SPECIFIC ALTERATIONS IN MOTOR NEURON MORPHOLOGY AND NISSL SUBSTANCE CONCENTRATION IN THE LOWER LUMBAR SPINAL SEGMENTS OF THE ALBINO RAT FOLLOWING SELECTED CHRONIC PHYSICAL ACTIVITY

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This is to certify that the

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ABSTRACT

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The purpose of this study was to determine the effects of seven chronic exercise programs on motor neuron morphology and Nissl substance concentration from the lower lumbar spinal segments of the adult-male, albino rat.

One hundred eighty-two, 72-day-old, normal, male, albino rats (Sprague-Dawley strain) were randomly assigned to seven treatment groups. After a twelve-day adjustment period, treatments began when the animals were 85 days of age. The 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); electric stimulus control (ESC); and long duration swimming (SWM). The animals had access to water and a commercial animal diet *ad libitum*. The treatments were conducted Monday through Friday under controlled environmental conditions.

Only those animals that met minimum training requirements and were subjectively determined to be in good health were selected for sacrifice. Animals within each treatment group were sacrificed prior to the commencement of treatments and at four-, eight-, and twelve-week durations after treatments began. The final sample consisted of 98 animals.

Animals were sacrificed under anesthesia with 6.48 percent sodium pentobarbital by intraperitoneal injection. The intact spinal cord from T10 to S2 was surgically exposed and removed. Subjectively, the lumbar enlargement was cut transversely between spinal segments L3 and L4. Caudal spinal segments L4 through S2 were fixed in 10 percent buffered formalin for 24 hours and then later embedded in paraffin. Serial, cranio-caudal cross-sections, 7μ thick, were mounted on 35-mm leader film and stained with Luxol Fast Blue and counterstained with Cresyl-echt Violet.

Nissl substance concentration was determined photometrically as percent light absorption. Using a microprojector to project the motor neuron at a magnification X1000, a two-dimensional structure with four lines intersecting each other at equal angles was used to make cross measurements of the soma, nucleus, and nucleolus. The Nissl substance concentration and the soma, nucleus, and nucleolus measurements were tested for distribution differences at the .05 level using chi-square analysis of contingency tables (ACT). Additional ACT were performed where significance was obtained.

A definite trend existed at eight weeks indicating the existence of an inverse relationship between the intensity (speed) of the controlled running wheel programs and the diameters of the soma, nucleus, and nucleolus. A direct relationship was found between the size of the motor neuron and Nissl substance concentration. The fact that this trend did not persist through the twelve-week duration indicates the animals may have been in the process of adapting to the exercise regimens. Following eight weeks of training the LON, MED, and VOL groups had significantly greater frequencies of motor neurons with larger morphological

characteristics and with higher Nissl substance concentrations than did the SHT, ESC, and SWM groups.

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By

Thomas B. Gilliam

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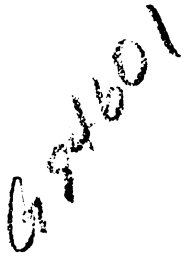

Dedication

To my wife, Elizabeth, and our children

ACKNOWLEDGEMENTS

A very special thank you is extended to both Dr. W. W. Heusner and Dr. W. D. VanHuss for their guidance, encouragement and support throughout the course of my graduate program and research.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ACT	Analysis of contingency tables
CDS	Cumulative duration shock. The duration of shock (seconds) received by experimental animals (SHT, MED, LON) and control animal (ESC) during all work periods of all bouts of a given training period.
CON	Sedentary control
CRW	Controlled running wheel
DNA	Deoxyribonucleic acid
EST	Expected swim time (minutes)
HCP	Histochemical photometer
L	Lumbar
LON	Long-duration, low-speed, endurance running exercise (Long CRW program)
ma	milliampere
MED	Medium-duration, moderate-speed, endurance running exercise (Medium CRW program)
mRNA	Messenger ribonucleic acid
NSC	Nissl substance concentration
PER	Percent expected revolutions; PER=100 TRR/TER
PET	Percent expected swim time; PET=100 STC/EST
PHOS	Phosphorylase
PSF	Percent shock free time; PSF=100-(100 CDS/TWT)
RNA	Ribonucleic acid
S	Sacral
SDH	Succinic dehydrogenase

SHT	Short-duration, high-speed, endurance running exercise (Short CRW program)
STC	Swim time completed
SWM	Long duration swimming exercise
T	Thoracic
TER	Total expected revolutions that an experimental animal (SHT, MED, LON) would run during all work periods of all bouts of a given training period, if he would run at the prescribed speed.
tRNA	Transfer ribonucleic acid
TRR	Total number of revolutions run by the experimental animal during all work periods of all bouts of a given training period (for animals in the SHT, MED, LON and VOL groups).
TWT	Total work time (seconds) during all work periods of all bouts for a given training period.
VOL	Voluntary running exercise

CHAPTER I

THE PROBLEM

A relative dearth of information is available at this time concerning the acute and chronic effects of physical activity of different intensities and durations upon motor neuron morphology. Early investigators using acute exercise (22,39,62) and other acute types of stress, i.e., axon crushing (5,8,10) and antidromic stimulation (2,33,39), have reported different patterns of morphological changes as well as different Nissl substance concentrations. Soma and nucleus sizes and Nissl substance concentrations have been found to increase in some acute studies and to decrease in others. No discernible pattern has been observed. The inconsistent results may be due to the different intensities and duration of the stress factors used in the various studies plus the difficulty of classifying the various stressors used, e.g., axon crushing.

Investigations concerning the effects of chronic exercise on the motor neuron are also few (23,29,40,76). The major conclusion of these studies is that the volumes of the soma and nucleus are unchanged whereas the nucleolus is increased in size. The increase in nucleolar volume is attributed to the high rate of protein synthesis occurring with chronic activity (29).

One study was conducted employing two chronic exercise programs of different intensities (40). The voluntary-forced group, which exercised at a higher intensity and longer duration than the sedentary-forced

group, demonstrated an increase in Nissl substance concentration with no significant change in nucleolar volume. The sedentary-forced group showed a decrease in Nissl substance concentration and an increase in nucleolar volume. It was felt that the voluntary-forced group was more capable of adapting metabolically to the increased functional activity of the motor neuron, since this group exercised for a longer duration than the sedentary-forced group within the same time period. This study was the first of its kind in that exercising at different intensities was shown to affect motor neuron morphology and Nissl substance concentration differentially.

Need for the Study

Additional research is needed to help clarify the effects of chronic physical activity of different intensities and durations upon the morphology and Nissl substance concentration of the motor neuron. Such investigations should involve a range of chronic exercise regimens including both aerobic and anaerobic activity. In this way, both the motor neuron adaptation to increased functional activity and the specific patterns of adaptation resulting from defined reproducible exercise regimens may be determined. Integration of the information obtained from this study with other neuromuscular data on chronic effects of specific regimens of physical activity may help to interpret changes that occur within the neuromuscular system as a result of chronic physical activity.

Statement of the Problem

This study was undertaken to determine if motor neuron morphology and Nissl substance concentration, in the lower lumbar spinal segments

of the albino rat, are differentially affected by selected chronic physical activities.

Rationale

The design of this study incorporporated seven treatments and four durations. The 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); electric stimulus control (ESC); and long duration swimming (SWM). Animals within each treatment group were killed prior to commencement of the treatments and following four-, eight-, and twelve-week treatment durations. Therefore, this study was purposely designed to include a variety of chronic exercise regimens in order to determine if there are differential effects of specific types, intensities, and durations of physical activity in the rat motor neuron.

The SHT, MED, and LON programs were implemented by using the controlled running wheel (83). Thus, controlled reproducible exercise regimens were utilized for three different intensities and durations. These three regimens, along with the long-duration swimming program and the voluntary-exercise program, represent specific types of physical activity with a variety of aerobic and anaerobic requirements. The maximum training period of 12 weeks was determined subjectively to be adequate to induce specific adaptive patterns in motor neuron morphology and Nissl substance concentration.

The male, albino rat was selected as the experimental model for this study for two reasons. First, the controlled running wheel and exercise programs were designed primarily for medium size rodents such

as the rat. Second, a companion study (18) on rats already was in progress. Therefore, the same animals were used for both studies to obtain parallel information.

Limitations of the Study

1. The results of this study may be specific to adult-male, albino rats (Sprague-Dawley strain) that are capable of meeting the requirements of the training methods employed.

2. The results may be specific to motor neurons located laterally in the right ventral horn of lower lumbar spinal segments.

3. The data on Nissl substance concentration reflect relative staining intensities, not quantitative concentrations.

CHAPTER II

REVIEW OF RELATED LITERATURE

This review centers on changes in Nissl substance concentration and morphological characteristics of motor neurons as their functional activity is altered from the resting state. Four sections are incorporated in this review: Nissl substance, morphological alterations, relationship between Nissl substance and morphological alterations, and the relationship between alpha motor neurons and muscle fiber type. The first two sections include discussions of the effects of axon crushing, electrical stimulation, and physical activity upon the motor neuron. A review of the literature dealing with these effects upon the motor-neuron is presented in Tables 1 and 2.

Nissl Substance

In 1899, Franz Nissl identified a tigroid substance easily stainable with aniline dyes in nerve cell bodies. This substance was later named "Nissl substance." Nissl substance is present in the cytoplasm and dendrites of nerve cell bodies but absent in the axon hillock and axon (54). Casperson (63) demonstrated that Nissl substance contains ribonucleic acid (RNA). Since then, nucleocytoplasmic reactions involving the formation of Nissl substance have been the subject of a number of studies (33,52,53,56). From these studies, it was postulated that desoxyribonucleic acid (DNA) in the nucleus serves as a template for messenger RNA (mRNA) in the nucleolus. Messenger RNA then passes to the

Table 1. Summary of the literature dealing with the effects of electrical stimulation, axon crushing, and physical activity upon the motor neuron[1]

Source	Soma	Nucleus	Nucleolus	RNA/Nissl
Electrical Stimulation				
Increased	39**, 43**		33	2*,52,66,67
Decreased	37,39, 50,51	37,39, 50,51		2,10,33
No change				
Axon Crushing				
Increased	5,10	5**,10**	33	8,10,33,52,75,85
Decreased				8,10,33,52,75,85
No change			6	
Acute Physical Activity				
Increased	22,29, 56,62, 76	62	56,62	3,12,22,21,67
Decreased	3,23, 39,67, 37	37,39		3,29,38,49,53,56, 62,65,76,77
No change	55	29,55	29,55	55
Chronic Physical Activity				
Increased			29,40	29
Decreased				40
No change	29,40, 76	29,40,76		23,76

[1]Each number presented in the table corresponds to the number identifying the literature in the list of references.

*Decrease after 1 min then increase.

**Initial increase then decrease.

Table 2. Summary of experimental methodology for the literature dealing with the effects of physical activity upon the motor neuron

Author	Experimental Model	Type of Activity	Duration of Activity
Aleksandrovskaia (3)	Rat	Swimming	40-min
Brumberg (12)	Mouse	Swimming	3-hr
Dolley (22)	Dog	Treadmill Walking	15-min 30-min 1-hr
Edstrom (29)	Guinea pig	Wheel Running	Group I 30-min Group II 32-hr over 29 days
Geinismann (37)	Rat	Swimming	40-min
Geinismann (38)	Rat	Swimming	40-min
Geinismann (39)	Rat	Swimming	50-min; 6-hr
Gerchman (40)	Rat	Swimming	Sed-forced 30-min/day for 52 days Vol-forced two 30 min/day for 52 days
Hochberg (49)	Rabbit	Track Running	Exhaustive
Hyden (52)	Guinea pig	Work Machine	2-hr
Hyden (53)	Guinea pig	Treadmill Running	Exhaustive
Kocher (55)	Rat	Wheel run Swimming	30-min 3-hr
Konecki (56)	Mouse	Swimming	50-min
Mann (62)	Dog	Treadmill Running	10-hr
Pevzner (67)	Mouse	Swimming	3-4-hr
Tumanov (76)	Rat	Swimming	Acute: one 4-hr period Chronic: 2-hr/day twice a week for 6 mos

cytoplasmic sites of protein synthesis, ribosomes, where its complementary nucleotide sequence is inserted into the growing polypeptide chain as amino-acyl transfer RNA (tRNA) (Figure 1). The eccentric location of the nucleolus during increased states of protein synthesis allows mRNA to pass directly from the nucleolus through the nuclear membrane into the cytoplasm (75) (Figure 1B). The turnover rate for mRNA is one-half to two hours and that for ribosomal RNA is less than twenty-four hours (63).

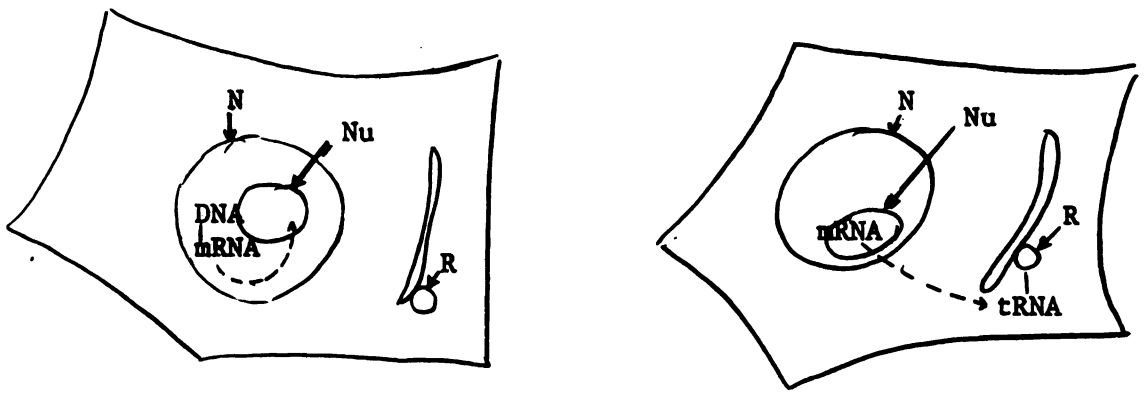

Figure 1. Nucleocytoplasmic reactions involving the formation of Nissl substance: A) DNA serves as template for mRNA. Messenger RNA then passes into nucleolus which is centrally located. B) Nucleolus moves eccentrically thus allowing mRNA to pass to the cytoplasmic sites of protein synthesis. (N=nucleus, Nu=nucleolus, R=ribosomes)

Different staining intensities of Nissl substance corresponded to the spectrum of functional activity of motor neurons (33). At one end is the extreme chromophilia cell which represents a state of active inhibition of prolonged duration. This cell stains very intensely (dark). At the other end of the continuum is the extreme chromophobia cell identified by little or no staining (light). This cell is in a state of severe functional stress or exhaustion. In the middle of the spectrum

is the chromoneutral cell which stains moderately. This cell is either at rest or in a state of normal activity.

A direct relationship has been established between the size of the resting nerve cell and the quantity of RNA and protein present (52). However, this relationship does not hold as the functional activity of the nerve cell is altered from the resting state (21).

During increased functional activity, cytoplasmic nucleoprotein substance increases immediately. This increase results from an increased protein synthesis which at first exceeds breakdown and makes the cell slightly chromophilic (77). As increased levels of functional activity continue, nucleoprotein substance decreases due to the breakdown of nucleoproteins which surpasses synthesis. This has been shown to make the cell chromophobic (78).

Axon Crushing

When the axon of the motor neuron is severed, a depletion of cytoplasmic Nissl substance occurs (8,10,33,52,75,85). The depletion results from the passing of cytoplasmic Nissl substance, necessary for nerve regeneration, to the damaged axon. Simultaneously, as indicated by the appearance of nuclear membrane nucleotides, there is an increase in protein metabolism within the motor neuron (52). Motor neuron chromatolysis is clearly visible three days after axon section and reaches its peak at ten days (8). Restoration of cytoplasmic Nissl substance begins after the tenth day and usually is completed two to three months following the onset of chromatolysis (8).

Electrical Stimulation

A number of investigators have reported alterations in Nissl substance (nucleoprotein content) in the motor neuron following antidromic electrical

stimulation of its ventral rootlets or the peripheral nerve (2,10,33, 38,50,52,66,67). After one minute of stimulation, there is a decrease in nucleoprotein content (2,10,33), whereas stimulation for three to six minutes results in an increase in nucleoproteins (2,52,66,67). Exhaustive stimulation (60 minutes) also causes a decrease in nucleoprotein substance (38,52,66). Given sufficient time to recover following stimulation, the nucleoprotein content returns to control levels.

It has been theorized that the immediate decrease in nucleoprotein content following one minute of stimulation is due to the time lag necessary to begin increased compensatory protein metabolism (2). Once this begins, the cell over-reacts resulting in an increased nucleoprotein content (2). With exhaustive stimulation, the sources necessary to maintain protein metabolism become depleted. This leads to a state of fatigue which is culminated by a complete reduction of nucleoprotein substance (66). That is, the nerve cell no longer is capable of transmitting a nerve signal.

Physical Activity

The findings of previous investigations dealing with the effects of acute and chronic exercise on the nucleoprotein content of the motor neuron are not consistent. Increased (3,12,22,21,67), decreased (3,29,38,49,53,56,62,65,76,77), and unchanged (55) levels of nucleoprotein substance due to acute exercise have been reported. The majority of the studies have favored a decreased while a minority have indicated an increase or no effect. Most of the investigators of the above studies (3,12,38,55,56,67,76) employed swimming, ranging from five minutes to four hours' duration, to bring about changes in nucleoprotein substance. These changes were not related to the duration of the swimming program.

That is, an increase in nucleoprotein substance was reported for animals that swam both for 40 minutes (3) and for three hours (12,67). Likewise, other studies which also used animals that swam for 40 minutes (38,56) and two hours (76) showed a decrease in nucleoprotein substance. It should be noted that most of the authors of the studies involving swimming did not indicate if the animals swam with an overload (i.e., weights attached to their tails). Those studies, not using swimming, used forced running programs such as treadmill running and wheel running as a means to alter nucleoprotein substance.

The elapsed time between the termination of the exercise period and the killing of the animal varied from a few minutes to 48 hours. There does not appear to be a linear relationship between the time of killing of the animal and the direction of change in nucleoprotein substance. For example, animals which were sacrificed immediately after exercise showed both an increase (3,12,22,67) and a decrease (29,38,56,76) in nucleoprotein substance.

It has been reported that nucleoprotein content usually returns to control levels within thirty-six to seventy-two hours following acute exercise (56,67). However, one investigator reported that normalization of RNA content occurred within four hours following a three-hour swimming period (12). Another investigator (3) reported that RNA content increased and decreased in a cyclic pattern for 20 days following acute exercise before normalization occurred.

