CHANGES OF NEURONES DIMENSIONS AS A POSSIBLE MORPHOLOGICAL CORRELATE OF THEIR INCREASED FUNCTIONAL ACTIVITY

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INTRODUCTION

The changes in the size of neurones, brought about by their functional activity, naturally attract the attention of neurohistologists. Establishment of modifications in nuclear and cytoplasmic dimensions because of a neurone's activation would give a reliable morphological correlate of the increased neuronal activity. Using it, the neurohistologist may determine the spatial distribution of the active neuronal ensembles responsible for carrying out some complex brain functions.

Is the increase of the neuronal activity always accompanied by changes of nuclear and cytoplasmic dimensions of the neurones involved? If so, what is the direction of the changes? The answers to these questions were given as early as the end of last century, when the general scheme of functionally determined changes in the size of nerve cells was propounded[24]. According to this scheme the dimensions of a neurone's nucleus and soma are increased with excitation, and decreased with 'fatigue' and 'exhaustion' caused by prolonged and intensive stimulation. This scheme was used for the interpretation of many experimental results, obtained later, but the analysis of the data existing in the literature undermines its validity.

Firstly, several authors[1,3,6,15,27,28,30,33,38] were unable to record any changes of neuronal size with activity. A great number of investigations, though, have shown modifications of the dimensions of a neurone's nucleus and soma with increased functional activity, but there is no agreement on the direction of these changes. Some workers found an increase in neuronal volume as a result of their stimulation[2,4,8,9,11,12,14,20,21,26,37,39,40,42]. Others, on the contrary, showed a decrease in the nuclear and somal sizes of the neurone with its activity[5,13,17,19,22,23,32,34,35]. Finally, some reports[7,10,25,29,31,41] described first an increase and then a decrease of the nuclear and somal dimensions in an active neurone.

Such a discrepancy of results obtained by different investigators prevents a clear conclusion about trends in the functionally determined changes of the dimensions of nerve cells. It might be thought that these contradictions were explained by the

use of various means of stimulating the neurones, and with the investigations of different structures of the nervous system on different experimental subjects. But conflicting results were achieved even in similar experiments conducted on the same species. For example, Vas[41] and Mann[31] found an increase in the nuclear and somal dimensions in the cells of the rabbit's superior cervical sympathetic ganglion after 15-min electrical stimulation, whereas Lambert[28] chould not detect such changes. Guerrini[19] showed that the nerve cells of the cerebral cortex and anterior horn of the spinal cord of pigeons decreased their dimensions after prolonged flight. But measurements made by Kocher[27] failed to demonstrate any changes.

The results described seemingly pointed to faulty methodology as a main reason for the heterogeneity of the results obtained by different authors. Hence, in the present investigation we aimed: (1) to work out an adequate method to study the functionally evoked changes of neuronal dimensions, and (2) to try to determine with the help of this method what are the changes, if any, in the neurone's nuclear and cytoplasmic volumes in the course of an increase of its functional activity.

MATERIAL AND METHODS

Big motoneurones were measured in the anterior horn (layer IX by Rexed[36]) of the 5–6th lumbar segments of the spinal cord of adult male albino rats, weighing 200–240 g. Eleven groups of control and 10 groups of experimental animals (one group for each period of stimulation) were used, each group consisting of 5 animals.

