ACTIVITY-INDUCED MORPHOMETRIC ALTERATIONS IN VENTRAL MOTONEURONS

Thesis for the Degree of M. A.
MICHIGAN STATE UNIVERSITY
Elizabeth Ann Leistikow
1977

ABSTRACT

ACTIVITY-INDUCED MORPHOMETRIC ALTERATIONS
IN VENTRAL MOTONEURONS

By

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Two physical-activity treatments of two durations were conducted to investigate the alterations in soma, nucleus, and nucleolus sizes of ventral motoneurons in 24 adult male albino rats (Sprague-Dawley strain). Endurance-running (END) and weight-lifting (WL) were selected as activity treatments and administered for eight-week (8-wk) and sixteen-week (16-wk) durations. Neurons located in the lumbar enlargement Lamina IX were selected for study. These neurons provided the motor innervation to the plantar-flexor muscles which were activated by the treatments.

Following the histological preparation of serial cross-sections, the motoneuron images were projected and sixty images were traced. The tracings of the motoneuron soma, nucleus, and nucleolus were measured by planimeter.

Two-way analyses of variance revealed significant ($P < .05$) differences in body weights of the experimental groups, but non-significant differences between experimental groups in soma, nucleus, and nucleolus areas. Both durations of the WL and END groups weighed less than the control animals. In effect, the two activity treatments

administered for both durations, either resulted in no distinguishable changes in motoneuron morphometry, or allowed for adaptation that was indistinguishable from control levels of motoneuron morphometry.

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF ARTS

Department of Health, Physical Education, and Recreation

1977

Dedicated to:

My parents:

The most wonderful people in the world, who have contributed so much in so many ways and still continue to grow.

My brothers:

For their camaraderie, efforts, hopes, and dreams.

My friends and relatives:

For their caring. To those of Uncle Cecil's living.

Waterloo, Iowa and West High School

Michigan State University

The Future

ACKNOWLEDGMENTS

I am appreciative of my advisor, Dr. Wayne D. Van Huss, for his scientific curiosity and discipline.

Much appreciation is due Dr. Roland R. Roy, Department of Biomechanics, for his intellectual stimulation during the course of study and patient guidance in the preparation of this manuscript.

A special word of thanks is extended to Dr. James H. Stapleton, Department of Statistics, for guidance with the statistical treatment of the data.

A word of appreciation to Dr. Kwok-Wai Ho, Department of Health, Physical Education, and Recreation, and Dr. James F. Taylor, Oklahoma College of Osteopathic Medicine and Surgery, for initiating the physical-activity investigation. Gratitude is due Dr. Rexford E. Carrow of the Departments of Anatomy and Pathology for use of the laboratory facilities and equipment, and to Richard Dey for instruction in histological techniques.

Special thanks are extended to Mrs. Barbara A. Wheaton and Mrs. Nina L. Miller, Department of Pathology, for their technical judgement, criticisms, and encouragements.

A very special word of thanks is extended to the Rutledges, Dr. Walter and Mrs. June Mack, Mrs. Nancy Marshall, Dr. Charles Sander, Mrs. Carolann Lyon, and Suzanne and Bill Wuerthele.

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CHAPTER I

THE PROBLEM

Present evidence for morphometric adaptations in ventral motoneurons to activity changes is inconclusive. Results of some experiments using chronic physical activity suggest that decreased size of structural elements (soma, nucleus, and nucleolus) accompanies increases in intensity and duration of the activity regimens (13, 14). Other chronic physical-activity treatments have resulted in no changes (11) or decreases (42) in motoneuron morphometry. Studies of morphometric alterations in motoneurons due to acute physical activity yielded conflicting results. Increases (6, 11, 29, 31, 42), decreases (1, 7, 12, 36), and no changes (11, 28) in the size of the soma, nucleus, and nucleolus all have been reported.

Need for the Study

Additional investigations of neuronal adaptations to chronic physical activity are needed. Changes induced by a power-type activity and after an extended treatment duration should be studied. Integration of the results from such investigations may help to explain the changes that have been observed within the neuromuscular system (See Chapter II) as a result of specific activity treatments.

Statement of the Problem

This study was undertaken to determine the effects of two different treatment programs after two durations on the size of the soma, nucleus, and nucleolus of ventral motoneurons innervating the working hindlimb musculature. One activity regimen was selected to require mainly aerobic metabolism (endurance running). The other activity was designed to require more strength and possibly more anaerobic metabolism (weight-lifting). Eight and sixteen weeks were selected for treatment durations.

Research Plan

Normal adult male rats were used as subjects. The activity treatments were selected to involve the neuromuscular units which comprise the triceps surae and plantaris muscles (hindlimb plantar flexors) and their spinal motor innervation in the lumbar enlargement. The neurons located in the right Lamina IX (37) of the lumbar enlargement were studied.

The activity treatments were designed so that one required primarily aerobic metabolism (endurance running), whereas the other possibly required more anaerobic metabolism (weight-lifting) within the motor units. In general, the treatments were administered daily, Monday through Friday, for eight- and sixteen-week durations. The treatments were administered in controlled-running wheels (43) and the controlled weight-lifting chambers (Appendix B).

The lumbar enlargements were fixed, dehydrated, cleared, and embedded. Cross sections were cut (7 micra) from the

paraffin-embedded tissue, mounted on leader film, stained in Luxol fast blue, and counterstained in cresyl echt violet (14, 41). Neuron images were projected, morphologic elements were traced, and areas of the elements were measured. The morphologic elements that were measured by compensating polar planimetry in each neuron were the soma, nucleus, and nucleolus.

Rationale for Method

The treatment wheels and chambers were designed for use with the rat. The endurance-running treatment has been shown to increase aerobic muscular capacity (21, 39) and was intended to simulate endurance-training for human performers. In this study the weight-lifting treatment was used for the first time.[1] The weight-lifting treatment was designed with the intent of increasing anaerobic muscular capacity of the experimental animals. The eight- and sixteen-week durations were selected to show the pattern of motoneuron-morphometric adaptation.

Limitations of the Study

1. The methods of formalin fixation and paraffin embedding have been criticized for causing variable shrinkage in cells (17).

2. The sacrifice time, i.e., the time between the last activity treatments and killing, varied by twenty-four hours between the two treatment durations.