One might speculate then that the lack of consistent results from earlier studies probably is due to dissimilarities in experimental methods involving the duration and intensity of the acute exercise programs used and the time element in killing the animal following exercise.

Literature regarding the effects of chronic exercise on nucleoprotein content is scant (23,29,40,76). Dolley (23) and Tumanov (76) concluded that chronic exercise has no effect on nucleoprotein content, whereas Edstrom (29) reported an increase. This increase is related to the high rate of protein synthesis associated with chronic activity.

In a previous study in this laboratory (40), albino rats were subjected to three chronic exercise regimens of different intensities. A sedentary group, a sedentary-forced group, and a voluntary-forced group were used. Each animal in the sedentary-forced group swam one 30-minute period per day with an attached tail weight equal to 3 percent of its body weight.[1] Each animal in the voluntary-forced group had access to a freely revolving activity wheel and swam two 30-minute periods each day with an attached tail weight equal to 4 percent of its body weight.[2] When compared to those of the sedentary group, motor neurons in the sedentary-forced group demonstrated a decrease in Nissl substance, whereas those in the voluntary-forced group showed an increase. From this study, it was concluded that continuous chronic activity[3] brings about a metabolic adaptation to the functional activity of the motor-neuron which is not apparent with short exercise periods[4] of chronic activity (40).

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[1]The author of this study (39) referred to this type of activity as short chronic activity.

[2]The author of this study (39) referred to this type of activity as continuous chronic activity.

[3]Two 30-minute swimming periods per day and voluntary activity in a freely revolving wheel (voluntary-forced group).

[4]One 30-minute swimming period per day (sedentary-forced group).

Morphological Alterations

The literature pertaining to morphological alterations in the motor neuron is discussed in the following sections.

Axon Crushing

Axon crushing results in morphological alterations of the motor neuron. An increase in cell volume ten days after crushing was reported by Barr (5) and Brattgard (10). In their studies, nuclear volume increased initially and then decreased below the values observed with no crushing. Nucleolar volume was found to increase (33) and to remain unchanged (5). It was theorized that water absorption from intercellular spaces causes an increase in cell volume (5,10). An initial increase of nuclear volume is an indication of increased protein metabolism associated with nerve regeneration. Subsequent decreases in nuclear volume may be due to loss of water to the cytoplasm which is undergoing increased osmotic tension with chromatolysis (5). One might speculate that the increase in nucleolar volume was also due to an increase in protein synthesis.

Electrical Stimulation

Experimentation involving both antidromic and orthodromic electrical stimulation as a means of increasing the functional activity of motor neurons has been conducted by a number of investigators. Electrical stimulation apparently causes a decrease in soma and nuclear volumes (37,39,50,51) and an increase in nucleolar volume (33). However, some investigators (39,43) have shown an initial gain in soma volume followed by a decrease as the duration of electrical stimulation increases. Thus, with electrical stimulation of an adequate duration to bring about morphological alterations, there is an inverse relationship between the sizes

of the soma and nucleus and the functional activity of the motor neuron (39).

Physical Activity

A number of experiments have been performed using physical activity as a means of altering the size (volume) of the motor neuron. Acute physical activity has been reported to increase (22,29,56,62), decrease (3,23,39,37), and not change (55) the volume of the soma. Similar conflicting results have been found for changes in nuclear volume. That is, the nucleus volume was reported to have increased (62), decreased (37,39), and remain unchanged (29,55) when subjected to various regimens of acute exercise. Nucleolar volume also was reported to increase (56,62) and to remain unchanged (29,55).

The types of acute physical activity used in these studies were swimming and forced running on a treadmill or a wheel. There does not appear to be any relationship between the type of physical activity used and the morphological alterations produced. That is, those authors (3,37,39) reporting a decrease in soma size used swimming as a means to bring about the decrease, whereas investigators reporting increases in soma size used both swimming (56,76) and forced wheel running (22, 29,62). The one investigator reporting no change in soma size due to acute physical activity used forced wheel running for 30 minutes followed by a swimming period of three hours (55).

The duration of exercise for the swimming studies ranged from five minutes to six hours. The authors of studies involving running reported exercise periods from 30 minutes to two hours in duration.

The time elapsed between the termination of the exercise period and the killing of the animal varied from a few minutes to 24 hours. There

does not appear to be a relationship between the time the animal was killed and the morphological alterations observed. For example, those animals killed immediately after exercise showed both increases (22, 29,56,76) and decreases (3,37,39,67) in soma size. Therefore, the lack of uniformity in the results of the studies involving acute physical activity might be attributed to the use of acute exercise programs of different types, intensities, and durations.

It has been reported that chronic physical activity causes no changes in soma and nuclear sizes (29,40,76) but does increase nucleolar size (29,40). In a study using two chronic swimming programs of different intensities, no significant differences were found between the two exercise groups and a control group with respect to either soma or nucleus area. Nucleolar area was significantly larger in the sedentary-forced group than in the control and voluntary-forced groups. The increase was thought to be due to the nucleolus' inability to adapt to short bouts of forced physical activity (40). The nucleolus in animals subjected to voluntary plus forced activity apparently adapted to the metabolic demands of the nerve cell (40).

Tumanov and Krivitskaya studied morphological changes in motor neurons of trained, untrained, and control animals (76). The trained animals swam two hours, twice a week for six months. The untrained animals swam only during the last exercise period, following which both groups were sacrificed. The sizes of the soma and nuclei of the trained animals were not significantly different from those of the control animals. However, the untrained animals had significantly larger soma and nuclei than did the control group.

Relationship between Nissl Substance and Morphological Alterations

Metabolic processes, involving quantitative changes in the nucleoprotein content of nerve cells, change as the motor and sensory activities of nerve cells change (53,56). Increased motor activity results in degradation of nucleotides and proteins in nerve cells which is identifiable by a chromophobic state and decreased soma size. A high rate of protein synthesis also occurs with increased motor activity to offset the loss of nucleoprotein content (29). This was confirmed by observations of increased nucleolar volume which is characteristic of increased protein synthesis (53,56,75). The increased protein metabolism also is accompanied by an increase in nuclear volume. It is difficult to explain the nuclear volume increase, since increased motor activity does not result in an increase in nuclear DNA content (2).

Relationship between Motor Neurons and Muscle Fiber Type

Alpha motor neurons are classified as tonic and phasic. These tonic and phasic motor neurons innervate slow and fast contracting muscle fibers, respectively (27,46). Phasic motor neurons are larger than tonic motor neurons, are capable of discharging more rapidly, and have a shorter hyperpolarization period (26,27,44). Since there is a direct relationship between axon diameter and soma size, the axon of the phasic motor neuron has the faster conduction velocity (16,44).

Campa, in a series of studies, attempted to classify motor neurons histochemically according to succinic dehydrogenase (SDH) and phosphorylase (PHOS) activities (14,15,16,17). Both the tonic and phasic motor neurons demonstrated high PHOS and low SDH activities, which suggests that all alpha fibers are dependent upon anaerobic metabolism. This is in sharp

contrast to the histochemical differences that have been found between red-slow (aerobic) and fast-white (anaerobic) muscle fibers innervated by efferent nerves from tonic and phasic motor neurons, respectively.

CHAPTER III

RESEARCH METHODS

This study was undertaken to determine the effects of seven exercise programs on motor neuron morphology and Nissl substance concentration from the lower lumbar spinal segments of the adult-male albino rat.

Experimental Animals

One hundred eighty-two normal, 72-day-old, male, albino rats (Sprague-Dawley strain)[1] were randomly assigned to seven treatment groups. Prior to the treatment period, each animal was allowed a 12-day adjustment period to adapt to laboratory conditions.

Treatment Groups

The seven treatment groups used in this study were:

Control (CON)

The control animals were housed in standard individual sedentary cages (24 cm long x 18 cm wide x 18 cm high) during both the adjustment and treatment periods and received no special treatment.

Voluntary (VOL)

The voluntary-exercise animals were housed in individual voluntary-activity cages for both the adjustment and treatment periods and received

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[1] Obtained from Hormone Assay Laboratory, Chicago, Illinois.

no special treatment. These cages differ from the sedentary cages in that each animal has access to a freely revolving activity wheel (13 cm wide x 35 cm in diameter). Individual records of total revolutions run (TRR) were recorded daily from revolution counters attached to the activity wheels.

Control Running Groups

These animals were housed in individual voluntary-activity cages during the adjustment period and individual sedentary cages during the treatment period. During the activity period, each animal was subjected to one of three interval-training programs in a controlled-running wheel (83).

The exercise intensity of these programs gradually increased until the 37th day. Thereafter, the exercise requirements did not change. The following descriptions of the three running programs are for the 37th and all following days of training (descriptions of these programs for each training day are in Appendix A, Tables A-1, A-2, and A-3).

Short (SHT). These animals were subjected to a short-duration, high-speed, endurance program consisting of eight bouts of exercise with 2.5 min of rest between bouts. Each bout consisted of six repetitions of a 10-sec work interval followed by a 40-sec rest interval. During the work intervals, the animals were expected to run at the relatively fast speed of 5.5 ft/sec.

Medium (MED). This group was subjected to a medium-duration, moderate-speed, endurance program consisting of five bouts of exercise with 5.0 min of rest between bouts. Each bout consisted of eight repetitions of 30-sec work intervals alternated with 30-sec rest intervals.

During the work intervals, the animals were expected to run at 4.0 ft/sec.

Long (LON). The animals in this group were expected to complete a long-duration, low-speed, endurance program consisting of four bouts of exercise with 2.5 min of rest between bouts. Each bout consisted of a continuous run lasting 12.5 min. This group was expected to run at 2.0 ft/sec.

Electrical Stimulus Control (ESC)

These animals were housed in individual voluntary-activity cages during the adjustment period and individual sedentary cages during the treatment period. Each animal was permanently paired with a SHT animal.

During the SHT activity period, each ESC animal was placed in a stimulus control cage (21 cm long x 14 cm wide x 10.5 cm high) adjacent to a controlled-running wheel (CRW). The ESC animals received electrical shock through a grid floor comparable to that of the CRW. Each ESC animal was exposed to the same light stimuli and electrical shock as its paired mate in the SHT group.

Swimming (SWM)

These animals were housed in individual voluntary-activity cages during the adjustment period and individual sedentary cages during the treatment period. Each animal swam in an individual cylindrical tank (76 cm high x 28 cm in diameter) in 70 cm of water (28-32°C). On the 37th day of training and thereafter, each animal was expected to swim continuously for 60 min with a weight attached to its tail equal to 3 percent of its body weight (see Appendix A, Table A-4).

Duration Groups

To provide chronological perspective of the treatment effects, animals were sacrificed after zero, four, eight, and twelve weeks of treatment. The animals designated as zero-week animals received no special treatment and were sacrificed following the adjustment period. The training requirements for each treatment group increased progressively from zero to eight weeks of training. Animals trained for twelve weeks followed the program from day 37 until day 60 (Appendix A).

Sample Size

A total of 14 animals were sacrificed at zero weeks. All of these animals were assigned to the CON treatment group (see Table 3). Eight animals were needed in each of the other treatment-duration cells for a companion study (18). However, the time required to collect and analyze

Table 3. Final cell frequencies by treatment and duration

Treatment	Duration			
	0-wk	4-wk	8-wk	12-wk
CON	14	4	4	4
SHT		4	4	4
ESC		4	4	4
SWM		4	4	4
VOL		4	4	4
MED		4	4	4
LON		4	4	4

the data for this study limited the sample size to four animals in each of the four-, eight-, and twelve-week cells. Therefore, from each group of eight animals sacrificed for the companion study, four were randomly selected to be used in this study. The final sample consisted of 98 animals (Table 3).

Treatment Procedures

Following the 12-day adjustment period, treatments began when all animals were 85 days of age. The SHT, MED, LON, ESC, and SWM treatments were conducted daily, Monday through Friday, in the Human Energy Research Laboratory, Michigan State University, East Lansing, Michigan. Body weights were recorded before and after each treatment period for the SHT, MED, LON, and ESC groups. Only pretreatment dry weight was recorded for the animals in the SWM group.

For each VOL animal, the total revolutions run (TRR) for the previous 24-hr period were recorded Tuesday through Friday between 10 a.m. and 11 a.m.

The SHT, MED, LON, and ESC treatment groups were trained in controlled-running wheels (83). During the first day of a three-day learning period, the animals ran chiefly in response to an electrical shock (1.2 ma). By the end of the third learning period, most animals were conditioned to run to a light stimulus which preceded the electrical shock. This enabled them to avoid the shock most of the time.

Each animal was placed in an individual controlled-running wheel (CRW). At the beginning of each running period, a brake was released and a light above each wheel was turned on to signal the start of a predetermined work interval. The light was turned off automatically if the animal reached a specified running speed during an initial interval

of time, the acceleration period. If the specified wheel speed was not reached during the acceleration period, the light stimulus was turned off and the animal was subjected to a mild electrical shock until the specified speed was attained. The shock was administered through a grid which formed the running surface of the wheel.

If the wheel speed dropped below the specified speed during the work interval, the light-shock sequence was repeated. Animals that reached the specified speed during the acceleration period and maintained that speed throughout the remainder of the work interval ran shock-free. At the end of each work interval, the wheel was braked to enforce a predetermined rest interval. A typical running program consisted of a preset number of alternating periods of work and rest.

After each treatment period, total revolutions run (TRR) and cumulative duration of shock (CDS) were recorded from a result unit attached to each CRW. These values, along with total expected revolutions run (TER) and total work time (TWT), were used to calculate percent expected revolutions (PER) and percent shock free time (PSF) (see Figures 4, 5, and 6). For the SWM groups, swim time completed (STC) was recorded and used with expected swim time (EST) to calculate percent expected swim time (PET) (see Appendix B, Table B-2).

Animal Care

Rats are normally more active at night than during daylight hours. Thus, the lights were off between 1 p.m. and 1 a.m. in the animal quarters. This allowed the animals to be trained during the active phase of their diurnal cycle and at a convenient time for the laboratory staff.

Standard laboratory procedures, such as daily animal handling, humidity and 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 a commercial animal diet[1] *ad libitum*.

Sacrifice Procedures

Sacrifices, each consisting of seven animals of the same duration group, were conducted biweekly from December 9, 1970, to February 14, 1972. The first two sacrifices included only zero-week animals. Subsequent sacrifices included one CON animal and two animals from each treatment group within one of the following sacrifice trios: SHT-ESC-SWM or VOL-MED-LON. All animals were sacrificed on Monday following their last exercise period on the previous Friday. Thus, 65-70 hours elapsed between the last exercise period and sacrifice. Only those animals subjectively determined to be in good health were sacrificed. Animals in the CRW programs were expected to meet a minimum criterion of 75 PER. Only those SHT, MED, and LON animals whose mean PER was 75 or higher were selected for sacrifice. Close proximity to a mean of 100 PET was established as the selection criterion for the SWM group, since most of those animals were able to meet the SWM program requirements.

The animals were weighed and then sacrificed under anesthesia which was accomplished by an intraperitoneal injection of 4 mg/100 g of body weight of 6.48 percent Halatal[2] (sodium pentobarbital).

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

[2]From Jensen-Salsberg Laboratories, Division of Richardson-Merrel, Inc., Kansas City, Missouri.

A laparotomy was performed to allow withdrawal of 1-2 ml of blood from the caudal vena cava. After barrel exchange, 4-5 ml of buffered Pelikan[1] ink (pH 7.2) was injected into the vascular system for subsequent capillary counts and capillary-per-fiber calculations in skeletal muscle. Following three minutes of *in vivo* circulation, the heart was excised and preserved for future study. Several muscles of both hind limbs and the soleus nerve of the left hind limb were taken for a companion study (18).

Removal of the spinal cord was effected using bone shears to make a transverse cut through the intervertebral disc between lumbar vertebrae three and four (L3, L4), which resulted in a transverse cut between spinal segments sacral two and three (S2, S3). The dorsal and ventral muscles and the supraspinous ligament were removed from thoracic vertebrae nine and ten (T9, T10) to free T9 from T10. Pulling vertebrae T10-L3 caudally exposed the lumbar enlargement and sacral spinal segments one and two (S1, S2) of the intact spinal cord. The spinal cord then was severed at spinal segment L1. Subjectively, the lumbar enlargement was cut transversely between spinal segments L3 and L4. Caudal spinal segments L4-S2 were fixed in 10 percent buffered formalin for 24 hours.

Tissue Analysis

The motor neurons from spinal segments L4-S2 were examined to obtain information parallel to that of an investigation of the right triceps surae and plantaris muscles. Motor neurons from L4-S2 are responsible for innervation of these plantar flexor muscles (10).

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[1]Obtained from John Henschel and Co., Farmington, Long Isle, New York.

Histologic Techniques

In preparation for paraffin impregnation, spinal cord segments L4-S2 were dehydrated with ethyl alcohol and cleared with terpinol (see Appendix D). The paraffin-embedded spinal cord segments were positioned on a rotary microtome for cutting. Serial, cranio-caudal cross-sections were cut at 7μ beginning with L4. A paper trough extending from the microtome blade, supported the paraffin strips. After collecting 10-15 sections, the paraffin strip was severed several sections below the blade and placed in a water bath (45-48°C) for subsequent mounting procedures. This procedure was repeated until enough sections were obtained to fill a five foot strip of leader film.[1]

To mount the tissue sections, a film transport device, modified after Wilson and Pickett (84), was used (20). This device supported a Kinderman guide and pick-up reel.[2] The transport system was placed in the water bath. Five feet segments of 35-mm leader film[1] were guided under the roller of the plexiglass transporter, through the Kinderman film guide, and secured in the pick-up reel. The film was continually coated with albumin-glycerol before entering the water bath to insure tissue adherence.

Each paraffin strip was positioned to join the center of the film as it emerged from the water onto the Kinderman guide. The pick-up reel was slowly turned as the paraffin strips were guided on the film. After drying at room temperature for 24 hours, the sections were stained with Luxol Fast Blue, and counterstained with Cresyl-echt Violet for

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[1]Processing Machine Leader 2988 Estar Base, Eastman Kodak Co., Rochester, New York.

[2]Ehrenreich Photo-optical Industries, Inc., Garden City, New York.

demonstration of morphological characteristics and Nissl substance concentration[1] (see Appendix D). Following the staining procedures, the strip of film was removed from the pick-up reel and secured to a horizontal-flat surface. Liquid plastic[2] was then brushed on the film to cover the tissue sections.