Spinal motoneurones were stimulated either by intensifying the natural motor activity or by giving electrical shocks to dorsal roots. In experiments with natural activation of motoneurones the rats were made to swim in water warmed to 33–35°C. These experiments included two series. In the 'A' series the rats swam with an attached load of 1/33 of their body weight. In the 'B' series the load was 3 times increased. The mean duration of swimming before the period of diving was 375 ± 55 min for the A, and 50 ± 7 min for the B series. These values were obtained for 20 rats in each series. Experimental animals were decapitated 5, 10, 20 and 40 min after beginning the swimming. The control rats were handled but did not have to swim at all. In experiments with orthodromic stimulation of the motoneurones the rat's spinal cord was preliminarily fully transected at the level of C_1–C_2. On the next day ventral and dorsal roots were cut bilaterally from L_4 to S_1. After 30 min the left dorsal roots of the L_5–L_6 segments were stimulated by rectangular pulses of current with a duration of 2 msec and a potential of 1 mV. The rate of the stimulation was 1 imp./sec and the intensity was supramaximal for evoking the combined mono- and polysynaptic reflex, which ensured the involvement of the majority of the motoneurones. The experimental rats were decapitated 10 and 40 min after the beginning of the stimulation. The control rats were treated exactly as the experimental ones, except for the stimulation.

The lumbar enlargements of the spinal cord were immersed for fixation in Carnoy's solution not later than 3 min after decapitation and kept there for 15 min at room temperature. This fixative was chosen as, according to Friede[16], it enables one to

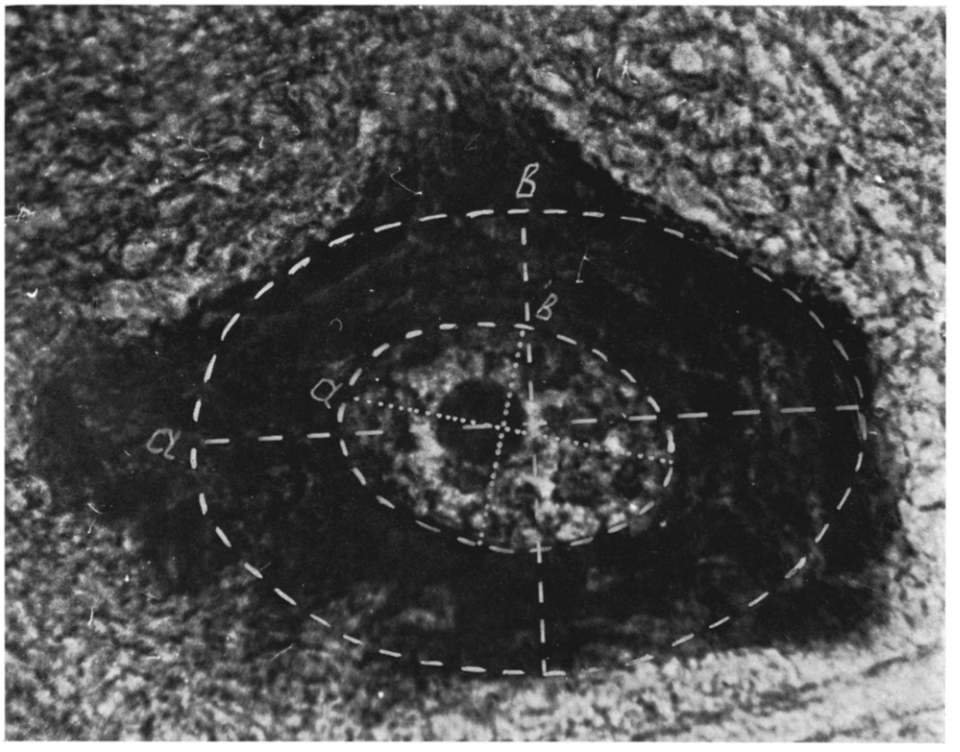

Fig. 1. Microphotograph of a spinal motoneurone taken in UV irradiation at 265 nm wavelength. Ellipses inscribed into the cell body or nucleus outlines are shown by a broken line. The 'a' and 'b' lines are major and minor axes of the ellipses. × 1500.