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[1] Human Energy Research Laboratory, Michigan State University, East Lansing, Michigan 48824.

3. The investigation did not include a control for the electrical shock that was used to motivate the animals. Previous investigation has suggested a direct relationship between the amount of shock received and the sizes of soma, nucleus, and nucleolus (14).

4. Due to practical considerations, the sample size was limited to four animals per cell.

5. The results may be specific to motoneurons in Lamina IX of the rat lumbar enlargement.

CHAPTER II

REVIEW OF RELATED LITERATURE

The review is divided into three sections. The first section includes a brief history of motoneuron-morphometric investigations with a rationale for a current neuromuscular approach to motoneuron-morphometric study. The second section is a discussion of previous comparable investigations emphasizing the one describing the most technically-comparable activity regimen. The significance of motoneuron size is considered in the third section.

Historical Overview of Motoneuron-Morphometric Studies

Motoneuron-morphometric investigation following functional activities has a long history. Specific, controlled physical activity as a motoneuron functional activity is a relatively-new development in the line of investigation. Hodge and his successors were among the first investigators of functional size changes (5, 6, 7, 8, 22, 23, 28, 31). Many functional activities were used, i.e., anemia, axon section, electrical stimulation, muscular fatigue, and shock. Morphometric changes varied from increases, to no changes, to decreases in cell size. The results require further definition in view of new technology and knowledge.

Many studies have been accumulating evidence for the effects of chronic physical activity on the skeletal muscle segment of the neuromuscular functional unit. The neuromuscular functional unit, the motor unit, is comprised of an α-motoneuron and the skeletal muscle fibers innervated by it. Structural (16, 27, 33), histochemical (3, 10), physiological (34), and biochemical (24, 25, 26) evidence has indicated shifts in the capacity of muscle as a result of changing functional demands. The question of what occurs in the neural segment during these shifts can be posed. It has been suggested that the shifts in muscular capacity may be mediated by altering functional demands on the neural segment of the motor unit (10). Shifts in the muscle fiber populations during growth (30) and experimental procedures, e.g., cross-innervation (18), also have been suggested to occur via the neural segment. Many neural mechanisms are being postulated and tested, such as axoplasmic flow, impulse conduction, transsynaptic transfer, nerve sprouting, and humoral factors. Thus far no proposed mechanism totally accounts for all observations, but the important concept remains that the neural elements are capable of influencing many characteristics of muscle fibers.

Previous Studies with Physical-Activity Treatments

Motoneuron morphometry has been studied following many types of functional activity. The effects of increased physical activity have been considered under both acute and chronic conditions. The neuronal-morphometric responses to acute activity, i.e., one

activity bout, have been variable. Soma increases (11), decreases (1, 12), and no changes (28) have been reported. Edstrom (11) ran guinea-pigs to exhaustion on a treadmill for 25-30 min and then killed them within 5 min. The observed cell bodies were 47% greater in volume than observed in control animals. The nuclei showed a slight, non-significant volume increase after the exhaustive activity. Aleksandrovskaya (1), Geinisman (12), and Kocher (28) used swimming as the means of neuronal activation. After 40 min of swimming, Aleksandrovskaya (1) reported a general decrease in the soma sizes with cyclic changes occurring through the 20th day following the activity. The cyclic changes decreased in amplitude during the 20-day period. Using 40 min of swimming of two intensities, Geinisman (12) reported decreases in soma size with the greater decreases in the more-intense activity group. No doubt some of the variation in all these investigative results are due to the diversity in experimental methods. The range in activities is readily apparent.

The chronic-activity investigations, i.e., long-term activity regimens, have inconclusive results, also. Edstrom (11) trained guinea-pigs for one month on a treadmill at 48 m/min pace. The motoneurons showed a non-significant size increase in the nucleolus. Tumanov (42) administered a 2-hr swimming treatment to rats bi-weekly for 6 months. The treated group, as well as an untrained group, were swam for 4 hr and then sacrificed with an unexercised control group included. The treated rats were observed

to have smaller soma sizes compared to the other two groups, and smaller nuclei compared to the acutely activated group.

Gerchman (13) compared the ventral-motoneuron morphometry in rats treated with three different physical-activity treatments for 52 days. The activity treatments consisted of: (A) sedentary housing with no forced activity; (B) sedentary housing with 30 min forced swimming daily while carrying 3% body weight; and (C) voluntary-activity housing with 30-min forced swimming twice daily while carrying 4% body weight. The results showed a non-significant decrease in soma sizes with increased activity loads. The nuclear sizes were not different between groups, but the nucleolar sizes of the B group were signficantly larger.

Gilliam (14) studied the pattern of motoneuron morphometry which resulted from treatments of various types, levels and durations of activities. The seven treatments were: sedentary control (CON); voluntary running (VOL), short-duration, high-intensity endurance running (SHT); medium-duration, moderate-intensity endurance running (MED); long-duration, low-intensity endurance running (LON); electrical stimulus control (ESC); and long-duration swimming (SWM). The LON treatment appears to be the most technically comparable to the END treatment of the present experiment. Both treatments involved a 36 m/min velocity in programs of a progressive nature. The four durations of treatment were: a pre-treatment control (0-wk); four treatment weeks (4-wk); eight treatment weeks (8-wk); and twelve treatment weeks (12-wk).

Gilliam measured the soma, nucleus, and nucleolus of sixty consecutive neurons per rat with four rats per treatment-by-duration group. The data were analyzed by analyses of contingency tables. The results showed some significant treatment and duration effects. The complexity of the seven-by-four experimental design and subsequent analyses of contingency tables complicated interpretation and discussion of the results. It appeared that there were specific motoneuron-morphometric responses to each treatment imposed. Gilliam categorized the experimental treatments for discussion purposes into two trios according to the morphometric alterations. The trios were: VOL, MED, and LON: and SHT, SWM, and ESC. Using these categories, he summarized the general treatment effect as larger motoneuron soma, nucleus, and nucleolus in the former trio (with the LON group having larger soma, nuclei, and nucleoli than the MED group) than the latter trio. Gilliam went on to summarize the general duration effect by noting in terms of soma, nucleus, and nucleolus sizes, the smallest at 0-wk, the largest after 4-wk, and a decrease after 8-wk followed by an increase after 12-wk. The complexity of the array of data analyses appeared to contrast the simplicity of the summaries.