For each animal, the first 60 motor neurons[3] located, that met the following two criteria, were used in this study:

1. Each motor neuron had to possess a nucleolus and a distinct nuclear membrane.

2. Each motor neuron had to be located in the lateral portion of the right ventral horn.

Photometric Techniques

The analysis of Nissl substance concentration (NSC) was performed on a Histochemical Photometer (HCP). This instrument has been described as consisting of "...A Prado projecting microscope, a photocell with associated circuits to measure light intensity and a digital readout" (82). The HCP was calibrated for each section of tissue so that a zero reading equaled zero light transmission or no light passing from the projector to the photocell. A maximum HCP readout of 40 percent represented 100 percent of the light transmitted through the 35-mm film and liquid plastic with

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[1]Nissl substance concentration is defined as the availability of cytoplasmic Nissl substance following a four-minute incubation period in Cresyl-echt Violet.

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

[3]A sample size of 60 motor neurons per animal was calculated to be necessary and sufficient to detect, as significant, any differences greater than or equal to 1.7σ when $\alpha = .05$ and $\beta = .20$.

no intervening tissue.[1] Each section was magnified X360 as verified by a calibration microscope slide. When a tissue section was in position, the percentage of light passing through the film, liquid plastic and tissue was recorded. This reading was then converted to a proportion of 100 percent light transmission by multiplying it by a constant of 2.5.

Three readings were taken of NSC for each motor neuron used in the study. The readings were performed randomly on the cytoplasmic Nissl substance. The readings then were transformed into percent light absorption by subtracting the converted values from 100. The calculated light absorption values were assumed to be directly related to the NSC.

Histometric Techniques

Using a Prado microprojector, the motor neurons were magnified X1000 and projected on white paper attached to a flat vertical surface. A two-dimensional triangulation structure, with four lines intersecting each other at equal angles, was permanently constructed on the nucleolus. Measurements of the distance across the soma and nucleus were made along each of the four lines (Figure 2, AB, ab). The nucleolus, being a sphere, required only one measurement (Figure 2, mn). Measurements were made using a millimeter rule (1 mm = 1μ).

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[1] Due to the high linear magnification (X360) of the projected motor neuron, a 100 percent readout was impossible to obtain.

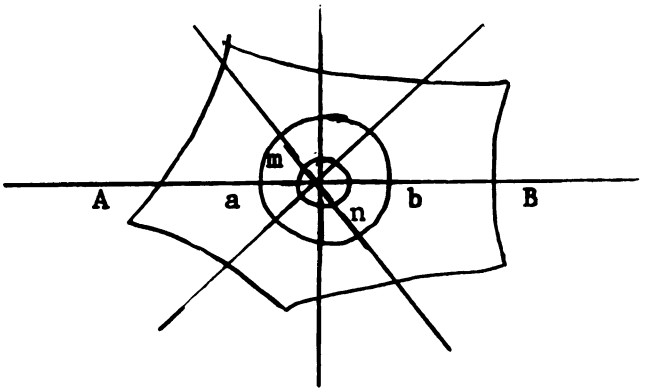


Figure 2. Triangulation showing one measurement of soma (AB), nucleus (ab), nucleolus (mn).

Statistical Procedures

The training data were analyzed by treatment groups and training days. Means and standard deviations were calculated for all variables of training performance, environmental conditions, and pre- and post-treatment body weights. Simple correlation coefficients were calculated between all possible pairs of these variables. Since the diameters of the wheels attached to the voluntary-activity cages were less than those of the CRW, the daily TRR values of the VOL animals were multiplied by a constant of 0.9163 to equate the TRR values of the VOL animals to those of SHT, MED, and LON animals.

For each motor neuron, mean values were calculated from the three readings of Nissl substance concentration and the four measurements across each of the soma and nucleus (Appendix C). These values, along with the single measurement across the nucleolus, were used in analyses of contingency tables (ACT).

An overall ACT was calculated to determine if there were any significant differences in distributions by either treatments or durations (see Table 3) for each dependent variable: Nissl substance concentration,

soma diameter, nucleus diameter, and nucleolus diameter. Providing significance was obtained in the overall analysis, subsequent ACT were calculated to detect significant differences within each category of each independent variable (e.g., CON is a category of the independent variable treatment). Upon obtaining significance within a category, additional ACT were calculated comparing individual cell distributions within the significant category (e.g., if CON was significant, then contrasts such as CON-0 wk vs. CON-4 wk and CON-0 wk vs. CON-8 wk were calculated). All possible category combinations within each independent variable were also tested for distribution differences (e.g., for the independent variable treatment, contrasts such as CON vs. SHT and MED vs. LON were calculated).

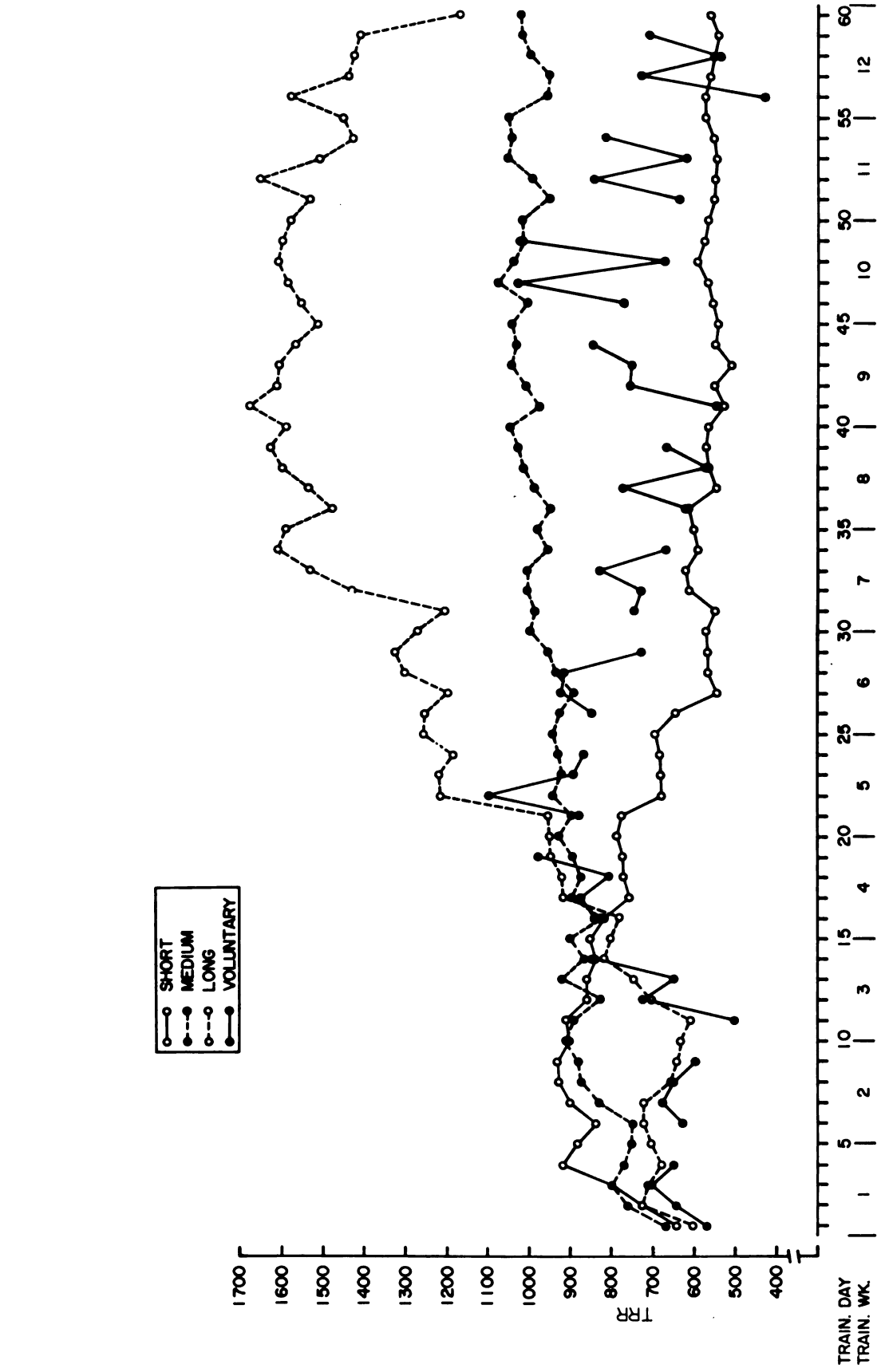
For the overall ACT and all subsequent analyses, an alpha level of .05 was selected. Modification of the alpha level for subsequent analyses was not necessary since each analysis was independent of all others.

The arcsine transformation was performed on the Nissl substance data (percent light absorption) in order to reduce extreme right skewness.

Treatment Results

On the basis of the TER for each CRW program (Appendix A), the LON group should have displayed the greatest mean increase in TRR, the MED animals a moderate increase, and the SHT group a slight increase followed by a gradual decrease. The mean daily TRR values shown in Figure 3 indicate that the LON, MED, and SHT treatment groups met their respective program requirements.

The mean daily TRR of the VOL animals tended to follow the TRR of the LON animals for the first four weeks of training. For the last eight weeks, the TRR values for the VOL animals were between those of the SHT



Mean Daily Total Revolutions Run (TRR) for VOLUNTARY and CRW SHORT, MEDIUM, and LONG

Figure 3

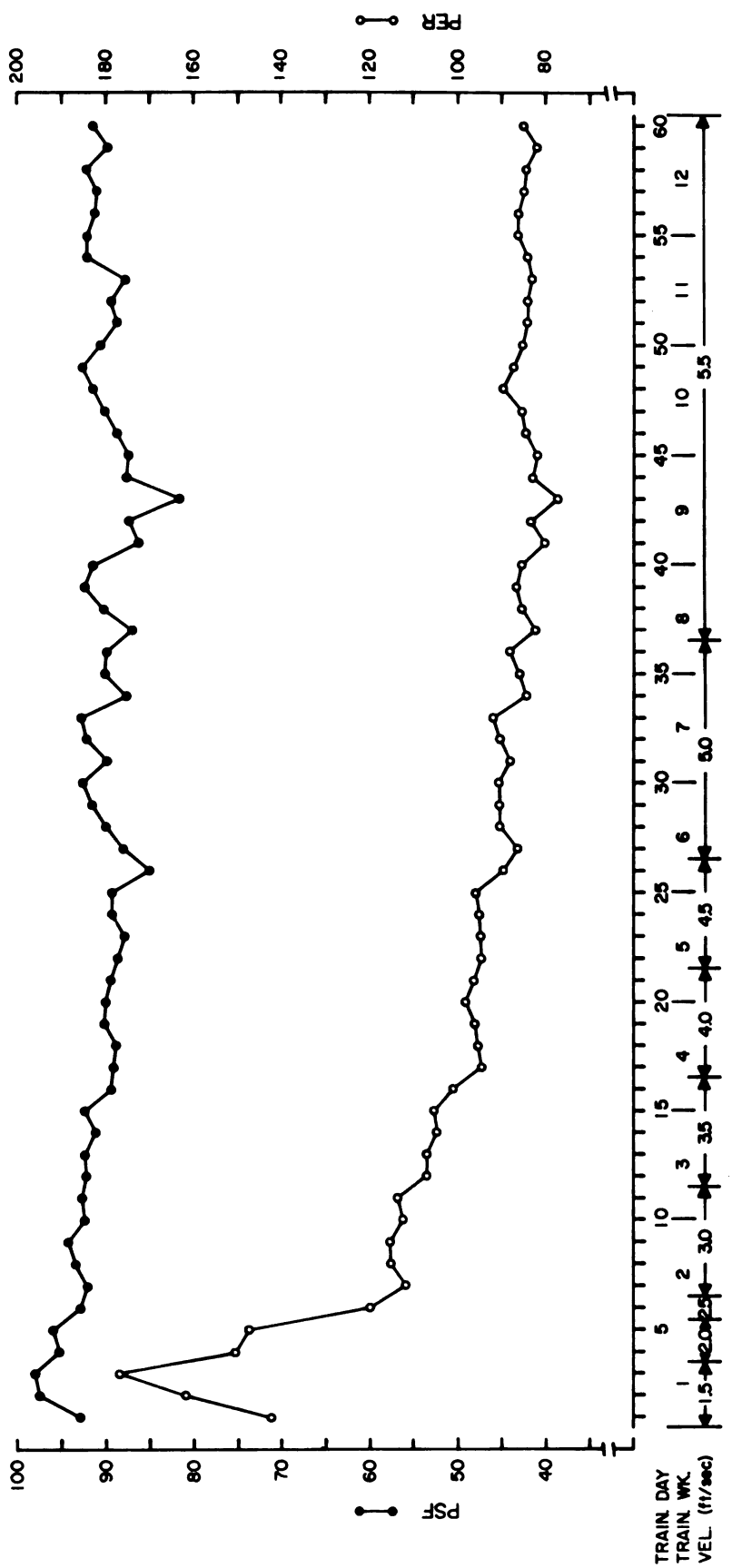
and MED groups (Figure 3). The interpretation of these observations is limited to total distance run. The speed of running by the VOL animals is unknown and cannot be equated to the speeds of the other running programs.

Figures 4, 5 and 6 show that the SHT, MED, and LON animals generally maintained percent expected revolutions (PER) values above 80, thus exceeding the PER criterion of 75 which was set as a minimum standard for execution of the CRW programs. This level of performance compares favorably with other groups of animals subjected to similar training programs (69,71,74). The high percent shock-free time (PSF) values (Figures 4, 5, and 6) indicate the animals generally responded to the light stimulus rather than to the electrical shock. The PSF values, when compared across treatment durations, show that the SHT animals and their paired ESC counterparts received the least amount of electrical shock. The LON animals received the most.

Almost without exception, the percent expected swim time (PET) values for the SWUm group were 100 (Table B-2, Appendix B). Therefore, PET was not plotted across duration for the SWM treatment.

Treatment Environment and Body Weight Results

The SHT, MED and LON animals exercised under relatively constant conditions of air temperature, humidity and barometric pressure. These values did not affect the PER and PSF values as indicated by the low correlations among the various parameters (Table B-1, Appendix B). Animals with relatively high body weights tended to display low PER values. A low positive correlation was obtained between percent body weight loss and PER and PSF. Thus, those animals showing relatively large weight losses tended to have higher PER and PSF values. A



Mean Daily Per Cent Shock Free Time (PSF) and Per Cent Expected Revolutions (PER) for CRW SHORT

Figure 4

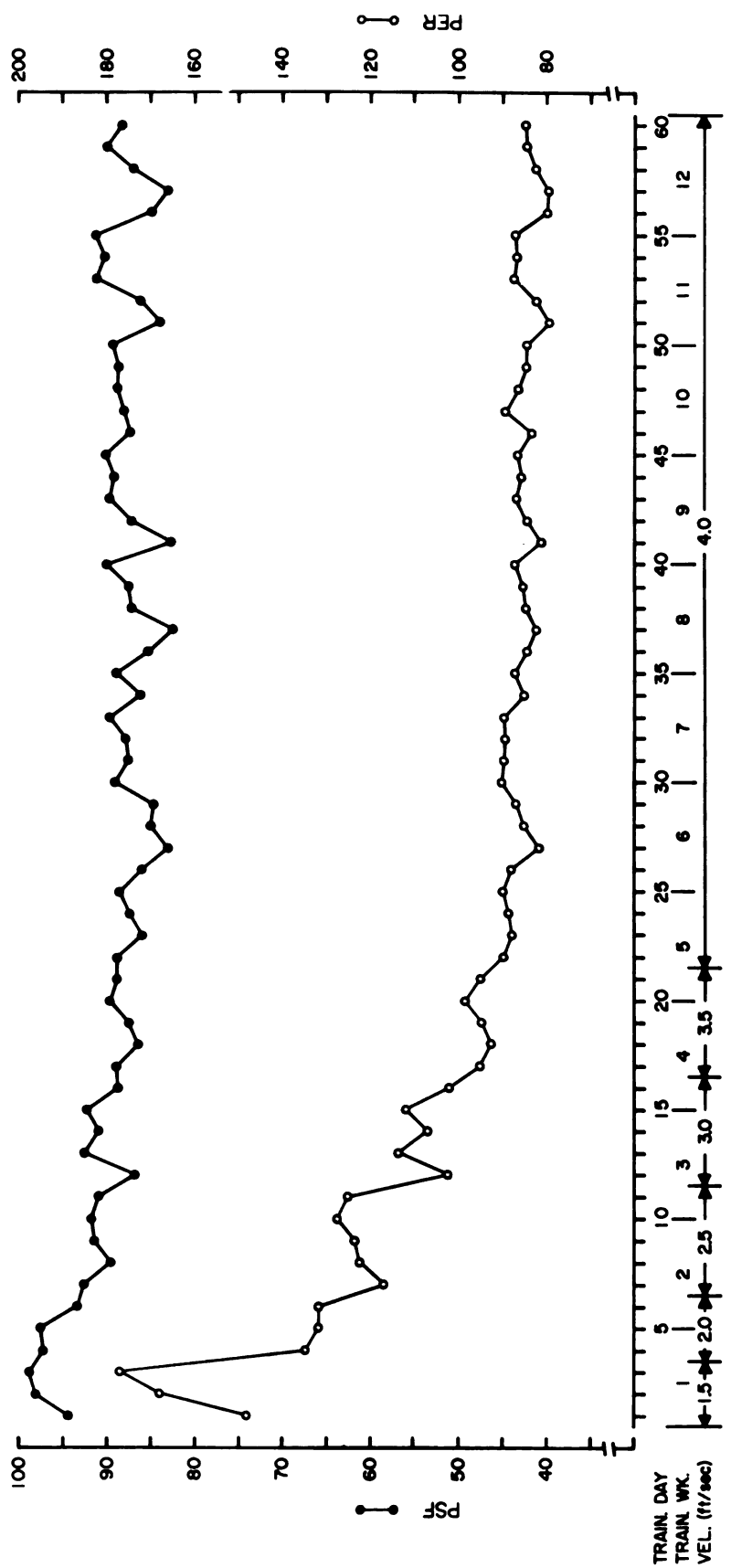

PER
PSF
TRAIN. DAY
TRAIN. WK.
VEL. (ft/sec)
Mean Daily Per Cent Shock Free Time (PSF) and Per Cent Expected Revolutions (PER) for CRW MEDIUM