record only vital changes of the cell volume and prevent artifacts of alterations post-mortem. After dehydration in alcohols of increasing concentrations (70, 96 and 100% ethanol for 30 min at room temperature) and extraction of lipids with chloroform (two changes for 30 min each at room temperature) the tissue was embedded in paraffin. The series of transverse sections of the spinal cord were made with a microtome adjustment to 5 μm. The sections were placed on quartz slides, and after removal of the paraffin, were mounted in glycerol under quartz coverslips. The sections were photographed under the ultraviolet microscope MUV-6 at 265 nm, using a quartz objective 50 × and quartz ocular 3 ×. On the 2300 times enlarged cell negative projections the ellipse was inscribed into the outlines of the cell body or nucleus. The largest (a) and, perpendicular to it, the smallest (b) axes of the ellipse were then measured (Fig. 1). The volume (V) was calculated from these linear dimensions according to the following formulae, deduced from an optical reconstruction:

$$V_{\text{cell body}} = 1/6\ \pi\ ab\ \frac{a+b}{3};\ V_{\text{nucleus}} = 1/6\ \pi\ ab^2;$$

$$V_{\text{cytoplasm}} = V_{\text{cell body}} - V_{\text{nucleus}}$$

TABLE I

SPINAL MOTONEURONAL NUCLEAR AND CYTOPLASMIC VOLUMES FOR DIFFERENT GROUPS OF CONTROL RATS

Group	*Number of neurones measured*	*Mean volume and standard error of the mean (cu. μm)*	
		Nucleus	*Cytoplasm*
1	151	2329 ± 44	23969 ± 591
2	155	2114 ± 43	20213 ± 554
3	150	1948 ± 40	17474 ± 499
4	153	1876 ± 42	16763 ± 444
5	161	1854 ± 35	16535 ± 444
6	152	1678 ± 34	15650 ± 445
7	150	1664 ± 35	15500 ± 408
8	153	1643 ± 30	15285 ± 386
9	156	1605 ± 30	14475 ± 405
Greatest difference		45.1%	65.6%
Level of significance		$P_{1-9} < 0.001$	$P_{1-9} < 0.001$

TABLE II

CYTOPLASMIC VOLUME, CYTOPLASMIC RNA CONCENTRATION AND CONTENT IN SPINAL MOTONEURONES FOR DIFFERENT GROUPS OF CONTROL RATS

Group	*Number of neurones studied*	*Mean values and standard errors of the mean*		
		Cytoplasmic volume (cu. μm)	*RNA concentration (pg/cu. μm)*	*RNA content (pg)*
1	151	23969 ± 591	0.0248 ± 0.0008	587 ± 19
2	155	20213 ± 554	0.0286 ± 0.0005	576 ± 16
3	150	17474 ± 499	0.0352 ± 0.0008	596 ± 18
4	153	16763 ± 444	0.0362 ± 0.0010	582 ± 18
9	156	14475 ± 405	0.0418 ± 0.0009	584 ± 18
Greatest difference		65.6%	68.6%	3.5%
Level of significance		$P_{1-9} < 0.001$	$P_{1-9} < 0.001$	$P_{2-3} > 0.4$

Measurements were taken only of motoneurones with the nucleolus in the plane of the section. The tissue shrinkage correction was not introduced into the calculations.

The numerical results obtained were treated statistically with the help of Student's *t* test, the empirical distributions being found not significantly different from normal.

RESULTS

In the course of investigations we observed that different groups of control rats varied widely in the nuclear and cytoplasmic volumes of their motoneurones

(Table I). The amplitude of the control parameter variation was so great that it might obscure the expected differences between control and experimental values. One of the possible reasons for such a wide scatter could be the error of measurement. But the random error of the measurements, expressed as the variation coefficient of 20 independent determinations of the same cell structure volume, did not exceed $\pm$ 5% either for the nucleus or for the cytoplasm of motoneurones.