In a subsequent report (15), the CON, LON, and SHT treatments at 0-wk and 12-wk were compared for effect on soma and nucleus sizes. In this simpler experimental comparison, the data showed significant distribution differences. The LON group, which was most technically comparable to the END group of this study, and the SHT group had a greater frequency of small somas and nuclei. The short group had the greatest number of small somas and nuclei.

Both acute- and chronic-activity treatments have produced inconsistent, and therefore, inconclusive effects on motoneuron morphometry. The types, intensities, and durations of activity varied greatly between investigations. This variation may have been responsible for the lack of consistent results. The activity treatment most comparable to the END group of this study resulted in increased numbers of small motoneuron somas and nuclei after twelve treatment weeks.

Motoneuron Size

The functional significance of motoneuron size has been explored electrophysiologically. To provide a predictor for motor unit output, a "size principle" for motoneuron recruitment has been proposed, elaborated, and delimited (2, 3, 19). Use of this principle summarized recruitment causal factors such as the synaptic input density, the qualitative and quantitative nature of various synaptic inputs, and the intrinsic neuron membrane properties. All of these features have been correlated with motoneuron size. Thus the "size principle" of motoneuron recruitment summarized a large amount of related information in a convenient simplification. A discussion of the forementioned causal factors is beyond the scope of this review, but has been presented elsewhere (2, 4, 46). In general, the larger motoneurons have been ascribed these properties: faster conduction velocities, larger spike potentials, higher thresholds of excitability, longer afterhyperpolarization times, increased accommodation and the ability to conduct more impulses per second. The properties

of smaller motoneurons have been contrasted directly to those of the larger motoneurons. Physiological evidence implied a size relationship of motoneurons to the motor unit muscle fiber type (9, 32, 45). In summary, motoneuron size has been interpreted as an indicator of motoneuron function and has been related to the total motor unit function.

CHAPTER III

RESEARCH METHODS

This study investigated morphometric alterations of ventral motoneurons resulting from two different physical-activity treatments. The activity regimens were designed to require motor units to function primarily aerobically or anaerobically. Treatment durations of eight and sixteen weeks were selected to follow possible neuronal adaptation patterns.

Treatment Groups

Thirty normal male albino rats[1] of the Sprague-Dawley strain were assigned randomly to three treatment groups following a standard 12-day period of adjustment to laboratory conditions. Treatments were started when the animals were 84 days old.

Weight-Lifting (WL) Treatment

The ten animals assigned to the weight-lifting (WL) treatment were housed in individual voluntary-activity cages during the adjustment period. Each cage (24 cm X 18 cm X 18 cm high) allowed access to a freely-revolving activity wheel (13 cm wide X 35 cm diameter). During the treatment period, the WL animals were housed

[1]Animals obtained from Hormonal Assay, Inc., Chicago, Illinois.

in individual sedentary cages (24 cm X 18 cm X 18 cm high). For the treatment administration, each animal was subjected to an interval-training program (Appendix A, Table A-1) in a controlled weight-lifting chamber (CWC, Appendix B). In this apparatus each animal was required to stand on his hind legs and thus to lift and support a weight suspended from a chain belt. Daily (5 days/week) each animal underwent alternating work and rest intervals until he individually accumulated a prescribed total work time. The WL program progressively was incremented by supported weight as it was decremented by total support time. The WL animals were engaged in highly-repetitive lifting until the eleventh week. Thereafter the WL animals had fewer repetitions during the lifting with longer rest intervals between work intervals. By eight weeks of treatment, each animal supported 80% body weight for a total of 6.5 min during 10-sec work intervals alternated with 10-sec rest intervals. The animals supported 180% body weight for a total of 50-70 sec during 10-sec work intervals alternated with 30-sec rest intervals from the 14th to the 16th treatment week.

Endurance-Running (END) Treatment

The endurance-running (END) animals also were housed in individual voluntary-activity cages during the adjustment period and individual sedentary cages during the treatment period. During the activity treatment, each animal was subjected to an endurance interval-training program (21, 39) in a controlled-running wheel (CRW). The CRW apparatus (43) was designed to produce participation

by small laboratory animals in highly-specified programs of reproducible activity. During the first forty days (eight weeks of treatments), the activity program gradually was increased to four 12.5-min bouts of running, at 36 m/min, with 2.5 min of rest between bouts (Appendix A, Table A-2). By the 62nd day, and thereafter, the activity consisted of 60 min/day of continuous running at 36 m/min.

Control (CON) Treatment

The animals of the control (CON) group were housed in individual sedentary cages throughout the adjustment and treatment periods. These animals received no special treatment.

Treatment Procedures

Treatments were initiated following the standard, 12-day adjustment period in the cages. The WL and END treatments were conducted daily Monday through Friday. The frequency of treatments was diminished to four times per week in the final weeks of the 16-week duration to allow some rest for the animals. The recovery day within the week served as a prophylactic for overtraining. All treatments were administered by the same technician. Body weights were recorded before and after each treatment for the WL and END animals.

Each WL animal was placed in an individual controlled weight-lifting chamber (CWC). A chain belt was attached around his midsection with an end of the chain lowered through the

floor grid. A weight was attached to the hanging portion of the chain such that (a) when the animal was in a normal quadruped position, the weight was lying upon the subfloor surface - unsupported by the animal (Appendix B, Figure B-2); (b) when the animal stood on his hind legs, the weight was supported entirely by the animal (Appendix B, Figure B-3). The animal was expected to stand on his hind legs and use one or both forepaw(s) to grasp a vertical rod which projected from the chamber ceiling. The rods were individually adjusted to require each animal to rise to a position of full ankle plantar flexion. Thus the position also entailed trunk, hip, and knee extension. At the beginning of each work interval, the rods were lowered into the chambers. The rods were raised out of sight at the start of each rest interval. The animals learned to stand by avoidance-response operant conditioning to a light stimulus that preceded an electrical shock (1.2 ma). Prior to the treatment administration, the daily treatment session was programmed on the master control unit (Appendix B, Figure B-1). Before each work interval, a light below the chamber was turned on as a warning signal for the animal. The light was turned off automatically if the animal stood and grasped the rod. If the rod was not grasped during the 3-sec lift time, the light remained on and the animal was subjected to the shock until the rod was grasped. If the animal released the rod during the work interval, the light-shock sequence was repeated. The shock was administered through a grid which formed the chamber floor. The WL performance criterion was established in

terms of the total support time each animal was required to accumulate daily on the master control unit.