Figure 5

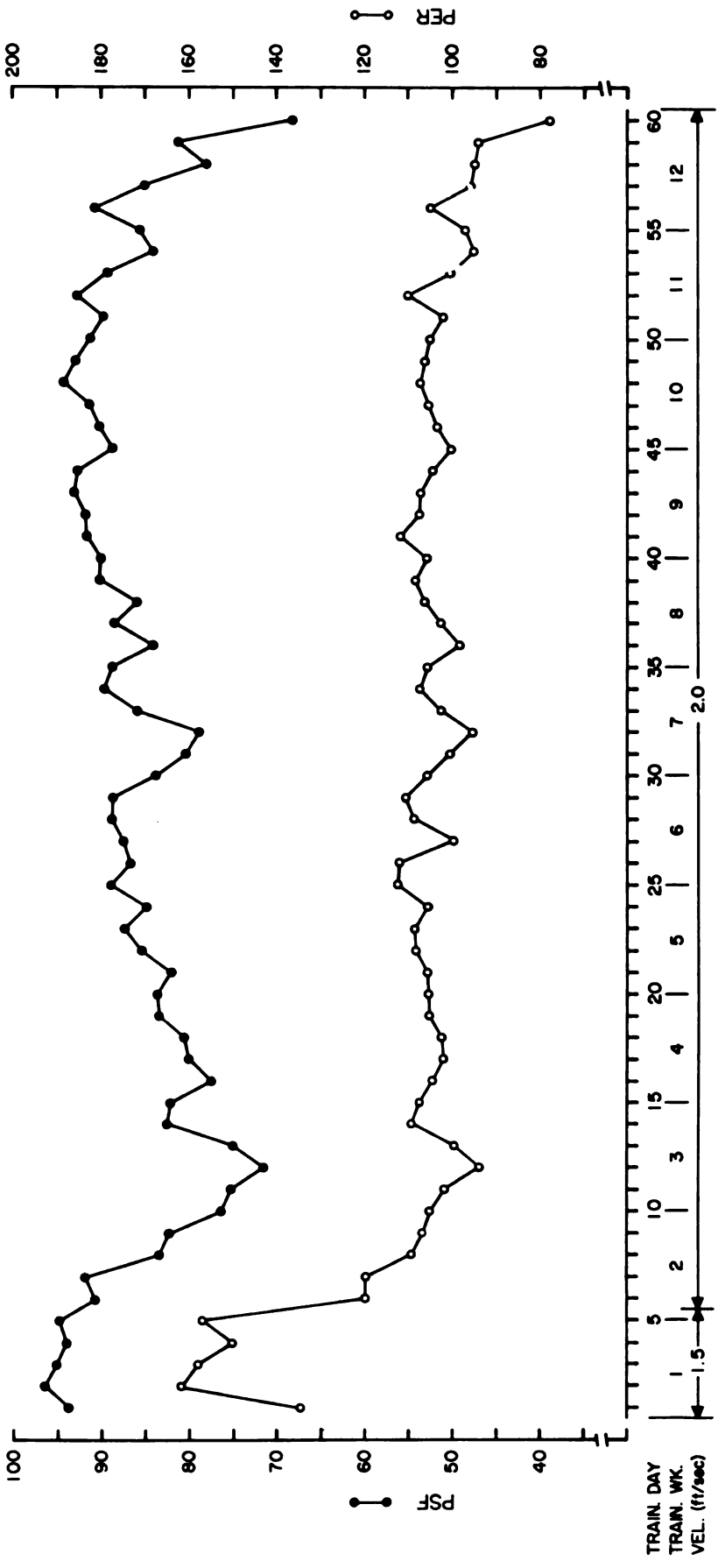

PER
PSF
200
180
160
140
120
100
80
100
90
80
70
60
50
40
TRAIN. DAY
TRAIN. WK.
VEL. (ft/sec)
1.5
2.0
Mean Daily Per Cent Shock Free Time (PSF) and Per Cent Expected Revolutions (PER) for CRW LONG

Figure 6

moderate correlation between PSF and PER confirms the near parallel plots of these two parameters (Figures 4, 5, and 6).

Training Results

The CON animals received no special treatment and were housed in sedentary cages; thus, these animals represented a level of low physical activity. Figure 3 shows definite differences in mean daily TRR for the SHT, MED, LON and VOL animals. The program expectations for the CRW groups and the SWM group were markedly different (Figures 4, 5, and 6 and Tables B-1 and B-2, Appendix B). The ESC animals cannot be equated to CON animals since they received a noxious stimulus. The chronic physical activity level of the ESC animals was not known. Therefore, based upon the treatment results, the seven treatment groups of animals appear to represent seven distinct types and levels of chronic physical activity.

CHAPTER IV

RESULTS AND DISCUSSION

Results of morphological measurements and Nissl substance concentration are presented in the first and second sections, respectively.

Morphological Results

The morphological results are presented for each dependent variable: soma diameter, nucleus diameter, and nucleolus diameter.

The chi-square values in Table 4 give evidence of overall significant treatment and duration effects at the .05 level for each dependent variable. Comparisons within each duration across all treatments

Table 4. Analyses of contingency tables for each independent variable with each dependent variable

Independent Variable	Dependent Variable	df	X^2	$X^2_{.05}$	
Treatment	Soma	252	487.5	297.5	S
	Nucleus	102	240.2	125.2	S
	Nucleolus	24	107.7	36.4	S
Duration	Soma	126	261.0	158.5	S
	Nucleus	51	155.6	68.7	S
	Nucleolus	12	119.3	21.0	S

S = significant at .05 level.

(Table 5) and within each treatment across all durations (Table 6) for each dependent variable indicate significance for all categories within both duration and treatment. Therefore, additional analyses were performed for each dependent variable to detect significant differences between treatments across all durations (Tables 7, 9, and 11) and between durations across all treatments (Tables 8, 10, and 12).

Table 5. Analyses of contingency tables within each duration across all treatments for each dependent variable

Dependent Variable	4 wk	8 wk	12 wk
Soma diameter	S	S	S
Nucleus diameter	S	S	S
Nucleolus diameter	S	S	S

S = significant at .05 level.

Table 6. Analyses of contingency tables within each treatment across all durations for each dependent variable

Dependent Variable	CON	SHT	ESC	SWM	VOL	MED	LON
Soma diameter	S	S	S	S	S	S	S
Nucleus diameter	S	S	S	S	S	S	S
Nucleolus diameter	S	S	S	S	S	S	S

S = significant at .05 level.

Soma

The summary results of the chi-square contingency analyses for soma diameter between treatments across all durations are presented in Table 7. It is evident from these results that there are significant shifts towards larger soma for the VOL, MED, and LON groups when the frequency

distributions of these groups are compared with that of the CON animals, whereas the SHT, ESC, and SWM groups showed significant increases in the frequency of smaller soma. Table 7 indicates there is an inverse relationship between soma size and the intensity (speed) of the controlled running wheel (CRW) programs. A direct relationship exists between soma size and the quantity of shock received by the LON, MED, SHT, and ESC groups (Table 7 and Appendix B).

Table 7. Summary of analysis of contingency tables, significant and nonsignificant relationships between treatments across all durations for soma diameter

	CON	SHT	ESC	SWM	VOL	MED
SHT	S-					
ESC	S-	S+				
SWM	S-	N	N			
VOL	S+	S+	S+	S+		
MED	S+	S+	S+	S+	S-	
LON	S+	S+	S+	S+	N	S+

Summary of significant relationships at .05 level:

SHT < ESC < CON < MED < LON

Summary of nonsignificant relationships:

MED < VOL < LON

SHT < SWM < ESC

N = not significant.

Contrasting row effect to column effect (e.g., SHT vs. CON).

S+ = significant distribution shift at the .05 level to the right in favor of the row effect in the contrast.

S- = significant distribution shift at the .05 level to the left in favor of the row effect in the contrast.

The frequency distributions for soma size between durations across all treatments indicate there were significantly smaller soma at zero weeks than at the four-, eight-, and twelve-week durations (Table 8). The greatest increase in soma size occurred at four weeks. A significant decrease in soma diameter occurred between four and eight weeks.

Table 8. Summary of analysis of contingency tables, significant, and nonsignificant relationships between durations across all treatments for soma diameter

	0 wk	4 wk	8 wk
4 wk	S+		
8 wk	S+	S-	
12 wk	S+	N	N

Summary of significant relationships at .05 level:

$$0\text{ wk} < \begin{matrix} 8\text{ wk} < 4\text{ wk} \\ 12\text{ wk} \end{matrix}$$

Summary of nonsignificant relationships:

8 wk < 12 wk < 4 wk

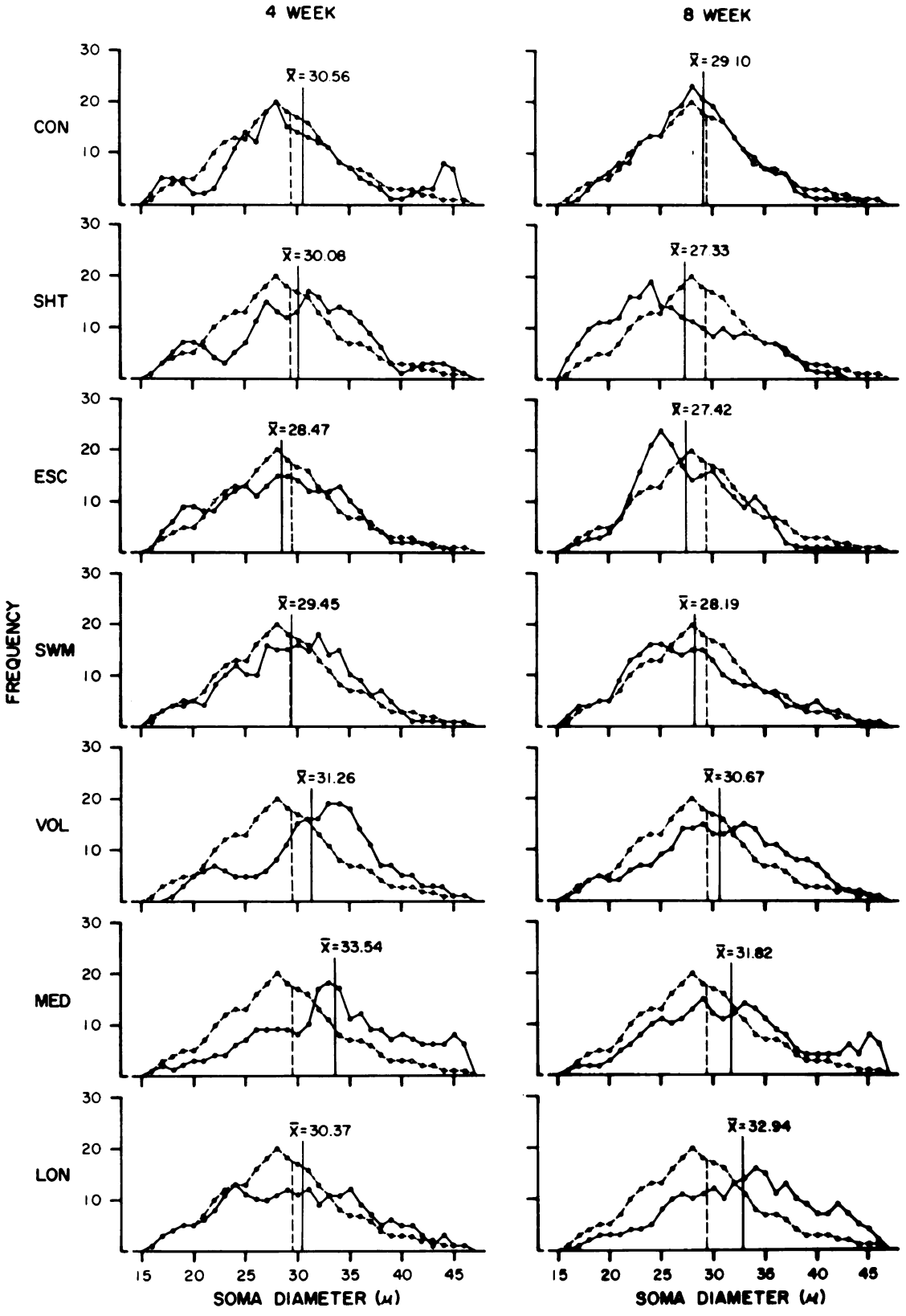
N = not significant.

Contrasting row effect to column effect (e.g., 4 wk vs. 0 wk).

S+ = significant distribution shift at the .05 level to the right in favor of the row effect in the contrast.

S- = significant distribution shift at the .05 level to the left in favor of the row effect in the contrast.

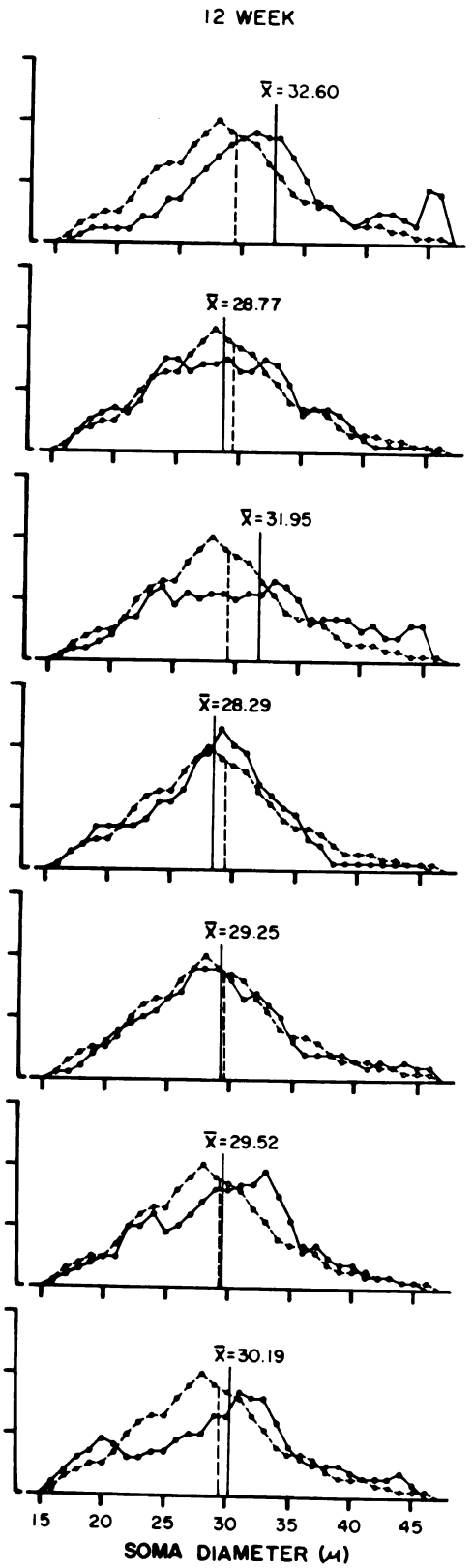
Additional chi-square analyses, between individual treatment cells within each duration (Figure 7), were performed for the significant comparisons found in Table 7. Inspection of these data reveals that the individual comparisons between treatment groups at eight weeks,



Soma Diameter (μ) for all Treatment-Duration Distributions

The broken line distribution represents the zero week distribution compared to each treatment-duration distribution (solid line). The vertical broken line is the mean (29.44μ) for the zero week distribution. The solid vertical line is the mean for the specific treatment-duration.

Figure 7



Summary of analyses of contingency tables between individual treatments within durations for soma diameter

Treatment distributions contrasted	Duration		
	4 wk	8 wk	12 wk
CON vs SHT	S +	S +	S +
CON vs ESC	S +	S +	S +
CON vs SWM	S +	S +	S +
CON vs VOL	S −	S −	S +
CON vs MED	S −	S −	S +
CON vs LON	N	S −	S +
SHT vs ESC	N	S −	S −
SHT vs SWM	− −	− −	− −
SHT vs VOL	S −	S −	N
SHT vs MED	S −	S −	N
SHT vs LON	S −	S −	S −
ESC vs SWM	− −	− −	− −
ESC vs VOL	S −	S −	S +
ESC vs MED	S −	S −	S +
ESC vs LON	S −	S −	N
SWM vs VOL	S −	S −	N
SWM vs MED	S −	S −	N
SWM vs LON	N	S −	S −
VOL vs MED	S −	N	N
VOL vs LON	− −	− −	− −
MED vs LON	S +	S −	N

Summary of analyses of contingency tables between individual durations within treatments for soma diameter.

Duration distributions contrasted	Treatment						
	CON	SHT	ESC	SWM	VOL	MED	LON
0wk vs 4wk	S −	S −	S +	S +	S −	S −	S −
0wk vs 8wk	N	S +	S +	S +	S −	S −	S −
0wk vs 12wk	S −	N	S −	S +	N	S −	S −
4wk vs 8wk	S +	S +	S +	S +	N	N	S −
4wk vs 12wk	− −	− −	− −	− −	− −	− −	− −
8wk vs 12wk	− −	− −	− −	− −	− −	− −	− −

N = not significant.
Contrasting from left to right (e.g. CON vs VOL).

S+ = significant distribution shift at the .05 level to the right in favor of the treatment or duration on the left in the contrast.

S− = significant distribution shift at the .05 level to the left in favor of the treatment or duration on the left in the contrast.

− − = overall analysis across all durations (top table) or treatments (bottom table) was not significant at .05 level. Therefore, analyses at individual treatment-duration cells were not performed.

Figure 7 (cont'd.)

except for the VOL vs. MED comparision, yield results which are identical to those of the overall comparisons across all durations. That is, the relationships found between soma diameter and treatment effect across all durations (Table 7) were prominent at eight weeks.

The results obtained at four weeks and twelve weeks, for certain treatment comparisons, differed from the pattern established between soma size and treatment effect at eight weeks. For example, the CON vs. LON comparison produced a nonsignificant result at four weeks. At eight weeks, the LON group had a significant increase in the frequency of larger soma; but, at twelve weeks, the effect was reversed in favor of the CON group. This is contrary to the results obtained across all durations.

Figure 7 also shows the results of the analyses between individual duration cells within each treatment. The soma tends to be smaller at zero weeks than at four, eight and twelve weeks for all treatment groups except the ESC, SWM and SHT. The greatest increase in soma size for the CON, VOL, and MED groups occurred at four weeks. The LON group had its greatest increase at eight weeks. Likewise, the SHT, ESC and SWM groups had their greatest decreases at eight weeks.

Nucleus

Table 9 shows the summary results of the chi-square analyses between treatment groups across all durations for nuclear diameter. A significant increase in the frequency of larger nuclei in the LON group is found when the results of that group are compared with those of the CON group, whereas the SHT, SWM, ESC, MED, and VOL groups show significant decreases in the frequency of larger nuclei. There are no significant differences between the MED, VOL, ESC, and SWM distributions or between

the SWM, ESC, and SHT distributions. However, the MED and VOL groups had larger nuclei than the SWM and ESC groups which had larger nuclei than the SHT group. The inverse relationship reported earlier between soma diameter and the intensity (speed) of the controlled running wheel programs also occurs with nucleus diameter.