The real reason for the divergence in mean nerve cell size among different groups of control rats emerged when the animals were studied for the RNA concentration and content in the cytoplasm of spinal motoneurones with the help of a photographic modification of ultraviolet cytospectrophotometry (the details of the photometric procedure will be published elsewhere[18]). As may be seen from Table II, the differences in motoneuronal cytoplasmic volume between control groups were accompanied by rather pronounced variations in cytoplasmic RNA concentration. In spite of that the RNA content of motoneuronal cytoplasm was practically the same for all groups of control animals. The observed stability of the cytoplasmic RNA content was connected with the reciprocal relation between cytoplasmic volume and its RNA concentration: the lower the average motoneuronal cytoplasmic volume of a certain group of rats, the higher the mean concentration of cytoplasmic RNA. Evidently, the degree of shrinkage of nerve cells under histological treatment was different for different groups of control rats. This is easily explained by the fact that the spinal cord preparations of various control groups were made in different seasons of the years 1962–1967. So the conditions of fixation, dehydration and lipid extraction were not identical. The main role, apparently, was played by the differences in room temperature as well as by the quality of the reagents.

The necessity for identical histological treatment of nervous tissue for obtaining comparable results in the study of neuronal size changes was emphatically displayed during special investigations of two groups of control male rats, in matched pairs of equal body weight. In the making of histological preparations one animal of a pair was allotted to group 10, the other to group 11. The lumbar enlargements of the rats making a pair were fixed, dehydrated together and embedded in the same paraffin block, from which sections of the spinal cords of both animals were made simultaneously. Thirty motoneurones from each animal were measured, and each group consisted of 5 rats. Comparison of these two groups of control animals showed that the mean values of their motoneuronal nuclear and cytoplasmic volumes were practically the same (Table III). The distribution histograms of the volume values were also similar, as proved by absence of significant differences by χ^2 criterion. Incidentally, the concentration of cytoplasmic RNA was also practically identical in these two control groups (Table III).

So the comparison itself of control and experimental groups of rats was conducted using the above-mentioned methodological precautions. Control and experimental rats were matched by sex, age and body weight. Histological treatment of the control and experimental preparations was conducted simultaneously, then 30 motoneurones were measured for each rat, and each group consisted of 5 animals. These methodological conditions guaranteed that biological and individual variability

TABLE III

NUCLEAR AND CYTOPLASMIC VOLUMES, CYTOPLASMIC RNA CONCENTRATION AND CONTENT IN SPINAL MOTONEURONES OF TWO GROUPS OF CONTROL RATS AFTER SIMULTANEOUS HISTOLOGICAL TREATMENT

Parameter	*Number of neurones*		*Mean values and standard errors of the mean*		*Difference (%)*	*P >*	
	Group 10	*Group 11*	*Group 10*	*Group 11*		*By t test*	*By χ^2 test*
Nuclear volume (cu. μm)	150	150	2201 ± 39	2239 ± 40	1.7	0.4	0.9
Cytoplasmic volume (cu. μm)			21285 ± 535	21798 ± 581	2.4	0.5	0.7
RNA concentration (pg/cu. μm)			0.0274 ± 0.0006	0.0265 ± 0.0006	3.4	0.2	0.7
RNA content (pg)			567 ± 18	565 ± 19	0.4	—	0.3