The END treatment group was trained in controlled-running wheels (CRW) with each animal being placed in an individual CRW. At the beginning of each work interval, a brake was released and a light above each wheel was turned on to signal the start of a predetermined running time. The light was turned off automatically if the animal reached a specified running speed during an initial acceleration period. If the specified wheel speed was not reached during the acceleration period, the light stimulus remained on and the animal was subjected to a shock (1.2 ma) until the specified speed was attained. The light-shock sequence was repeated if the wheel speed dropped below the specified speed during the work interval. Animals that reached the specified speed during the acceleration period and maintained that speed throughout the remainder of the work interval ran shock-free. The shock was administered through a grid-like running surface. The wheel was braked at the end of each work period to enforce a predetermined rest interval. A typical running treatment consisted of a preset number of alternating work and rest intervals. The intervals were preset on a master control unit which was analogous to the CWC master control unit. During the first day of a three-day learning period, the animals ran primarily in response to the electrical shock. By the end of the third day of the learning period, most animals were conditioned to run to the light stimulus which preceded the shock. Thus they were able to avoid the shock most of the time.

Performance parameters were recorded for each of the END animals. After each treatment period, total meters run (TMR) and cumulative duration shock (CDS) were recorded from a result unit of each CRW. Performance indices of percentage of expected meters (PEM) and percentage of shock-free time (PSF) were calculated from TMR, total expected meters (TEM), CDS, and total work time (TWT). Performance criteria of 75 PEM and 75 PSF were set for the END treatment.

Treatment Durations

The WL and END treatments were administered for eight-week (8-wk) and sixteen-week (16-wk) durations. The intensity of work in the WL program was increased progressively through the fourteenth week and thereafter was maintained. A progressive increase of the END total work time occurred within the first thirteen weeks. Thus the END program was held constant during the terminal three weeks.

Research Design

Originally ten animals were assigned to each of the three treatment groups. The animals selected for each sacrifice were successful in meeting the performance criteria and subjectively were determined to be in good general health. In the final sample, the tissues of four animals represented each treatment per duration group.

Animal Care

Since rats tend to run more during the early part of their active cycle, the diurnal cycle of the animals was regulated automatically by lighting the animal quarters between 1:00 am and 1:00 pm. The animals were trained during the active phase of their diurnal cycle from 11:00 am to 4:00 pm. Thus the active period of these nocturnal animals occurred at a convenient time for the technician who administered the treatments.

Standard laboratory procedures such as temperature control and regular cage cleaning were observed to maintain a relatively constant environment for the animals. Throughout the experiment the animals had access to water and commercial diet[1] ad libitum.

Sacrifice Procedures

To avoid the acute effects of activity, the animals were killed from three to five days following the last treatment period of the WL and END groups. The sacrifice order for the animals was determined randomly, except that animals from all three groups were killed each day. Before sacrifice all animals were coded. All subsequent procedures were done without knowledge of experimental groups until all raw data were obtained.

Each animal was weighed and then sacrificed under anesthesia which was accomplished by an intraperitoneal injection of 6.48% sodium pentabarbitol (4 mg/100 g body weight). The right triceps

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[1]Wayne Laboratory Blox, Allied Mills, Inc., Chicago, Illinois.

surae and plantaris muscles were removed and rapidly frozen for histochemical analysis. A thoracotomy then was performed to allow a vinyl acetate perfusion of the coronary trees. Following perfusion, the heart was excised and preserved for future study. The left triceps surae and plantaris muscles were removed and individual muscle weights were obtained.

Spinal cord removal was effected by an intervertebral incision between the third and fourth lumbar vertebrae (L_3, L_4) which resulted in cord severance between the second and third sacral segments (s_2, s_3). The dorsal and ventral muscles as well as the supraspinatus ligament were removed from the ninth and tenth thoracic vertebrae (T_9, T_{10}) to free T_9 and T_{10}. Caudal pulling of vertebrae T_{10} - L_3 exposed the lumbar enlargement and sacral spinal segments s_1 and s_2 of the intact spinal cord. The spinal cord then was severed at spinal segment 1_1. After subjective identification, the lumbar enlargement was excised and fixed in 10% buffered formalin.

Histological Techniques

Each enlargement was dehydrated and cleared in an Autotechnicon[1] following 16-17 days of formalin fixation. The lumbar enlargements were embedded in paraffin and then sectioned on a rotary microtome set at seven micra. The paraffin sections were

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[1]Autotechnicon Ultra from Technicon Instruments Corporation, Tarrytown, New York 10591.

mounted with albumin glycerol on leader film.[1] A film transport device modified by Gilliam (14) from Wilson and Pickett (44) was used to transport the film-mounted sections onto a reel.

The film-mounted sections were allowed to dry at room temperature for 24 hours. They were stained with Luxol fast blue and counterstained with cresyl echt violet for demonstration of morphologic characteristics (14, 41). Immediately following staining, the sections were covered by dipping the film in liquid plastic.[2] The film was removed from the reel for subsequent drying.

Measurement of Neurons

The first sixty cephalic motoneurons of each animal that met the following criteria were used for the study: (a) each motoneuron had to be located in the right Lamina IX (37); (b) each motoneuron had to possess a distinct nuclear membrane and an identifiable nucleolus (14).

A Prado microprojector was used to project (X1000) an image of the motoneurons onto a sheet of paper. The soma, nucleus, and nucleolus perimeters were traced. Area measures of the tracings were taken by a compensating polar planimeter. Measures were made in square centimeters ($1\ cm^2 = 100\ \mu^2$).

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[1] Processing machine leader from Eastman Kodak Co., 2988 Estar Base, Rochester, New York.

[2] Liquid plastic from Lab-line Instruments, Inc., Melrose Park, Illinois.

Bias between sampling order and soma size was tested by computing the correlation in four randomly-selected animals. The results are displayed in Table 1.