Table 9. Summary of analysis of contingency tables, significant, and nonsignificant relationships between treatments across all durations for nucleus diameter

	CON	SHT	ESC	SWM	VOL	MED
SHT	S-					
ESC	S-	N				
SWM	S-	N	N			
VOL	S-	S+	N	N		
MED	S-	S+	N	N	N	
LON	S+	S+	S+	S+	S+	S+

Summary of significant relationships at .05 level:

$$\text{SHT} < \begin{matrix}\text{MED}\\ \text{VOL}\end{matrix} < \text{CON} < \text{LON}$$

Summary of nonsignificant relationships:

$$\text{SHT} < \begin{matrix}\text{SWM}\\ \text{ESC}\end{matrix} < \begin{matrix}\text{MED}\\ \text{VOL}\end{matrix}$$

N = not significant.

Contrasting row effect to column effect (e.g., SHT vs. CON).

S+ = significant distribution shift at the .05 level to the right in favor of the row effect in the contrast.

S- = significant distribution shift at the .05 level to the left in favor of the row effect in the contrast.

The comparisons between durations across all treatment groups indicate there were significantly smaller nuclei diameters at zero weeks than at the four-, eight-, and twelve-week durations (Table 10). It

appears that the greatest increase in frequency of larger nuclei occurs at four weeks. These results parallel those obtained for the soma diameter.

Table 10. Summary of analysis of contingency tables, significant, and nonsignificant relationships between durations across all treatments for nucleus diameter

	0 wk	4 wk	8 wk
4 wk	S+		
8 wk	S+	S-	
12 wk	S+	N	N

Summary of significant relationships at .05 level:

$$0 \text{ wk} < \begin{matrix} 8 \text{ wk} \\ 12 \text{ wk} \end{matrix} < 4 \text{ wk}$$

Summary of nonsignificant relationships:

8 wk < 12 wk < 4 wk

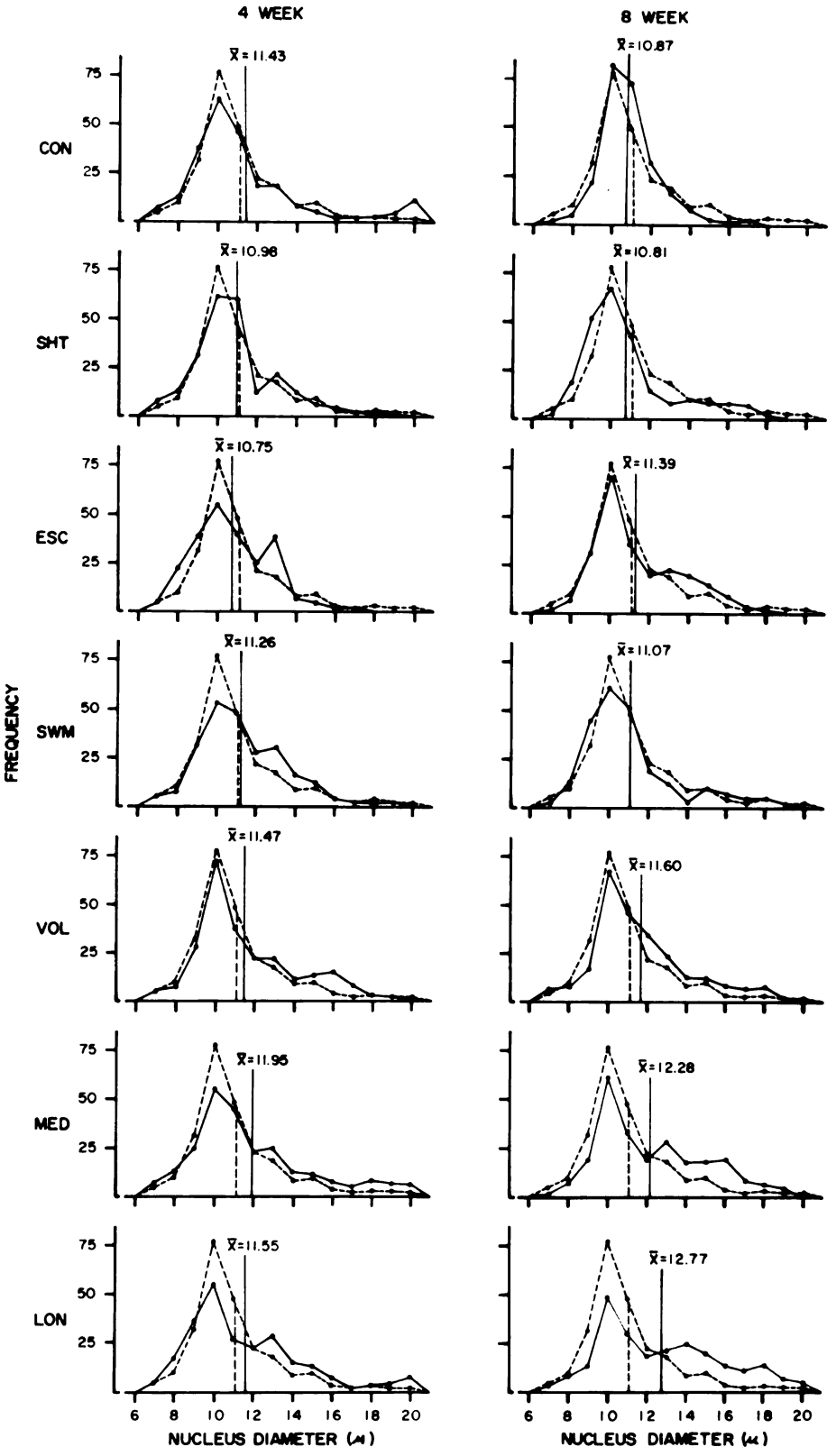
N = not significant.

Contrasting row effect to column effect (e.g., 4 wk vs. 0 wk).

S+ = significant distribution shift at the .05 level to the right in favor of the row effect in the contrast.

S- = significant distribution shift at the .05 level to the left in favor of the row effect in the contrast.

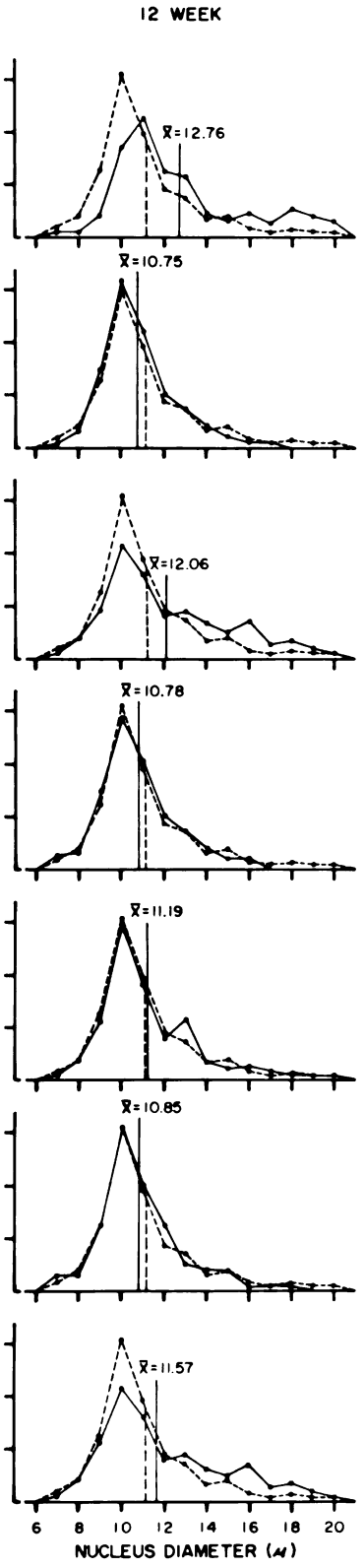
Figure 8 summarizes the results of the comparisons between individual treatment cells within each duration. The inverse relationship between nucleus size and intensity of the CRW programs exists for individual treatment comparisons within certain durations. For example, the nuclei for the MED group were not significantly different from those of the LON group at four and eight weeks. However, at twelve weeks, the LON



Nucleus Diameter (μ) for all Treatment - Duration Distributions

The broken line distribution represents the zero week distribution compared to each treatment - duration distribution (solid line). The vertical broken line is the mean (11.08μ) for the zero week distribution. The solid vertical line is the mean for the specific treatment - duration.

Figure 8



Summary of analyses of contingency tables between individual treatments within durations for nucleus diameter

Treatment distributions contrasted	Duration		
	4 wk	8 wk	12 wk
CON vs SHT	S +	S +	S +
CON vs ESC	S +	S +	S +
CON vs SWM	S +	S +	S +
CON vs VOL	N	S -	S +
CON vs MED	N	S -	S +
CON vs LON	S +	S -	S +
SHT vs ESC	- -	- -	- -
SHT vs SWM	- -	- -	- -
SHT vs VOL	N	S -	N
SHT vs MED	S -	S -	N
SHT vs LON	S -	S -	S -
ESC vs SWM	- -	- -	- -
ESC vs VOL	- -	- -	- -
ESC vs MED	- -	- -	- -
ESC vs LON	S -	S -	N
SWM vs VOL	- -	- -	- -
SWM vs MED	- -	- -	- -
SWM vs LON	N	S -	S -
VOL vs MED	- -	- -	- -
VOL vs LON	N	S -	N
MED vs LON	N	N	S -

Summary of analyses of contingency tables between individual durations within treatments for nucleus diameter

Duration distributions contrasted	Treatment						
	CON	SHT	ESC	SWM	VOL	MED	LON
Owk vs 4wk	S -	N	S +	S +	S -	S -	S -
Owk vs 8wk	S +	S +	S +	S +	S -	S -	S -
Owk vs 12wk	S -	N	S -	N	N	N	S -
4wk vs 8wk	S +	S +	S -	S +	N	N	S -
4wk vs 12wk	- -	- -	- -	- -	- -	- -	- -
4wk vs 12wk	- -	- -	- -	- -	- -	- -	- -

N = not significant.
Contrasting from left to right (e.g. CON vs VOL)

S+ = significant distribution shift at the .05 level to the right in favor of the treatment or duration on the left in the contrast.

S- = significant distribution shift at the .05 level to the left in favor of the treatment or duration on the left in the contrast.

-- = overall analysis across all durations (top table) or treatments (bottom table) was not significant at .05 level. Therefore, analyses at individual treatment - duration cells were not performed.

Figure 8 (cont'd.)

group had significantly more of the larger nuclei than did the MED group. The relationship established between nucleus size and treatment effect (Table 9) holds true primarily at eight weeks with some differences occurring at four and twelve weeks (i.e., at twelve weeks the CON group had significantly larger nuclei than did the LON group).

A summary of the chi-square analyses between individual durations within each treatment also is presented in Figure 8. The nucleus increases in diameter with increasing duration for all treatment groups except the SHT and SWM groups. This increase in size is not linear across durations. The greatest distribution shifts toward smaller nuclei for the SHT and SWM groups occur at eight weeks. The VOL, MED, and LON groups showed their greatest increases in nuclear size at eight weeks. The ESC and CON distributions are the only two to show shifts toward both larger and smaller nuclei. The ESC distribution shows a significant decrease in the frequency of larger nuclei at four weeks and then an increase at eight weeks. The CON distribution has a significant increase in the frequency of larger nuclei at four weeks and a decrease at eight weeks.

Nuelcolus

The summary results of the analyses between treatment groups across all durations are presented in Table 11. It is evident that the SHT, ESC, SWM, MED, and LON groups had significant decreases in the frequency of larger nucleoli when the results of these groups are compared with those of the CON group. The number of larger nucleoli of the VOL group also decreased, but not significantly. The LON, MED, and SHT distributions are not significantly different from each other, but an inverse relationship between the intensity (speed) of the CRW programs and the nucleolus diameter does occur. The ESC distribution displays the greatest decrease in nucleolus diameter.

Table 11. Summary of analysis of contingency tables, significant, and nonsignificant relationships between treatments across all durations for nucleolus diameter

	CON	SHT	ESC	SWM	VOL	MED
SHT	S-					
ESC	S-	N				
SWM	S-	N	N			
VOL	N	S+	S+			
MED	S-	N	S+	N	S-	
LON	S-	N	S+	S+	N	N

Summary of significant relationships at .05 level:

$$\begin{matrix}\text{SWM}\\ \text{ESC}\end{matrix} < \begin{matrix}\text{LON}\\ \text{MED}\\ \text{SHT}\end{matrix} < \text{CON} \qquad \begin{matrix}\text{MED}\\ \text{SHT}\\ \text{ESC}\\ \text{SWM}\end{matrix} < \text{VOL}$$

Summary of nonsignificant relationships:

ESC < SWM < SHT < MED < LON < VOL < CON

N = not significant.

Contrasting row effect to column effect (e.g., SHT vs. CON).

S+ = significant distribution shift at the .05 level to the right in favor of the row effect in the contrast.

S- = significant distribution shift at the .05 level to the left in favor of the row effect in the contrast.

The comparisons between durations across all treatment groups are presented in Table 12. The zero-week animals had the smallest nucleoli and the four-week animals the largest. The difference between the eight- and twelve-week distributions was not significant.

Figure 9 summarizes the chi-square analyses between individual treatment cells within each duration. The overall relationship between nucleolus diameter and treatment effect (Table 11) is most evident at

Table 12. Summary of analysis of contingency tables and significant relationships between durations across all treatments for nucleolus diameter

	0 wk	4 wk	8 wk
4 wk	S+		
8 wk	S+	S-	
12 wk	S+	S-	N

Summary of significant relationships at .05 level:

$$0 \text{ wk} < \begin{matrix} 8 \text{ wk} \\ 12 \text{ wk} \end{matrix} < 4 \text{ wk}$$

N = not significant.

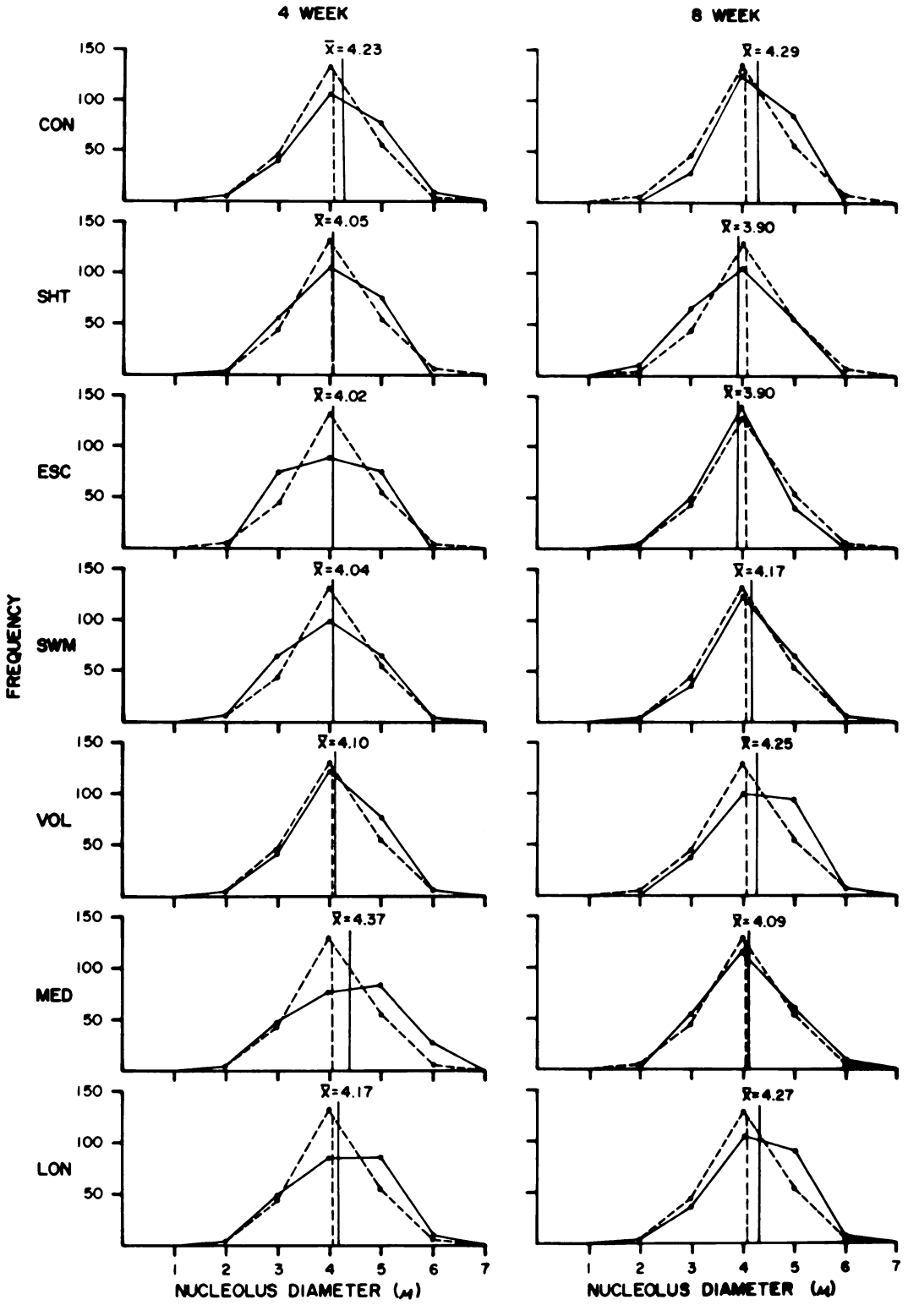
Contrasting row effect to column effect (e.g., 4 wk vs. 0 wk).

S+ = significant distribution shift at the .05 level to the right in favor of the row effect in the contrast.

S- = significant distribution shift at the .05 level to the left in favor of the row effect in the contrast.

eight weeks. The results obtained at four weeks and twelve weeks for certain comparisons differed from the results presented in Table 11. For example, at four weeks, the MED group had significantly larger nucleoli than did the VOL group. This is in disagreement with the results obtained at eight and twelve weeks.

