated that although normal rat liver does not contain NGNA, this form of sialic acid was observed in hepatomata induced by the feeding of *p*-dimethylaminobenzene[3] and in several rat ascites hepatoma[4].

These specific chemical changes associated with malignancy, and the recent discovery of NGNA[5] in Hela S_3 cells (a line of cells of human cancer origin) prompted this qualitative and quantitative study of the sialic acids in human cancer tissue.

Materials and methods. Human cancer tissues were obtained from the autopsies of cancer victims while normal

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