Table 1.--Pearson Product-Moment Correlation between Sampling Order and Soma Size

Animal Number	Treatment	Duration	Correlation Coefficient
18	WL	8-wk	-0.12
2	END	16-wk	0.00
24	CON	8-wk	-0.02
21	CON	8-wk	-0.28

Statistical Methods

Data were analyzed using a SPSS computer system. A two-way, fixed-effects ANOVA model was used with rat a nested factor. Significance was set at $P < .05$.

The observations of each experimental group were analyzed for independence from a rat effect. Since the observations were shown to not be independent of the rat effect, pooling of the neuronal observations within experimental groups was rejected. The F-ratio and probability of the rat effect occuring simply by chance are displayed in Table 2.

Table 2.--F-Ratio and Probability of Significant Differences between Rats within Experimental Groups by Dependent Variable.

Duration	Dependent Variable	Treatment					
		CON		END		WL	
		F-Ratio	Prob.	F-Ratio	Prob.	F-Ratio	Prob.
8-wk	Soma	4.012	0.008	2.814	0.040	0.850	0.468
	Nucleus	4.358	0.005	1.183	0.317	1.756	0.156
	Nucleolus	15.241	0.005	5.531	0.001	6.923	0.000
16-wk	Soma	18.222	0.000	6.940	0.000	3.885	0.010
	Nucleus	17.621	0.000	28.985	0.000	7.415	0.000
	Nucleolus	28.632	0.000	25.227	0.000	6.428	0.000

CHAPTER IV

RESULTS AND DISCUSSION

This chapter is organized into four main sections. The first section deals with the treatment results from the endurance-running (END) treatment. Body-weight results are presented next. The third part is devoted to the morphometric results. Finally, a discussion is attempted to relate the present findings to those reported in the literature.

Treatment Results

The endurance (END) controlled-running wheel (CRW) treatment is presented in Appendix A, Table A-2. This treatment was a modified version of a standard program used routinely in this laboratory (21, 39). The treatment was modified to hopefully stimulate aerobic metabolic processes in individual motor units. The performance of the END animals was evaluated using the percentage of expected meters (PEM) and the percentage of shock-free time (PSF) as criterion measures.

The END group performance data are presented in Figure 1. The PEM values were 73 or higher on all but three days with the mean PEM value at 85. The PSF values were 68 or higher on all but two days with the mean value at 84. These results indicate that the

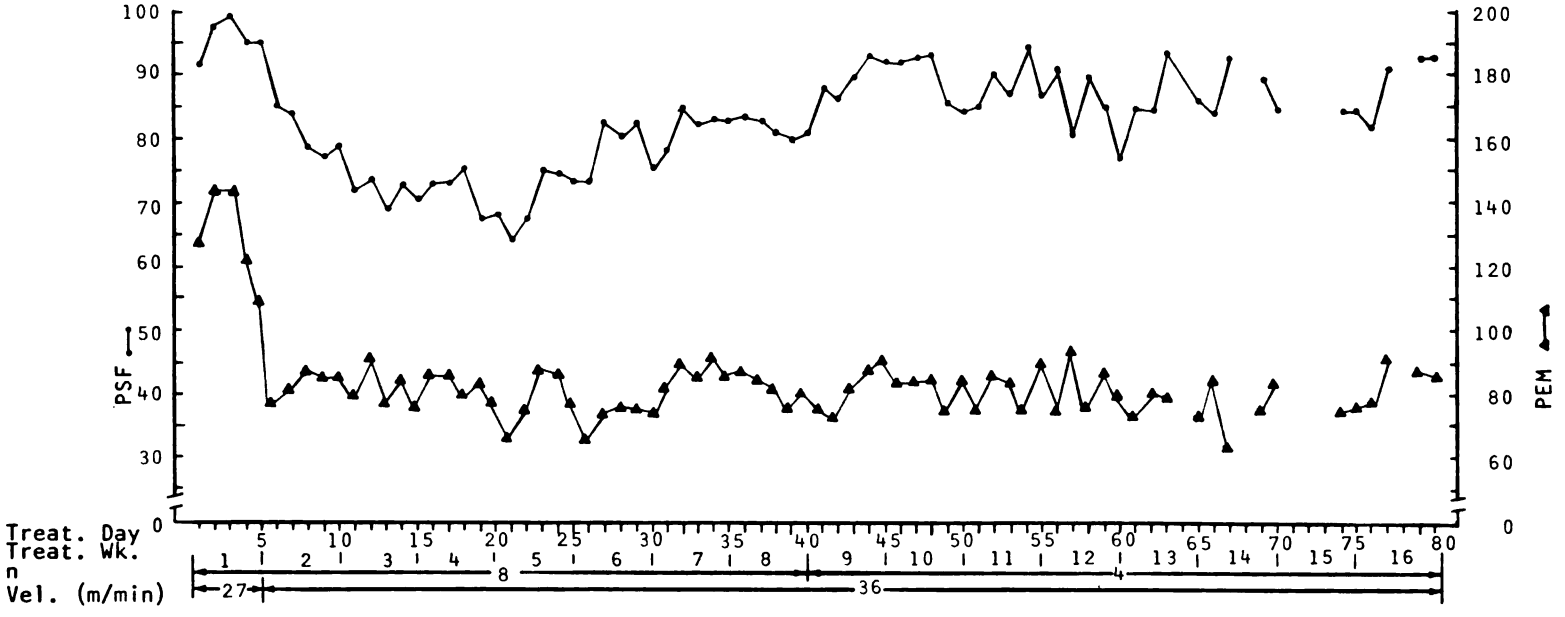

Figure 1.--Mean Daily Percentage of Shock-Free Time (PSF) and Percentage of Expected Meters (PEM) for the Endurance-Running (END) Group.

animals were able to maintain the daily activity requirements fairly well. The relatively-high PSF values indicate that the animals tended to respond to the conditioned light stimulus rather than the shock stimulus. The END animals ran at the relatively-low velocity of 36 m/min. The periods of continuous running were gradually increased to 60 min by the thirteenth week of treatment and were maintained at this level for the remainder of the 16-wk treatment. See Appendix A, Table A-2.