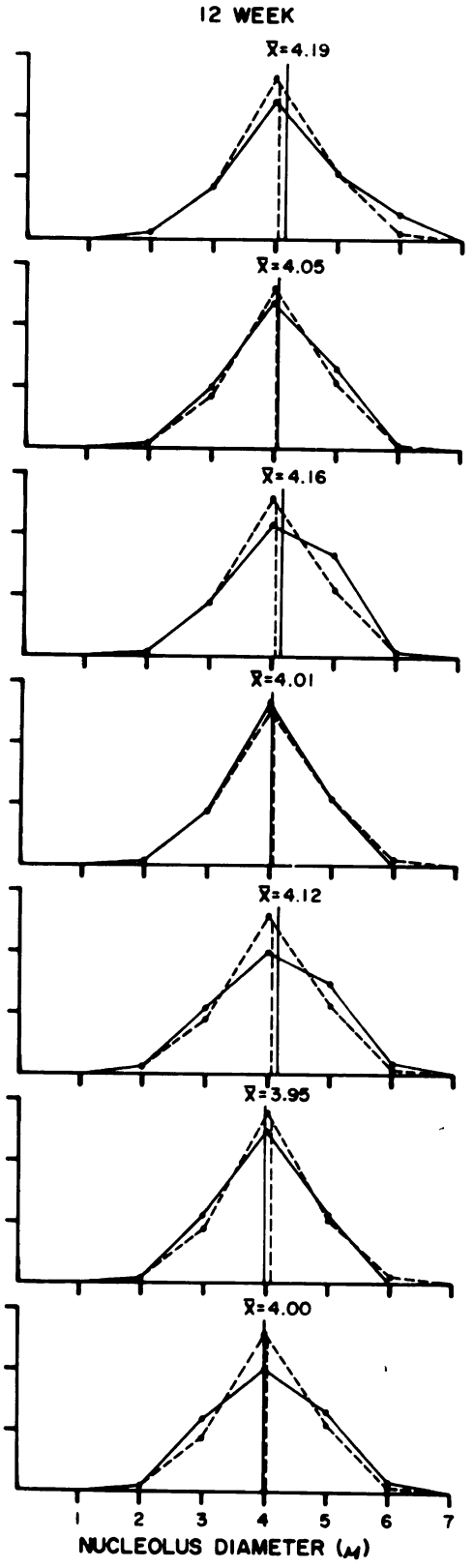
It is difficult to determine a specific trend from the analyses between individual durations within each treatment (Figure 9). It appears that the greatest significant increase in nucleolus diameter for each of the SHT, MED, and LON groups occurred at four weeks, whereas the VOL and SWM groups had their greatest increase at eight weeks.



Nucleolus Diameter (μ) for all Treatment-Duration Distributions

The broken line distribution represents the zero week distribution compared to each treatment-duration distribution (solid line). The vertical broken line is the mean (4.03μ) for the zero week distribution. The solid vertical line is the mean for the specific treatment-duration.

Figure 9



Summary of analyses of contingency tables between individual treatments within durations for nucleolus diameter

Treatment distributions contrasted	Duration		
	4 wk	8 wk	12 wk
CON vs SHT	N	S+	N
CON vs ESC	S+	S+	N
CON vs SWM	N	N	S+
CON vs VOL	--	--	--
CON vs MED	S-	S+	N
CON vs LON	N	N	S+
SHT vs ESC	--	--	--
SHT vs SWM	--	--	--
SHT vs VOL	N	S-	N
SHT vs MED	--	--	--
SHT vs LON	--	--	--
ESC vs SWM	--	--	--
ESC vs VOL	S-	S-	N
ESC vs MED	S-	S-	S+
ESC vs LON	N	S-	S+
SWM vs VOL	N	N	S-
SWM vs MED	--	--	--
SWM vs LON	N	N	S-
VOL vs MED	S-	S+	S+
VOL vs LON	--	--	--
MED vs LON	--	--	--

Summary of analyses of contingency tables between individual durations within treatments for nucleolus diameter

Duration distributions contrasted	Treatment						
	CON	SHT	ESC	SWM	VOL	MED	LON
0wk vs 4wk	S-	S-	S+	S+	N	S-	S-
0wk vs 8wk	S-	S+	S+	S-	S-	N	S-
0wk vs 12wk	S-	N	S-	N	S-	N	S+
4wk vs 8wk	N	N	S+	S-	S-	S+	N
4wk vs 12wk	N	N	S-	S-	N	S+	S+
8wk vs 12wk	--	--	--	--	--	--	--

N = not significant.
Contrasting from left to right (e.g. CON vs VOL).

S+ = significant distribution shift at the .05 level to the right in favor of the treatment or duration on the left in the contrast.

S- = significant distribution shift at the .05 level to the left in favor of the treatment or duration on the left in the contrast.

-- = overall analysis across all durations (top table) or treatments (bottom table) was not significant at .05 level. Therefore, analyses at individual treatment-duration cells were not performed.

Figure 9 (cont'd.)

General Pattern of Morphological Results

The morphological results for the soma, nucleus, and nucleolus give evidence of significant treatment and duration effects. There was a definite trend at eight weeks indicating the existence of an inverse relationship between the intensity (speed) of the CRW programs and the sizes of the soma, nucleus, and nucleolus. That is, as the intensity of training increases, decreases in the diameters of the soma, nucleus, and nucleolus occur. The fact that this trend did not persist through the twelve-week duration perhaps indicates the beginning of an adaptation to exercise. A direct relationship was found between the amount of electrical shock received by the LON, MED, SHT, and ESC groups and the sizes of the soma, nucleus, and nucleolus.

From the treatment effects presented in this chapter, it is believed that animals trained on VOL, MED, and LON programs have larger soma, nuclei, and nucleoli than do those trained on SHT, ESC, and SWM programs. It has been observed that the total revolutions run (TRR) by the VOL animals was between the TRR values of the MED and LON groups. In addition, one might speculate that the intensity of running for the VOL group was low in that the TRR was recorded for a 24-hour period. Therefore, the similarities in results found in the VOL, MED, and LON groups are not surprising.

Photometric Results

The chi-square values for the overall treatment and duration effects upon Nissl substance concentration are presented in Table 13. Since both of these values are significant at the .05 level, additional analyses were conducted to determine category effects within each treatment across all durations (Table 14) and within each duration across all treatments (Table 15). Each of these analyses yielded significant results.

Table 13. Analyses of contingency tables for each independent variable with Sin^{-1} Nissl substance

Independent Variable	Dependent Variable	df	X^2	$X^2_{.05}$	
Treatment	Nissl substance	189	458.9	218.5	S
Duration	Nissl substance	90	609.3	113.2	S

S = significant at .05 level.

Table 14. Analyses of contingency tables within each treatment across all durations for Sin^{-1} Nissl substance

Dependent Variable	CON	SHT	ESC	SWM	VOL	MED	LON
Sin^{-1} Nissl substance	S	S	S	S	S	S	S

S = significant at .05 level.

Table 15. Analyses of contingency tables within each duration across all treatments for Sin^{-1} Nissl substance

Dependent Variable	4 wk	8 wk	12 wk
Sin^{-1} Nissl substance	S	S	S

S = significant at .05 level.

Therefore, additional analyses were performed to detect significant differences between treatments across all durations (Table 16) and between durations across all treatments (Table 17).

Table 16. Summary of analysis of contingency tables and significant relationships between treatments across all durations for $\sin^{-1}$ Nissl substance

	CON	SHT	ESC	SWM	VOL	MED
SHT	S-					
ESC	S-	S-				
SWM	S-	S-	S-			
VOL	S+	S+	S+	S+		
MED	N	S+	S+	S+	S-	
LON	S+	S+	S+	S+	S-	N

Summary of significant relationships at .05 level:

$$\text{SWM} < \text{ESC} < \text{SHT} < \text{CON} < \begin{matrix}\text{MED} \\ \text{LON}\end{matrix} < \text{VOL}$$

N = not significant.

Contrasting row effect to column effect (e.g., SHT vs. CON).

S+ = significant distribution shift at the .05 level to the right in favor of the row effect in the contrast.

S- = significant distribution shift at the .05 level to the left in favor of the row effect in the contrast.

It is evident from Table 16 that the LON and MED distributions are shifted to the right (an increase in Nissl substance concentration) as compared to the SHT distribution. The inverse relationship, established between intensity (speed) of the running programs and soma, nucleus, and nucleolus diameters appears to be reflected, at least in part, in Nissl substance concentration. That is, the SHT group tended to have less Nissl substance than did the MED and LON groups. The LON and MED groups were not significantly different.

The VOL group had the greatest number of cells with a high Nissl substance concentration. The lowest concentration of Nissl substance occurred in the SWM group. From Table 16, it is apparent that the VOL,

LON, and MED groups had high concentrations of Nissl substance, whereas the SHT, ESC, and SWM groups had low concentrations.

The comparisons between durations across all treatments (Table 17) indicates the greatest concentration of Nissl substance occurred at zero weeks. It is apparent from the relationships shown in Table 17 that Nissl substance concentration decreased significantly with time until eight weeks, after which there was a significant increase.

Table 17. Summary of analysis of contingency tables and significant relationships between durations across all treatments for Sin^{-1} Nissl substance

	0 wk	4 wk	8 wk
4 wk	S-		
8 wk	S-	S-	
12 wk	S-	S-	S+

Summary of significant relationships at .05 level:

8 wk < 12 wk < 4 wk < 0 wk

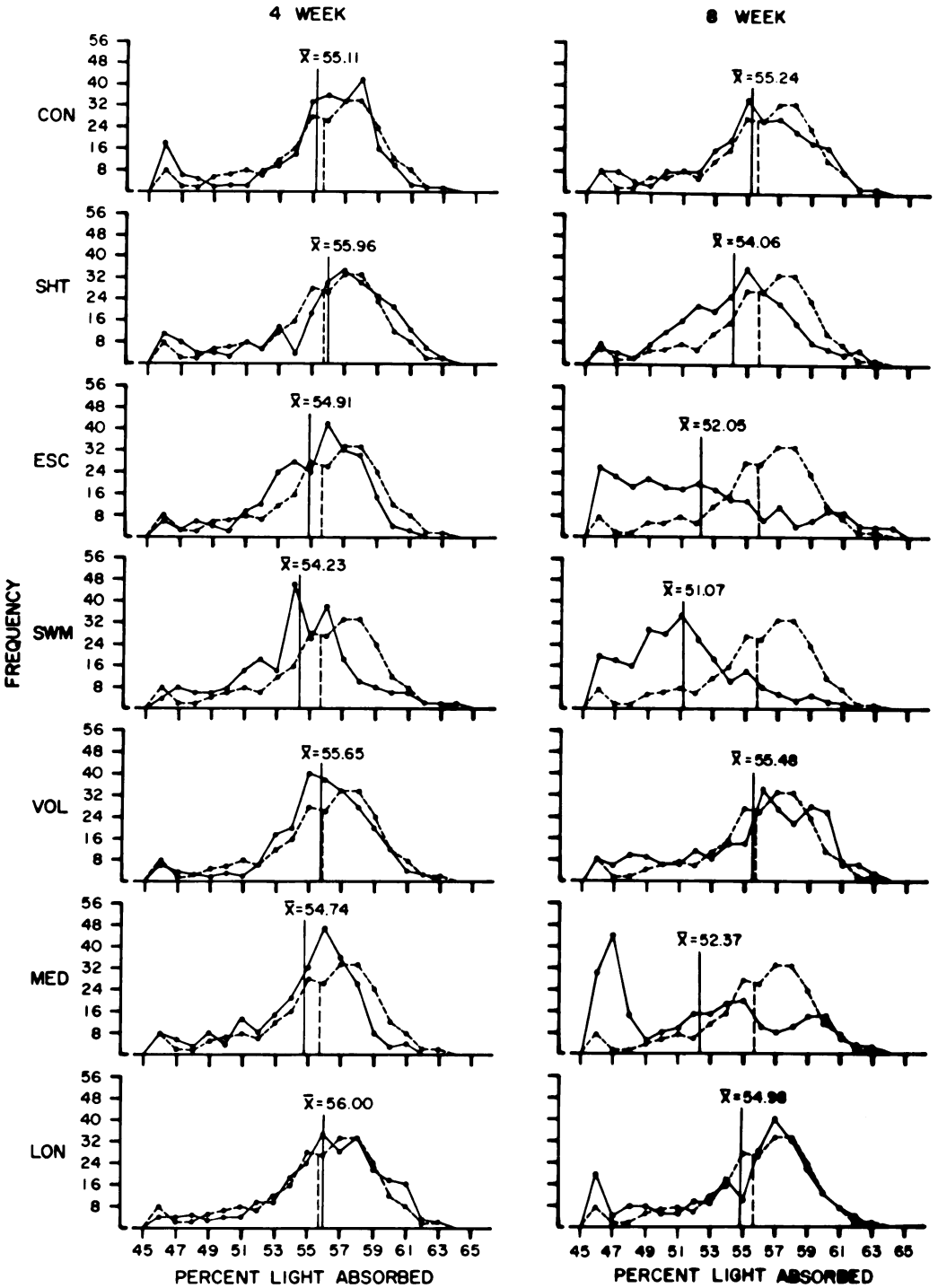
N = not significant.

Contrasting row effect to column effect (e.g., 4 wk vs. 0 wk).

S+ = significant distribution shift at the .05 level to the right in favor of the row effect in the contrast.

S- = significant distribution shift at the .05 level to the left in favor of the row effect in the contrast.

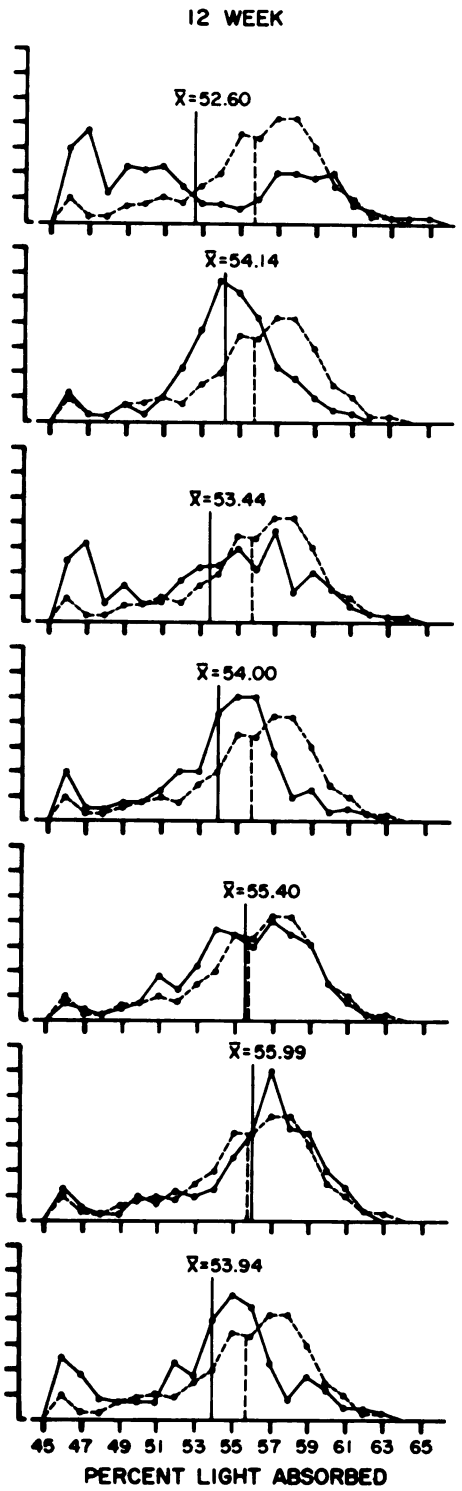
Figure 10 summarizes the results of the comparisons between individual treatment cells within each duration. The results of the individual comparisons, except the SHT vs. MED and VOL vs. LON, at eight weeks are identical to those of the comparisons between treatments across all durations. That is, the overall relationship established between Nissl



Nissl Substance Concentration (percent light absorbed) for all Treatment-Duration Distributions

The broken line distribution represents the zero week distribution compared to each treatment-duration distribution (solid line). The vertical broken line is the mean (55.76 percent) for the zero week distribution. The solid vertical line is the mean for the specific treatment-duration.

Figure 10



Summary of analyses of contingency tables between individual treatments within durations for $\sin^{-1}$ Nissl

Treatment distributions contrasted	Duration		
	4 wk	8 wk	12 wk
CON vs SHT	S−	S+	S−
CON vs ESC	S+	S+	S−
CON vs SWM	S+	S+	S−
CON vs VOL	N	S−	S−
CON vs MED	−−	−−	−−
CON vs LON	S−	S−	S−
SHT vs ESC	S+	S+	S+
SHT vs SWM	S+	S+	N
SHT vs VOL	S+	S+	S+
SHT vs MED	S+	S+	S−
SHT vs LON	N	S−	S−
ESC vs SWM	S+	S+	S−
ESC vs VOL	S−	S−	S−
ESC vs MED	N	S−	S−
ESC vs LON	S−	S−	N
SWM vs VOL	S−	S−	S−
SWM vs MED	S−	S−	S−
SWM vs LON	S−	S−	N
VOL vs MED	S+	S+	S−
VOL vs LON	N	N	S+
MED vs LON	−−	−−	−−

Summary of analyses of contingency tables between individual durations within treatments for $\sin^{-1}$ Nissl

Duration distributions contrasted	Treatment						
	CON	SHT	ESC	SWM	VOL	MED	LON
Owk vs 4wk	S+	N	S+	S+	N	S+	N
Owk vs 8wk	N	S+	S+	S+	S−	S+	S+
Owk vs 12wk	S+	S+	S+	S+	N	N	S+
4wk vs 8wk	S−	S+	S+	S+	S−	S+	S+
4wk vs 12wk	S+	S+	S+	N	N	S−	S+
8wk vs 12wk	S+	S+	S−	S−	S+	S−	S+

N = not significant.
Contrasting from left to right (e.g. CON vs VOL).

S+ = significant distribution shift at the .05 level to the right in favor of the treatment or duration on the left in the contrast.

S− = significant distribution shift at the .05 level to the left in favor of the treatment or duration on the left in the contrast.

−− = overall analysis across all durations (top table) or treatments (bottom table) was not significant at .05 level. Therefore, analyses at individual treatment-duration cells were not performed.

Figure 10 (cont'd.)

substance concentration and treatment effect across durations (Table 16) existed at eight weeks. Some variations occurred at four and twelve weeks. For example, the SHT group had significantly higher Nissl substance concentrations at four and eight weeks than did the MED group. This is contrary to the results presented in Table 16. At twelve weeks, the MED group did have a significantly higher Nissl substance. Therefore, the significantly higher concentration obtained across all durations in favor of the MED group can be attributed to the significance obtained at twelve weeks.

With the exception of a few discrepancies, the results of the analyses between individual duration cells within each treatment (Figure 10) are very similar to the results obtained between durations across all treatments (Table 17). The comparisons show that the majority of the treatment groups had lower concentrations of Nissl substance at four, eight, and twelve weeks than they did at zero weeks. The treatment groups also tended to have significantly higher Nissl substance concentration at four weeks than at eight or twelve weeks.