TABLE IV

SPINAL MOTONEURONAL NUCLEAR AND CYTOPLASMIC VOLUMES IN CONTROL AND EXPERIMENTAL RATS

Type of experiment	*Stimulation duration (min)*	*Number of neurones measured*		*Nucleus*				*Cytoplasm*			
				Mean volume and standard error of the mean (cu. μm)		*Per cent of control*	*P*	*Mean volume and standard error of the mean (cu. μm)*		*Per cent of control*	*P*
		Control	*Exptl.*	*Control*	*Exptl.*			*Control*	*Exptl.*		
Swimming, A series	—	156	—	1605 ± 30	—	—	—	14475 ± 405	—	—	—
	5	—	162	—	1717 ± 29	107.0	< 0.01	—	14762 ± 448	102.0	> 0.6
	10	—	154	—	1676 ± 27	104.3	> 0.05	—	14257 ± 332	98.5	> 0.6
	20	—	185	—	1682 ± 26	104.8	> 0.05	—	15362 ± 380	106.1	> 0.1
	40	—	184	—	1601 ± 27	99.8	> 0.9	—	13292 ± 369	92.7	< 0.05
Swimming, B series	5	153	159	1643 ± 30	1594 ± 24	97.0	> 0.2	15285 ± 386	15640 ± 499	102.3	> 0.5
	10	152	151	1678 ± 34	1737 ± 39	103.5	> 0.2	15650 ± 445	14886 ± 408	95.1	> 0.2
	20	150	150	1664 ± 35	1659 ± 34	99.7	> 0.9	15500 ± 408	15951 ± 424	102.9	> 0.4
	40	161	156	1854 ± 35	1504 ± 25	81.1	< 0.001	16535 ± 444	12605 ± 351	76.2	< 0.001
Orthodromic stimulation	10	153	146	1876 ± 42	1876 ± 40	100.0	> 0.9	16763 ± 444	18483 ± 497	110.3	< 0.01
	40	150	156	1948 ± 40	1750 ± 31	89.8	< 0.001	17474 ± 499	15292 ± 411	87.5	< 0.001

effects, as well as measurement errors, being small and not statistically significant for the groups of control animals under comparison, would not obscure the experimental results.

The study of the changes in the spinal motoneuronal nuclear volume with the increase of their functional activity showed (Table IV) that comparatively mild motor activity (swimming, series A) led to an increase in nuclear size during the earlier period of stimulation (5 min). With continued swimming the values gradually returned to normal, and 40 min after the beginning of the experiments the motoneuronal nuclear volume of experimental animals did not differ from the control values. Severe motor activity (swimming, series B) and orthodromic electrical stimulation evoked similar changes in the motoneuronal nuclear dimensions. In the beginning of the stimulation no significant changes were found, but with the longest duration of the activation (40 min) the nuclear volume significantly decreased.

The measurements of the cytoplasmic volume revealed (Table IV) that, in experiments with swimming, series A, statistically significant changes were obtained only 40 min after the beginning of motor activity and consisted in a decrease in comparison with the control. Similar changes were recorded with the swimming, series B, and more intensive motor activity in this series was accompanied by a more pronounced decrease of motoneuronal cytoplasmic volume. Orthodromic electrical stimulation of 10 min duration resulted in an increase of cytoplasmic volume, which was replaced by a decrease 40 min after the beginning of the stimulation.

DISCUSSION

The observations reported here are of a certain interest because they help us to understand the reasons for contradictory results obtained by different authors, who investigated functionally produced neuronal size changes. Relative changes of the nuclear and cytoplasmic volumes, evoked by experimental conditions, could be detected with enough reliability only by strictly following certain methodological precautions. The main precautions were: (1) control and experimental animals must not differ in sex, age and body weight; (2) histological treatment of control and experimental material must be conducted simultaneously; (3) sufficient neurones from each animal, as well as enough animals in the control and experimental groups, must be studied to lessen the effect of biological and individual variability and measurement errors on the final results. Unfortunately, the formulated conditions were not kept in mind by many investigators interested in the problem. This may be one of the reasons for the divergence of results reported by various authors. But analysis of the results presented in this paper reveal another cause of the contradictoriness.

The consideration of our experimental data demonstrates the dependence of neuronal size changes on the duration, intensity and character of stimulation (Table IV, Fig. 1). The duration of the functioning determined the direction of the neuronal size changes. Relatively short stimulation brought about an increase in the volume of the nucleus (swimming, series A) and of the cytoplasm (orthodromic stimulation) in