The single 60-min bout of activity was determined subjectively to result in daily physical exhaustion of the END animals. Repeated exposure to this exhaustion could have resulted in a mild state of overtraining. To avoid the possibility of overtraining, the animals were given a weekly recovery day during the thirteenth week and thereafter. Similarly, the WL animals were observed and subjectively determined to be stressed to the point of approaching an over-stressed state. Beginning in the eleventh week, the WL animals were assigned recovery days within the progressively-loaded treatment program.

Body-weight data were recorded before and after each treatment. The percentage of body-weight loss was another indicator used to avoid overtraining. The percentage of body-weight loss, environmental factors, and the END performance criteria were used to calculate basic statistics to provide insight into the activity treatments. See Appendix D.

Body-Weight Results

At both sacrifices, 8-wk and 16-wk, the treated animals were significantly lighter than the control group. See Table 3. The body-weight difference between the END and WL animals was not significantly different. These results are in agreement with those of previous studies using the CRW (21, 38, 40) and support the general observation that strenuous activity slows the usual gain in body weight seen over time in the rat. Usually the slower weight gain associated with activity is attributed to either increased caloric expenditure, reduced caloric intake, or a combination of both factors. These parameters were not monitored in the present study.

Table 3.--Analysis of Variance for Overall Treatment Effects and Student-Newman-Keul's Tests of Paired Comparisons for Body Weight.

Dependent Variable	Treatment Means CON	END	WL	F-Ratio	Prob.	SNK Test **
8-wk Body Weight (g)	463	418	403	10.286	0.005*	C > E, W
16-wk Body Weight (g)	505	434	440	9.175	0.007*	C > E, W

*Significant overall treatment effect at the .05 level.
**SNK tests were run at the .05 level of significance.

Morphometric Results

The soma, nucleus, and nucleolus areas were obtained from the first 60 cephalic motoneurons in the lumbar enlargement right Lamina IX (37). To have been selected for measurement, the Lamina IX motoneuron had to possess a distinct nuclear membrane and a nucleolus. The areas were measured with a compensating polar planimeter. The mean areas for each animal are presented in Appendix C for each morphologic element.

The results of the two-way Analyses of Variance show no overall significant differences between groups for any of the three morphologic elements. These analyses are presented in Tables 4, 5, and 6. Since no overall significance ($P < .05$) was observed for any of the three dependent variables, no subsequent analysis was permissable.

Table 4.--Two-Way Analysis of Variance of Soma Area.

Source	SS	df	MS	F-Ratio	Prob.
A (Treatment)	10.127	2	5.063	2.181	0.142
B (Duration)	0.770	1	0.770	0.332	0.572
AB (Treatment-Duration)	0.420	2	0.210	0.090	0.914
ERROR	41.786	18	2.321		
TOTAL	53.102	23			

Table 5.--Two-Way Analysis of Variance of Nucleus Area.

Source	SS	df	MS	F-Ratio	Prob.
A (Treatment)	0.077	2	0.039	0.554	0.584
B (Duration)	0.007	1	0.007	0.093	0.764
AB (Treatment-Duration)	0.005	2	0.003	0.036	0.964
ERROR	1.256	18	.070		
TOTAL	1.345	23			

Table 6.--Two-Way Analysis of Variance of Nucleolus Area.

Source	SS	df	MS	F-Ratio	Prob.
A (Treatment)	0.002	2	0.001	1.624	0.225
B (Duration)	0.000	1	0.000	0.102	0.753
AB (Treatment-(Duration)	0.000	2	0.000	0.196	0.823
ERROR	0.014	18	0.001		
TOTAL	0.016	23			

Discussion

The treatment parameters compared favorably to previously-imposed activity treatments (14, 38) in body-weight results, percentage of expected meters (PEM) and percentage of shock-free time (PSF). The high PEM and PSF parameters indicate the END animals adapted well to the activity treatments. The WL group was the first attempt of this laboratory to simulate the power activity of human weight-lifters in small animals. At eleven weeks the sharp decrease in total support

time with the increase in the rest interval reflected the subjective judgment of the program designer. It was observed that the animals were participating in a highly-repetitive, probably more aerobic-type of activity than intended the first eleven weeks of treatment. As a result, the WL data should be interpreted with this aerobic component in mind. The goal to design a treatment requiring mainly anaerobic metabolism was probably not accomplished. In the last weeks of the WL treatment, the animals were able to achieve lifts of 180% body weight and then to maintain that level of training the last three weeks of treatment.

The lack of significant differences between experimental groups of soma and nuclei sizes supported the findings of Gerchman (13). The non-significant differences in nucleoli sizes opposed the previous findings (13, 14). Speculation about the lack of significant differences in the morphometric data may lead to two interpretations. In effect, the two activity treatments, administered for both durations, may have resulted in either no distinguishable size changes in soma, nuclei (11, 13, 42), and nucleoli, or morphometric adaptation (3) that was indistinguishable from control levels. If the resolution of the histological technique was insufficient to detect real morphometric changes (17), any experimental effect may have been indistinguishable. Likewise if the variation in sacrifice times between durations was critical (14, 29, 35), the actual experimental effect may have been masked.

A physiological interpretation of these experimental results is not possible at this time. It is evident that significant

experimental changes were not observed. The results may have demonstrated adaptation or lack of plasticity of ventral-motoneuron morphometry to the END and WL treatments.

CHAPTER V

SUMMARY, CONCLUSION, AND RECOMMENDATIONS

Summary

The purpose of this study was to determine the effects of two activity treatments on rat ventral-motoneuron morphometry after two treatment durations. The Lamina IX motoneurons were studied at the lumbar enlargement.

Thirty normal adult male albino rats were assigned randomly to: a control (CON) treatment; an endurance-running (END) treatment; and a weight-lifting (WL) treatment. Monday through Friday, the treatments were conducted for eight and sixteen weeks under controlled laboratory conditions. The final sample consisted of four animals per experimental group.

The animals were anesthetized, lumbar enlargements were removed, and standard histological techniques were observed. Paraffin-embedding and cresyl echt violet counterstaining with Luxol fast blue were used. The prepared sections were projected so the motoneuron images could be traced. The soma, nucleus, and nucleolus areas were measured on the traced motoneurons. The morphometric data were analyzed using a nested, two-way, fixed-effects ANOVA model.