Discussion

It is evident that each of the experimental groups responded specifically to the various training programs imposed. However, for purposes of discussion, those groups can be categorized according to changes in morphological characteristics and Nissl substance concentration into two trios: VOL, LON, and MED; and SHT, ESC, and SWM. The VOL, LON, and MED groups had larger soma, nuclei, and nucleoli and higher concentrations of Nissl substance than did the SHT, ESC, and SWM groups. Within the LON, MED, and VOL groups, the LON group had larger morphological characteristics and a higher Nissl substance concentration than did the MED group.

In looking at the duration effect, the zero-week animals had the greatest number of small soma, nuclei, and nucleoli with the highest concentration of Nissl substance. The four-week animals had the greatest frequency of large soma, nuclei, and nucleoli and, excluding the zero-week animals, had the highest concentration of Nissl substance. A decrease in soma, nucleus, and nucleolus size and a decrease in Nissl substance concentration occurred after eight weeks of training. Between eight and twelve weeks of training, the soma, nucleus, and nucleolus sizes and the Nissl substance concentration increased. It should be pointed out that the training programs progressively increased in exercise intensity during the first eight weeks of training and then leveled off. Thus, one might speculate that the increases observed from eight to twelve weeks could indicate the beginning of adaptations to the several exercise regimens.

The LON and SHT programs were designed to simulate aerobic and anaerobic activity, respectively. One might speculate, then, that the LON group should have a greater number of small motor neurons than would the SHT group (14,26,27,44). The results at eight weeks indicate that the LON group had a greater number of large motor neurons than did the SHT group. However, at twelve weeks, there was a decrease in the number of large motor neurons for the LON group and an increase in small motor neurons for the SHT group. Perhaps if the training period was extended, the results would continue to show a decrease in motor neuron size for those animals in the LON group and an increase for those animals in the SHT group. If these changes would continue to a point where the SHT group would have a greater number of large motor neurons than would the LON group, then the results could reflect aerobic and anaerobic activity.

The significant training changes obtained in soma, nucleus, and nucleolus sizes do not confirm the results of earlier studies which showed no changes in size for the soma and nucleus (29,40,76) and an increase in size for nucleolus (40). The morphological changes in the soma and nuclei of the ESC group also compare favorably with the results of earlier studies (37,39,50,51). However, those studies were conducted as acute experiments, not chronic. Results from an earlier investigation (40) conducted in this laboratory using swimming as a training method are similar to the results for the soma size and Nissl substance concentration obtained on the SWM group in this investigation. That is, the soma decreased in size and there was a decrease in Nissl substance concentration.

Previous investigations indicate motor neurons return to "normal"[1] activity thirty-six to seventy-two hours following increased functional activity (67,56). Since sacrifices in this study were performed approximately sixty-five to seventy-two hours after the last exercise period, the motor neurons should have been in a state of "normality" or a stabilized internal environment. It is apparent from the morphological and Nissl substance concentration results that, if in fact the motor neurons do return to "normality" within 72 hours, this state is specific to each exercise regimen after eight weeks of training.

A direct relationship was observed between Nissl substance concentration and soma, nucleus, and nucleolus size which is in agreement with previous investigation (54,56). Results of earlier studies (53,54) also

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[1]Normal activity is defined as a state in which the internal environment of the cell has returned to a stabilized condition. In other words, the cell has recovered from the acute effects of the increased functional activity.

show a direct relationship between functional activity and protein synthesis. Thus, a large motor neuron in a state of "normality" should have a greater concentration of Nissl substance, due to an increase in functional activity, than would a small motor neuron.

Values of the total revolutions run (TRR) for the CRW groups indicate that the LON group ran the greatest distance followed by the MED group and then the SHT group. The greatest group differences in both TER (total expected revolutions) and TRR values occur during the seventh and eighth weeks of training. Therefore, it appears that the significant differences between the CRW groups at eight weeks can be attributed to specific training effects. This line of reasoning can be carried one step further. If TRR is a reflection of functional activity, then a direct relationship exists between Nissl substance concentration, motor neuron size, and functional activity. This relationship would explain the large morphological characteristics and the high Nissl substance concentration found in the LON group.

Since the VOL results are similar to those of the MED and LON groups, one can speculate that the intensity of wheel running for the VOL group was similar to that of the LON and MED groups. It was thought that the training responses of the SWM and LON groups should have been similar, in that both training programs probably require aerobic activity. The results obtained for the SWM and LON groups were clearly dissimilar. The differences cannot be resolved from the present data. However, the muscle results of a companion study (18) indicated that the SWM and LON groups were similar.

A physiologic interpretation of the results of this study is not possible at this time. It is evident that specific differences have been observed for the treatments and durations used. That is, it appears

that motor neurons in a state of "normality" reflect patterns of change which are specific to various chronic exercise regimens. With few exceptions, the motor neurons at eight weeks were altered according to the functional requirements of the chronic exercise treatments during the experimental period. On the basis of the twelve-week results, it would appear likely that if the programs were extended a reversal in motor neuron size and Nissl substance concentration could occur. If this hypothesis is correct, the results presented herein may reflect adaptive changes which occur prior to the "true" changes produced by the various programs of chronic exercise.

CHAPTER V

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

Summary

The purpose of this study was to determine the effects of seven chronic exercise programs on motor neuron morphology and Nissl substance concentration from the lower lumbar spinal segments of the adult-male, albino rat.

One hundred eighty-two, 72-day-old, normal, male, albino rats (Sprague-Dawley strain) were randomly assigned to seven treatment groups. After a twelve-day adjustment period, treatments began when the animals were 85 days of age. The 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); electric stimulus control (ESC); and long duration swimming (SWM). The animals had access to water and a commercial animal diet *ad libitum*. The treatments were conducted Monday through Friday under controlled environmental conditions.

Only those animals that met minimum training requirements and were subjectively determined to be in good health were selected for sacrifice. Animals within each treatment group were sacrificed prior to the commencement of treatments and at four-, eight-, and twelve-week durations after treatments began. The final sample consisted of 98 animals.

Animals were sacrificed under anesthesia with 6.48 percent sodium pentobarbital by intraperitoneal injection. The intact spinal cord from T10 to S2 was surgically exposed and removed. Subjectively, the lumbar enlargement was cut transversely between spinal segments L3 and L4. Caudal spinal segments L4 through S2 were fixed in 10 percent buffered formalin for 24 hours and then later embedded in paraffin. Serial, cranio-caudal cross-sections, 7μ thick, were mounted on 35-mm leader film and stained with Luxol Fast Blue and counterstained with Creysl-echt Violet.

Nissl substance concentration was determined photometrically as percent light absorption. Using a microprojector to project the motor neuron at a magnification X1000, a two-dimensional structure with four lines intersecting each other at equal angles was used to make cross measurements of the soma, nucleus, and nucleolus. The Nissl substance concentration and the soma, nucleus, and nucleolus measurements were tested for distribution differences at the .05 level using chi-square analysis of contingency tables (ACT). Additional ACT were performed where significance was obtained.

A definite trend existed at eight weeks indicating the existence of an inverse relationship between the intensity (speed) of the controlled running wheel programs and the diameters of the soma, nucleus, and nucleolus. A direct relationship was found between the size of the motor neuron and Nissl substance concentration. The fact that this trend did not persist through the twelve-week duration indicates the animals may have been in the process of adapting to the exercise regimens. Following eight weeks of training, the LON, MED, and VOL groups had significantly greater frequencies of motor neurons with larger

morphological characteristics and with higher Nissl substance concentrations than did the SHT, ESC, and SWM groups.

Conclusions

The following conclusions can be drawn from the results of this study:

1. The size of the soma, nucleus, and nucleolus and the Nissl substance concentration of the motor neuron are affected by chronic physical activity.

2. Motor neurons in a state of "normality" reflect patterns of change which are specific to various chronic exercise regimens following eight weeks of training.

3. A direct relationship exists between the size of the motor neuron and Nissl substance concentration following eight weeks of training.

Recommendations

1. It appears that one of the major factors contributing to the patterns of change obtained in this study was the time of sacrifice in relationship to the last exercise period. Therefore, additional studies should be performed altering the time interval between the last exercise period and the time of sacrifice.

2. A histochemical study is needed to investigate the roles of aerobic metabolism and anaerobic glycolysis of motor neurons subjected to different levels of chronic physical activity.

3. A microelectrode implant technique should be developed to monitor changes in the neurophysiological characteristics of spinal cord motor neurons which result from chronic physical activity.

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APPENDICES

APPENDIX A

TRAINING PROGRAMS

TABLE A-1.-- Standard eight-week, short-duration, high-speed endurance training program for postpubertal and adult male rats in controlled running wheels.

Wk.	Day of Wk.	Day of Tr.	Acceleration Time (sec)	Work Time (min: sec)	Rest Time (sec)	Repetitions per Bout	No. of Bouts	Time Between Bouts (min)	Shock (ma)	Run Speed (ft/ sec)	Total Time of Prog. (min: sec)	Total Exp. Revolutions *TER*	Total Work Time (sec) *TWT*
0	4=T	-2	3.0	40:00	10	1	1	5.0	0.0	1.5	40:00	---	---
	5=F	-1	3.0	40:00	10	1	1	5.0	0.0	1.5	40:00	---	---
1	1=M	1	3.0	00:10	10	40	3	5.0	1.2	1.5	49:30	450	1200
	2=T	2	3.0	00:10	10	40	3	5.0	1.2	1.5	49:30	450	1200
	3=W	3	3.0	00:10	10	40	3	5.0	1.2	1.5	49:30	450	1200
	4=T	4	2.5	00:10	10	40	3	5.0	1.2	2.0	49:30	600	1200
	5=F	5	2.0	00:10	10	40	3	5.0	1.2	2.0	49:30	600	1200
2	1=M	6	1.5	00:10	10	28	4	5.0	1.2	2.5	51:40	700	1120
	2=T	7	1.5	00:10	15	27	4	5.0	1.2	3.0	59:00	810	1080
	3=W	8	1.5	00:10	15	27	4	5.0	1.2	3.0	59:00	810	1080
	4=T	9	1.5	00:10	15	27	4	5.0	1.2	3.0	59:00	810	1080
	5=F	10	1.5	00:10	15	27	4	5.0	1.2	3.0	59:00	810	1080
3	1=M	11	1.5	00:10	15	27	4	5.0	1.2	3.0	59:00	810	1080
	2=T	12	1.5	00:10	20	23	4	5.0	1.2	3.5	59:40	805	920
	3=W	13	1.5	00:10	20	23	4	5.0	1.2	3.5	59:40	805	920
	4=T	14	1.5	00:10	20	23	4	5.0	1.2	3.5	59:40	805	920
	5=F	15	1.5	00:10	20	23	4	5.0	1.2	3.5	59:40	805	920
4	1=M	16	1.5	00:10	20	23	4	5.0	1.2	3.5	59:40	805	920
	2=T	17	1.5	00:10	25	20	4	5.0	1.0	4.0	60:00	800	800
	3=W	18	1.5	00:10	25	20	4	5.0	1.0	4.0	60:00	800	800
	4=T	19	1.5	00:10	25	20	4	5.0	1.0	4.0	60:00	800	800
	5=F	20	1.5	00:10	25	20	4	5.0	1.0	4.0	60:00	800	800
5	1=M	21	1.5	00:10	25	20	4	5.0	1.0	4.0	60:00	800	800
	2=T	22	1.5	00:10	30	16	4	5.0	1.0	4.5	55:40	720	640
	3=W	23	1.5	00:10	30	16	4	5.0	1.0	4.5	55:40	720	640
	4=T	24	1.5	00:10	30	16	4	5.0	1.0	4.5	55:40	720	640
	5=F	25	1.5	00:10	30	16	4	5.0	1.0	4.5	55:40	720	640
6	1=M	26	1.5	00:10	30	16	4	5.0	1.0	4.5	55:40	720	640
	2=T	27	2.0	00:10	35	10	5	5.0	1.0	5.0	54:35	625	500
	3=W	28	2.0	00:10	35	10	5	5.0	1.0	5.0	54:35	625	500
	4=T	29	2.0	00:10	35	10	5	5.0	1.0	5.0	54:34	625	500
	5=F	30	2.0	00:10	35	10	5	5.0	1.0	5.0	54:35	625	500
7	1=M	31	2.0	00:10	35	10	5	5.0	1.0	5.0	54:35	625	500
	2=T	32	2.0	00:10	35	7	8	2.5	1.0	5.0	54:50	700	560
	3=W	33	2.0	00:10	35	7	8	2.5	1.0	5.0	54:50	700	560
	4=T	34	2.0	00:10	35	7	8	2.5	1.0	5.0	54:50	700	560
	5=F	35	2.0	00:10	35	7	8	2.5	1.0	5.0	54:50	700	560
8	1=M	36	2.0	00:10	35	7	8	2.5	1.0	5.0	54:50	700	560
	2=T	37	2.0	00:10	40	6	8	2.5	1.0	5.5	52.10	660	480
	3=W	38	2.0	00:10	40	6	8	2.5	1.0	5.5	52:10	660	480
	4=T	39	2.0	00:10	40	6	8	2.5	1.0	5.5	52:10	660	480
	5=F	40	2.0	00:10	40	6	8	2.5	1.0	5.5	52:10	660	480

This standard program was designed using male rats of the Sprague-Dawley strain. All animals were between 70 and 170 days-of-age at the beginning of the program. The duration and intensity of the program were established so that 75 per cent of all such animals should have *PSP* and *PER* scores of 75 or higher during the final two weeks. Alterations in the work time, rest time, repetitions per bout, number of bouts, or time between bouts can be used to affect changes in these values. Other strains or ages of animals could be expected to respond differently to the program.

All animals should be exposed to a minimum of one week of voluntary running in a wheel prior to the start of the program. Failure to provide this adjustment period will impose a double learning situation on the animals and will seriously impair the effectiveness of the training program.

Standard short-duration, high-speed endurance maintenance program for postpubertal and adult male rats in controlled running wheels.

Acceleration Time (sec)	Work Time (min: sec)	Rest Time (sec)	Repetitions per Bout	No. of Bouts	Time Between Bouts (min)	Shock (ma)	Run Speed (ft/ sec)	Total Time of Prog. (min: sec)	Total Exp. Revolutions *TER*	Total Work Time (sec) *TWT*
1.5	00:30	30	6	3	5.0	1.0	4.0	26:30	540	540

TABLE A-2.-- Standard eight-week, medium-duration, moderate-speed endurance training program for postpubertal and adult male rats in controlled running wheels.

Wk.	Day of Wk.	Day of Tr.	Acceleration Time (sec)	Work Time (min: sec)	Rest Time (sec)	Repetitions per Bout	No. of Bouts	Time Between Bouts (min)	Shock (ma)	Run Speed (ft/ sec)	Total Time of Prog. (min: sec)	Total Exp. Revolutions *TER*	Total Work Time (sec) *TWT*
0	4=T	-2	3.0	40:00	10	1	1	5.0	0.0	1.5	40:00	---	---
	5=F	-1	3.0	40:00	10	1	1	5.0	0.0	1.5	40:00	---	---
1	1=M	1	3.0	00:10	10	40	3	5.0	1.2	1.5	49:30	450	1200
	2=T	2	3.0	00:10	10	40	3	5.0	1.2	1.5	49:30	450	1200
	3=W	3	3.0	00:10	10	40	3	5.0	1.2	1.5	49:30	450	1200
	4=T	4	2.5	00:15	15	19	4	5.0	1.2	2.0	52:00	570	1140
	5=F	5	2.5	00:15	15	19	4	5.0	1.2	2.0	52:00	570	1140
2	1=M	6	2.0	00:15	15	19	4	5.0	1.2	2.0	52:00	570	1140
	2=T	7	2.0	00:15	15	19	4	5.0	1.2	2.5	52:00	712	1140
	3=W	8	1.5	00:15	15	19	4	5.0	1.2	2.5	52:00	712	1140
	4=T	9	1.5	00:15	15	19	4	5.0	1.2	2.5	52:00	712	1140
	5=F	10	1.5	00:15	15	19	4	5.0	1.2	2.5	52:00	712	1140
3	1=M	11	1.5	00:15	15	19	4	5.0	1.2	2.5	52:00	712	1140
	2=T	12	1.5	00:15	15	18	4	5.0	1.2	3.0	50:00	810	1080
	3=W	13	1.5	00:15	15	18	4	5.0	1.2	3.0	50:00	810	1080
	4=T	14	1.5	00:15	15	18	4	5.0	1.2	3.0	50:00	810	1080
	5=F	15	1.5	00:15	15	18	4	5.0	1.2	3.0	50:00	810	1080
4	1=M	16	1.5	00:15	15	18	4	5.0	1.2	3.0	50:00	810	1080
	2=T	17	1.5	00:15	15	18	4	5.0	1.0	3.5	50:00	945	1080
	3=W	18	1.5	00:15	15	18	4	5.0	1.0	3.5	50:00	945	1080
	4=T	19	1.5	00:15	15	18	4	5.0	1.0	3.5	50:00	945	1080
	5=F	20	1.5	00:15	15	18	4	5.0	1.0	3.5	50:00	945	1080
5	1=M	21	1.5	00:15	15	18	4	5.0	1.0	3.5	50:00	945	1080
	2=T	22	1.5	00:15	15	14	5	5.0	1.0	4.0	53:45	1050	1050
	3=W	23	1.5	00:15	15	14	5	5.0	1.0	4.0	53:45	1050	1050
	4=T	24	1.5	00:15	15	14	5	5.0	1.0	4.0	53:45	1050	1050
	5=F	25	1.5	00:15	15	14	5	5.0	1.0	4.0	53:45	1050	1050
6	1=M	26	1.5	00:15	15	14	5	5.0	1.0	4.0	53:45	1050	1050
	2=T	27	1.5	00:20	20	11	5	5.0	1.0	4.0	55:00	1100	1100
	3=W	28	1.5	00:20	20	11	5	5.0	1.0	4.0	55:00	1100	1100
	4=T	29	1.5	00:20	20	11	5	5.0	1.0	4.0	55:00	1100	1100
	5=F	30	1.5	00:20	20	11	5	5.0	1.0	4.0	55:00	1100	1100
7	1=M	31	1.5	00:20	20	11	5	5.0	1.0	4.0	55:00	1100	1100
	2=T	32	1.5	00:25	25	9	5	5.0	1.0	4.0	55:25	1125	1125
	3=W	33	1.5	00:25	25	9	5	5.0	1.0	4.0	55:25	1125	1125
	4=T	34	1.5	00:25	25	9	5	5.0	1.0	4.0	55:25	1125	1125
	5=F	35	1.5	00:25	25	9	5	5.0	1.0	4.0	55:25	1125	1125
8	1=M	36	1.5	00:25	25	9	5	5.0	1.0	4.0	55:25	1125	1125
	2=T	37	1.5	00:30	30	8	5	5.0	1.0	4.0	57:30	1200	1200
	3=W	38	1.5	00:30	30	8	5	5.0	1.0	4.0	57:30	1200	1200
	4=T	39	1.5	00:30	30	8	5	5.0	1.0	4.0	57:30	1200	1200
	5=F	40	1.5	00:30	30	8	5	5.0	1.0	4.0	57:30	1200	1200

This standard program was designed using male rats of the Sprague-Dawley Strain. All animals were between 70 and 170 days-of-age at the beginning of the program. The duration and intensity of the program were established so that 75 per cent of all such animals should have *PSF* and *PER* scores of 75 or higher during the final two weeks. Alterations in the rest time, repetitions per bout, number of bouts, or time between bouts can be used to affect changes in these values. Other strains or ages of animals could be expected to respond differently to the program.