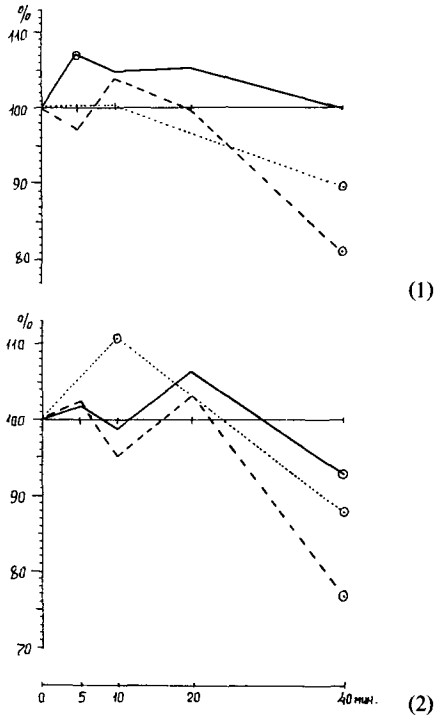

Fig. 2. Changes in motoneuronal nuclear (1) and somal (2) size, during swimming, of 'A' series (unbroken line), 'B' series (broken line) and orthodromic stimulation (dotted line). On the ordinate are shown deviations of mean dimensional level in percentage of the control. On the abscissa is plotted the duration of the experiments in minutes. The statistically significant shifts are indicated with circles.

motoneurones. Prolongation of the stimulation resulted in a decrease of the motoneuronal nuclear and cytoplasmic dimensions. The intensity of the stimulation influenced the extent of the changes. With the same duration of swimming (40 min) the motoneuronal cytoplasmic volume depended on the intensity of motor activity: in series A the cytoplasmic size decreased by 7.3%, and with 3 times enhancement of the load (series B), it decreased 23.8%. The stimulation character influenced the pattern of neuronal volume changes. Artificial activation of spinal motoneurones by electrical orthodromic stimulation led first to an increase (10 min) and then to a decrease (40 min) of the cytoplasmic volume of these nerve cells. On the other hand, the natural activation of spinal motoneurones by swimming evoked no significant changes in the earlier period of the stimulation.

So, duration, intensity and character of the stimulation determine the direction, extent and general pattern of the neuronal volume changes. This makes evident another reason for the ambiguity of the data reported by different investigators concerned with functionally evoked changes in the volume of nerve cells. As most of the authors employed, as a rule, only one means of neuronal activation, often without varying either duration or intensity of the stimulation, they happened to

witness sometimes an increase, sometimes a decrease of the neuronal volume, sometimes even an alternation of these changes.

In the present study the effects of the stimulation are compared by their duration, intensity and character, which made it possible to detect a certain tendency in the spinal motoneuronal volume changes with an increase of their functional activity. This trend is the progressive decrease of nuclear and cytoplasmic volumes with increase in duration and intensity of a single applied functional load. Therefore it may be supposed that the decrease of the motoneuronal nuclear and cytoplasmic volumes may be considered a morphological correlate of their increased functional activity. Further investigations will show how general is this morphological correlate of an active state of nerve cells, *i.e.*, whether it also characterizes the neurones of other structures of the nervous system and other ways of stimulation.

SUMMARY

Effects of increased functional activity of spinal motoneurones were studied morphologically on rats in experiments with swimming under different loads and with orthodromic electrical stimulation of 5–40 min duration. The volume of lumbar motoneurones was calculated from major and minor axes of ellipses inscribed into cell body or nuclear outlines on cells' negative projections. Formulae for volume calculations were derived from an optical reconstruction.

Both mild and intensive natural motor activity, as well as orthodromic stimulation, resulted in a decrease of the motoneuronal cytoplasmic volume in the longest duration (40 min) of the experiments. The changes of cytoplasmic dimensions were accompanied by a decrease of the nuclear volume after 40 min intensive swimming and orthodromic stimulation. The increase of motoneuronal size was registered only in earlier periods of activation: 5 min after the beginning of mild swimming for the nucleus, and 10 min after the beginning of orthodromic stimulation for motoneuronal cytoplasm. Thus the motoneuronal size changes depended on the duration, intensity and character of the stimulation. The results obtained make it seem that the decrease of the spinal motoneuronal nuclear and cytoplasmic volume might be considered a morphological correlate of their increased functional activity.

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