None of the observed motoneuron features varied overall ($P < .5$) due to treatments, durations, or treatment-duration interactions.

The body weight and the treatment data did compare favorably with the data from previous activity-treatment studies. The activity groups had lighter weights at both durations.

Conclusion

The following conclusion can be drawn from this study. The mean sizes of motoneuron soma, nucleus, and nucleolus were not affected differentially by the two durations of the two activity treatments.

Recommendations

The following recommendations are suggested:

1. Histochemical and electrophysiological studies of ventral motoneurons subjected to various activities should be undertaken to supplement morphometric studies.

2. Techniques for more precise location of the neural segment of motor units should be developed for specific muscle motor units.

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APPENDICES

APPENDIX A

APPENDIX A

TREATMENT PROGRAMS

Table A-1.--Weight-Lifting Treatment Program for Postpubertal and Adult Male Rats in Controlled Weight-Lifting Cages.

Week	Day of Training	Lift Time (sec)	Work Interval (sec)	Rest Interval (sec)	Shock (ma)	Work Load (%Body Weight)	Total Support Time (min:sec)
1	1-5	3	25	10	1.2	0	25:00
2	6-11	3	10	10	1.2	0	14:00
3	12-15	3	10	10	1.2	20	12:00
4	16-20	3	10	10	1.2	30	10:00
5	21-22	3	10	10	1.2	40	10:00
	23-25	3	10	10	1.2	50	10:00
6	26-30	3	10	10	1.2	60	8:00
7	31-35	3	10	10	1.2	70	6:30
8	36-40	3	10	10	1.2	80	6:30
9	41-45	3	10	10	1.2	100	4:30
10	46-48	3	10	10	1.2	110	3:20
	49-50	3	10	10	1.2	120	2:30
11	51-52	3	10	30	1.2	140	1:40
	54-55	3	10	30	1.2	160	1:40
12	57-60	3	10	30	1.2	160	:50-75
13	61-63	3	10	30	1.2	170	:50-75
	65	3	10	30	1.2	170	:50-75
14	66-67	3	10	30	1.2	180	:50-75
	69-70	3	10	30	1.2	180	:50-75
15	73	3	10	30	1.2	180	:50-75
16	76-77	3	10	30	1.2	180	:50-75
	79-80	3	10	30	1.2	180	:50-75

Table A-2.--Endurance-Running Treatment Program for Postpubertal and Adult Male Rats in Controlled-Running Wheels.

Week	Day of Training	Acceleration Time (sec)	Work Interval (min:sec)	Rest Interval (sec)	Repetitions per Bout	No. of Bouts	Time Between Bouts (min)	Shock (ma)	Run Speed (m/min)	Total Work Time (sec)
1	1-3	3.0	:10	:10	40	3	5.0	1.2	27	1200
	4	2.5	:20	:10	30	2	5.0	1.2	27	1200
	5	2.5	:30	:15	20	2	5.0	1.2	27	1200
2	6	2.0	:40	:20	15	2	5.0	1.2	36	1200
	7	2.0	:50	:25	12	2	5.0	1.2	36	1200
	8	1.5	1:00	:30	10	2	5.0	1.2	36	1200
	9	1.5	2:30	:60	4	2	5.0	1.2	36	1200
/3	10-11	1.0	2:30	:60	4	2	5.0	1.2	36	1200
/4	12-16	1.0	5:00	0	1	5	2.5	1.2	36	1500
/5	17-21	1.0	7:30	0	1	4	2.5	1.0	36	1800
/6	22-26	1.0	7:30	0	1	5	2.5	1.0	36	2250
/7	27-31	1.0	10:00	0	1	4	2.5	1.0	36	2400
/8	32-36	1.0	10:00	0	1	5	2.5	1.0	36	3000
/9	37-41	1.0	12:30	0	1	4	2.5	1.0	36	3000
/10	42-46	1.0	12:30	0	1	5	2.5	1.0	36	3750
	47-50	1.0	15:00	0	1	4	2.5	1.0	36	3600
11	51	1.0	15:00	0	1	3	1.0	1.0	36	2700
	52	1.0	15:00	0	1	3	1.0	1.0	36	3000
	53	1.0	15:00	0	1	3	1.0	1.0	36	3300
/12	54-56	1.0	15:00	0	1	4	1.0	1.0	36	3600
	57-59	1.0	30:00	0	1	2	2.5	1.0	36	3600
/13	60-61	1.0	30:00	0	1	2	1.0	1.0	36	3600
	62-63	1.0	60:00	0	1	1		1.0	36	3600
/14	65-67	1.0	60:00	0	1	1		1.0	36	3600
/15	69-72	1.0	60:00	0	1	1		1.0	36	3600
/16	74-77	1.0	60:00	0	1	1		1.0	36	3600
	79-80	1.0	60:00	0	1	1		1.0	36	3600

APPENDIX B

APPENDIX B

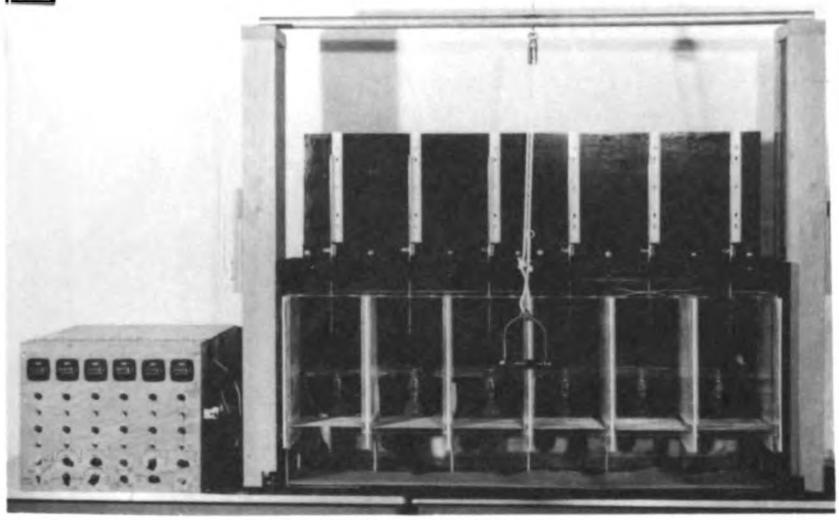
PICTURES OF CONTROLLED WEIGHT-LIFTING CHAMBERS (CWC)

Figure B-1.--Controlled Weight-Lifting Chambers (CWC) and Control Unit. The WL performance criterion was established in terms of the total support time each animal was required to accumulate daily on the master control unit (located on the left).

Figure B-2.--Rest Interval in Controlled Weight-Lifting Chambers (CWC). A weight was attached to the hanging portion of the chain belt such that when the animal was in a normal quadruped position, the weight was lying upon the subfloor surface - unsupported by the animal. The ceiling rods were raised out of sight at the start of each rest interval.

Figure B-3.--Work Interval in Controlled Weight-Lifting Chambers (CWC). At the beginning of each work interval, a light below the chamber was turned on as a warning signal for the animal. The light was turned off automatically if the animal stood and grasped the ceiling rod. When the animal stood on his hind legs, the weight was supported entirely by the animal.

1



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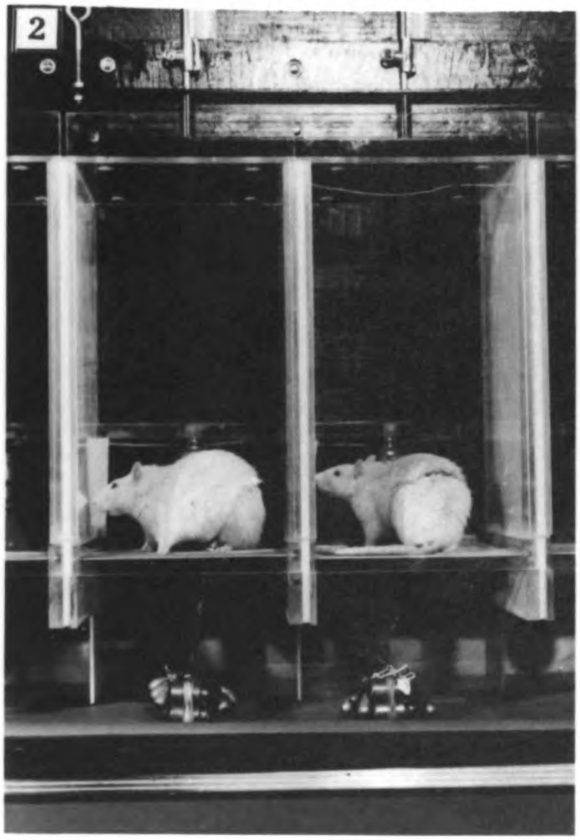

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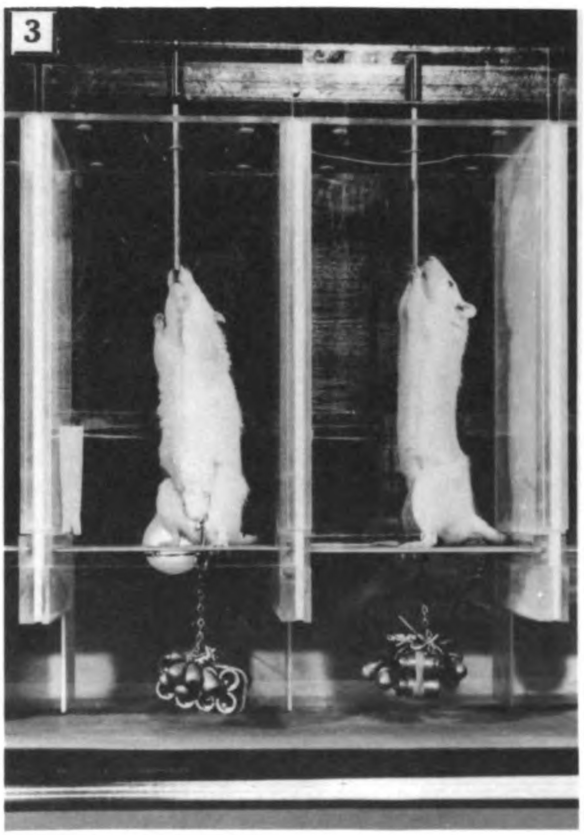

APPENDIX C

APPENDIX C

ANIMAL MEAN DATA BY TREATMENT AND DURATION

Table C-1.--Animal Mean Data of Soma Area ($100\ \mu^2$) by Treatment and Duration.

Duration	Treatment		
	CON	END	WL
8-wk	12.28	12.13	12.88
	10.58	13.28	13.90
	11.62	14.58	13.28
	13.57	12.62	12.22
16-wk	8.03	14.30	13.37
	13.42	11.47	11.20
	11.03	11.82	12.05
	12.78	14.83	14.47

Table C-2.-- Animal Mean Data of Nucleus Area ($100\ \mu^2$) by Treatment and Duration.

Duration	Treatment		
	CON	END	WL
8-wk	1.77	2.03	2.21
	1.99	2.21	2.08
	2.08	2.22	1.95
	2.17	2.24	2.01
16-wk	1.47	2.66	2.33
	2.22	1.79	2.02
	2.05	1.77	1.74
	2.25	2.19	2.09

Appendix C--Continued

Table C-3.-- Animal Mean Data of Nucleolus Area ($100\,\mu^2$) by Treatment and Duration.

Duration	Treatment		
	CON	END	WL
8-wk	0.177	0.160	0.168
	0.141	0.187	0.177
	0.211	0.187	0.186
	0.200	0.201	0.139
16-wk	0.121	0.239	0.167
	0.214	0.163	0.135
	0.185	0.173	0.156
	0.179	0.187	0.173

APPENDIX D

APPENDIX D

BASIC STATISTICS FOR TREATMENT DATA

Table D-1.--Basic Statistics for the Percentage of Body-Weight Loss, Environmental Factors, and END Performance Criteria.

Variable	N*	Mean	Standard Deviation
Air Temp. (°F)		73.5	2.87
Percent Humidity		25.2	9.28
Bar. Press.(mm Hg)		741.2	6.39
WL Percent Body-Wgt. Loss	488	1.25	0.57
END Percent Body-Wgt. Loss	426	2.47	0.53
PEM	426	85	13.69
PSF	426	84	8.25

* Total treatment days for all animals.