All animals should be exposed to a minimum of one week of voluntary running in a wheel prior to the start of the training program. Failure to provide this adjustment period will impose a double learning situation on the animals and will seriously impair the effectiveness of the training program.

Standard medium-duration, moderate-speed endurance maintenance program for postpubertal and adult male rats in controlled running wheels.

Acceleration Time (sec)	Work Time (min: sec)	Rest Time (sec)	Repetitions per Bout	No. of Bouts	Time Between Bouts (min)	Shock (ma)	Run Speed (ft/ sec)	Total Time of Prog. (min: sec)	Total Exp. Revolutions *TER*	Total Work Time (sec) *TWT*
2.0	00:10	40	4	6	2.5	1.0	5.5	28:30	330	240

TABLE A-3.-- Standard eight-week, long-duration, low-speed endurance training program for postpubertal and adult male rats in controlled running wheels.

Wk.	Day of Wk.	Day of Tr.	Acc-eler-ation Time (sec)	Work Time (min: sec)	Rest Time (sec)	Repe-ti-tions per Bout	No. of Bouts	Time Bet-ween Bouts (min)	Shock (ma)	Run Speed (ft/ sec)	Total Time of Prog. (min: sec)	Total Exp. Revo-lu-tions *TER*	Total Work Time (sec) *TWT*
0	4=T	-2	3.0	40:00	10	1	1	5.0	0.0	1.5	40:00	---	---
	5=F	-1	3.0	40:00	10	1	1	5.0	0.0	1.5	40:00	---	---
1	1=M	1	3.0	00:10	10	40	3	5.0	1.2	1.5	49:30	450	1200
	2=T	2	3.0	00:10	10	40	3	5.0	1.2	1.5	49:30	450	1200
	3=W	3	3.0	00:10	10	40	3	5.0	1.2	1.5	49:30	450	1200
	4=T	4	2.5	00:20	10	30	2	5.0	1.2	1.5	34:40	450	1200
	5=F	5	2.5	00:30	15	20	2	5.0	1.2	1.5	34:30	450	1200
2	1=M	6	2.0	00:40	20	15	2	5.0	1.2	2.0	34:20	600	1200
	2=T	7	2.0	00:50	25	12	2	5.0	1.2	2.0	34:10	600	1200
	3=W	8	1.5	01:00	30	10	2	5.0	1.2	2.0	34:00	600	1200
	4=T	9	1.5	02:30	60	4	2	5.0	1.2	2.0	31:00	600	1200
	5=F	10	1.0	02:30	60	4	2	5.0	1.2	2.0	31:00	600	1200
3	1=M	11	1.0	02:30	60	4	2	5.0	1.2	2.0	31:00	600	1200
	2=T	12	1.0	05:00	0	1	5	2.5	1.2	2.0	35:00	750	1500
	3=W	13	1.0	05:00	0	1	5	2.5	1.2	2.0	35:00	750	1500
	4=T	14	1.0	05:00	0	1	5	2.5	1.2	2.0	35:00	750	1500
	5=F	15	1.0	05:00	0	1	5	2.5	1.2	2.0	35:00	750	1500
4	1=M	16	1.0	05:00	0	1	5	2.5	1.2	2.0	35:00	750	1500
	2=T	17	1.0	07:30	0	1	4	2.5	1.0	2.0	37:30	900	1800
	3=W	18	1.0	07:30	0	1	4	2.5	1.0	2.0	37:30	900	1800
	4=T	19	1.0	07:30	0	1	4	2.5	1.0	2.0	37:30	900	1800
	5=F	20	1.0	07:30	0	1	4	2.5	1.0	2.0	37:30	900	1800
5	1=M	21	1.0	07:30	0	1	4	2.5	1.0	2.0	37:30	900	1800
	2=T	22	1.0	07:30	0	1	5	2.5	1.0	2.0	47:30	1125	2250
	3=W	23	1.0	07:30	0	1	5	2.5	1.0	2.0	47:30	1125	2250
	4=T	24	1.0	07:30	0	1	5	2.5	1.0	2.0	47:30	1125	2250
	5=F	25	1.0	07:30	0	1	5	2.5	1.0	2.0	47:30	1125	2250
6	1=M	26	1.0	07:30	0	1	5	2.5	1.0	2.0	47:30	1125	2250
	2=T	27	1.0	10:00	0	1	4	2.5	1.0	2.0	47:30	1200	2400
	3=W	28	1.0	10:00	0	1	4	2.5	1.0	2.0	47:30	1200	2400
	4=T	29	1.0	10:00	0	1	4	2.5	1.0	2.0	47:30	1200	2400
	5=F	30	1.0	10:00	0	1	4	2.5	1.0	2.0	47:30	1200	2400
7	1=M	31	1.0	10:00	0	1	4	2.5	1.0	2.0	47:30	1200	2400
	2=T	32	1.0	10:00	0	1	5	2.5	1.0	2.0	60:00	1500	3000
	3=W	33	1.0	10:00	0	1	5	2.5	1.0	2.0	60:00	1500	3000
	4=T	34	1.0	10:00	0	1	5	2.5	1.0	2.0	60:00	1500	3000
	5=F	35	1.0	10:00	0	1	5	2.5	1.0	2.0	60:00	1500	3000
8	1=M	36	1.0	10:00	0	1	5	2.5	1.0	2.0	60:00	1500	3000
	2=T	37	1.0	12:30	0	1	4	2.5	1.0	2.0	57:30	1500	3000
	3=W	38	1.0	12:30	0	1	4	2.5	1.0	2.0	57:30	1500	3000
	4=T	39	1.0	12:30	0	1	4	2.5	1.0	2.0	57:30	1500	3000
	5=F	40	1.0	12:30	0	1	4	2.5	1.0	2.0	57:30	1500	3000

This standard program was designed using male rats of the Sprague-Dawley strain. All animals were between 70 and 170 days-of-age at the beginning of the program. The duration and intensity of the program were established so that 75 per cent of all such animals should have *PSF* and *PER* scores of 75 or higher during the final two weeks. Alterations in the work time, number of bouts, or time between bouts can be used to affect changes in these values. Other strains or ages of animals could be expected to respond differently to the program.

All animals should be exposed to a minimum of one week of voluntary running in a wheel prior to the start of the program. Failure to provide this adjustment period will impose a double learning situation on the animals and will seriously impair the effectiveness of the training programs.

Standard long-duration, low-speed endurance maintenance program for postpubertal and adult male rats in controlled running wheels.

Acc-eler-ation Time (sec)	Work Time (min: sec)	Rest Time (sec)	Repe-ti-tions per Bout	No. of Bouts	Time Bet-ween Bouts (min)	Shock (ma)	Run Speed (ft/ sec)	Total Time of Prog. (min: sec)	Total Exp. Revo-lu-tions *TER*	Total Work Time (sec) *TWT*
1.0	12:30	0	1	2	2.5	1.0	2.0	27:30	750	1500

TABLE A-4.-- Standard eight-week, endurance, swimming training program for postpubertal and adult male rats.

Wk.	Day of Wk.	Day of Tr.	Per Cent Tail Weight	Expected Swim Time (min) *EST*
1	1=M	1	0	30
	2=T	2	0	40
	3=W	3	C*	50
	4=T	4	C	60
	5=F	5	C	60
2	1=M	6	2	40
	2=T	7	2	40
	3=W	8	2	40
	4=T	9	2	45
	5=F	10	2	50
3	1=M	11	3	30
	2=T	12	3	30
	3=W	13	3	30
	4=T	14	3	35
	5=F	15	3	35
4	1=M	16	3	35
	2=T	17	3	40
	3=W	18	3	40
	4=T	19	3	40
	5=F	20	3	40
5	1=M	21	3	40
	2=T	22	3	45
	3=W	23	3	45
	4=T	24	3	45
	5=F	25	3	45
6	1=M	26	3	45
	2=T	27	3	50
	3=W	28	3	50
	4=T	29	3	50
	5=F	30	3	50
7	1=M	31	3	50
	2=T	32	3	55
	3=W	33	3	55
	4=T	34	3	55
	5=F	35	3	55
8	1=M	36	3	55
	2=T	37	3	60
	3=W	38	3	60
	4=T	39	3	60
	5=F	40	3	60

*C = clothes pin only.

This standard program was designed using male rats of the Sprague-Dawley strain. All animals were between 70 and 90 days-of-age at the beginning of the program. The duration and intensity of the program were established so that 75 per cent of all such animals should have *PET* scores of 75 or higher during the final two weeks. Alterations in the per cent tail weight or expected swim time can be used to affect changes in these values. Other strains or ages of animals could be expected to respond differently to the program.

All animals should be exposed to a minimum of one week of voluntary running in a wheel prior to the start of the program. Failure to provide this adjustment period will impose a severe, sudden exercise stress upon the animals and will seriously impair the effectiveness of the training program.

Standard endurance swimming maintenance program for postpubertal and adult male rats.

Per Cent Tail Weight	Expected Swim Time (min) *EST*
2	40

APPENDIX B

ENVIRONMENTAL CONDITIONS AND BODY WEIGHT VALUES

Table B-1. Treatment environmental and body weight values for SHT, MED, and LON

Variable	N[1]	Mean	Stan. Dev.	Simple Correlations					
				Air Temp.	% Humidity	Bar. Press.	Pre-treat. Body Wgt.	% Body Wgt. Loss	PER
Air Temp. (°C)	1341	22.8	2.18						
% Humidity	1341	37.5	14.74	0.253					
Bar. Press (mm Hg)	1341	741.0	4.92	-0.168	-0.037				
Pre-Treat. Body Wgt. (grams)	1341	366.5	33.38	-0.078	0.288	0.006			
% Body Wgt. Loss	1341	2.3	0.86	-0.007	0.083	0.021	-0.012		
PER	1341	105	28.12	0.078	-0.034	0.006	-0.517	0.229	
PSF	1341	89.0	8.93	-0.049	-0.137	-0.007	-0.161	0.239	0.511

[1]Total training days for all animals.

Table B-2. Treatment environmental and body weight values for SWM

Variable	N[1]	Mean	Stan. Dev.	Simple Correlations: Water Temp.	Air Temp.	% Humidity	Bar. Press.	Pre-Treat. Body Wgt.
Water Temp. (°C)	477	31.8	0.42					
Air Temp. (°C)	477	22.8	1.65	-0.011				
% Humidity	477	36.4	14.0	-0.100	0.088			
Bar. Press. (mm Hg)	477	740.7	4.65	0.014	-0.214	0.122		
Pre-Treat. Body Wgt. (grams)	477	384.5	41.11	-0.001	-0.095	0.045	0.005	
PET	477	99.9	2.93	-0.018	0.007	-0.140	0.010	0.016

[1]Total training days for all animals.

APPENDIX C

FREQUENCY DISTRIBUTIONS, MEANS AND STANDARD DEVIATIONS FOR EACH DEPENDENT VARIABLE

Table C-1. Soma diameter frequencies for treatments within durations

Treatment	Soma Diameter (μ)																																	
	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	Mean	S.D.
Duration 0* weeks																																		
CON	2	4	12	34	34	36	30	76	96	76	108	90	156	131	136	110	101	120	58	36	52	46	28	16	22	22	12	16	10	4	6	29.44	5.53
VOL																																		
SHT																																		
MED																																		
LON																																		
ESC																																		
SWM																																		
Duration 4 weeks																																		
CON	2	5	8	2	1	4	0	5	15	14	13	8	25	20	14	11	13	15	8	9	6	5	5	3	0	0	3	2	3	4	17	30.56	7.29
VOL	0	1	2	5	8	5	7	7	2	6	8	3	13	17	14	18	17	21	18	14	10	10	8	2	10	3	2	6	0	2	1	31.26	6.11
SHT	3	1	6	7	7	8	4	4	6	7	9	18	18	4	16	22	14	13	13	15	10	9	7	4	1	1	3	5	2	2	1	30.08	6.46
MED	2	0	3	1	3	4	3	6	3	10	8	9	11	7	8	9	14	29	11	10	12	14	2	12	6	7	7	5	5	7	12	33.34	7.05
LON	0	3	6	2	7	5	5	13	14	13	16	6	12	12	9	16	7	13	7	14	13	9	6	6	4	7	5	2	3	0	5	30.37	6.91
ESC	2	1	8	9	9	9	7	8	17	10	11	13	16	17	13	13	9	15	12	12	7	6	3	3	3	1	3	2	1	0	0	28.47	6.13
SWM	2	3	4	6	1	7	5	13	13	9	9	11	19	14	12	23	11	20	12	14	5	8	6	6	3	0	1	1	0	1	1	29.45	5.82
Duration 8 weeks																																		
CON	0	0	1	1	6	8	4	13	8	15	15	10	29	17	23	20	14	14	12	7	9	4	5	1	1	2	0	1	0	0	0	29.10	4.73
VOL	2	0	5	8	1	4	6	7	8	6	14	11	16	14	16	11	12	20	12	11	11	10	6	8	10	3	2	4	1	0	1	30.67	6.26
SHT	0	2	8	10	11	13	10	12	26	11	21	10	12	14	6	11	7	14	6	9	10	3	7	4	0	1	1	0	1	0	0	27.33	5.89
MED	1	1	4	1	2	6	6	6	13	11	10	9	15	15	16	6	12	17	14	8	12	7	4	3	4	4	3	4	7	0	19	31.82	7.33
LON	1	0	3	3	2	4	3	5	3	8	12	9	11	10	11	14	6	20	16	12	16	6	16	7	5	8	8	10	4	2	5	32.44	6.65
ESC	2	2	3	4	3	6	11	15	22	26	25	13	12	17	16	14	8	10	10	12	4	0	1	2	0	1	0	1	0	0	0	27.42	4.82
SWM	2	3	8	1	7	9	14	17	12	20	15	11	17	16	13	9	8	11	4	10	7	2	3	7	3	4	2	2	1	1	1	28.19	6.32

Table C-1 (cont'd.)

Treatment	Some Diameter (μ) 16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	Mean	S.D.
Duration 12 weeks																																	
CON	0	0	2	4	1	2	4	6	3	11	6	12	17	12	20	19	14	19	18	5	8	5	4	4	2	5	7	4	1	3	22	32.60	6.90
VOL	0	3	1	3	8	7	10	9	12	12	16	15	22	18	15	15	10	16	9	6	3	4	5	4	3	2	0	6	1	1	4	29.25	6.14
SHT	1	3	4	7	6	7	4	14	18	14	14	11	18	12	15	11	14	20	7	7	5	9	8	2	3	1	0	1	2	1	1	28.77	5.99
MED	1	2	4	4	4	8	3	18	10	7	9	13	14	15	19	15	16	20	20	4	10	5	5	6	0	6	0	0	1	0	1	29.52	5.71
LON	4	1	8	9	4	14	5	8	6	5	10	7	11	11	9	20	10	21	16	12	8	5	6	5	4	5	2	3	4	2	5	30.19	7.23
ESC	2	0	4	2	4	6	0	14	6	14	15	8	10	13	9	12	10	12	10	18	9	4	5	12	5	5	6	6	1	5	13	31.95	7.38
SWM	1	3	4	6	10	5	6	10	7	15	12	13	29	16	25	20	11	10	15	5	7	3	1	0	1	3	0	1	1	0	0	28.29	5.20

*Pooled zero week animals.

Table C-2. Nucleus diameter frequencies for treatment within durations

Treatment	Nucleus Diameter (μ) 7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	Mean	S.D.
Duration 0* weeks																	
CON	30	66	214	540	335	152	123	64	70	34	10	22	14	6	0	11.08	2.25
VOL																	
SHT																	
MED																	
LON																	
ESC																	
SWM																	
Duration 4 weeks																	
CON	6	11	37	64	47	18	19	8	6	1	3	3	5	10	2	11.43	3.06
VOL	6	9	28	68	37	22	21	11	13	15	7	2	1	0	0	11.47	2.51
SHT	8	12	31	62	61	13	22	13	7	6	3	1	1	0	0	10.98	2.17
MED	6	12	20	55	44	22	25	12	11	8	5	8	6	4	2	11.95	3.04
LON	6	16	36	55	26	24	28	15	13	7	0	2	4	6	2	11.55	2.94
ESC	6	22	38	55	40	25	39	7	5	2	0	0	1	0	0	10.75	1.97
SWM	5	9	32	54	48	27	30	16	12	3	1	2	0	1	0	11.26	2.15
Duration 8 weeks																	
CON	1	5	21	82	71	31	16	8	2	1	2	0	0	0	0	10.87	1.47
VOL	7	9	18	68	43	22	24	13	12	8	7	8	1	0	0	11.60	2.57
SHT	4	19	51	63	43	16	8	10	9	9	7	1	0	0	0	10.81	2.33
MED	2	7	14	62	33	19	29	17	19	20	8	6	4	0	0	12.28	2.71
LON	4	9	14	49	30	18	21	25	20	13	11	14	7	5	0	12.77	3.17
ESC	3	8	31	70	36	20	23	20	15	9	4	1	0	0	0	11.39	2.27
SWM	3	13	45	62	51	19	13	3	10	9	5	5	2	0	0	11.07	2.46
Duration 12 weeks																	
CON	3	1	10	42	56	31	29	10	9	11	6	14	10	4	4	12.76	3.11
VOL	4	10	28	74	47	19	29	8	5	6	4	3	1	2	0	11.19	2.32
SHT	3	7	37	79	54	25	17	10	5	2	0	0	1	0	0	10.75	1.71
MED	7	7	32	75	51	31	13	10	10	1	2	1	0	0	0	10.85	1.89
LON	7	14	29	52	48	15	24	10	12	14	3	8	3	1	0	11.57	2.78
ESC	4	10	23	54	40	20	22	16	12	18	6	8	5	2	0	12.06	2.90
SWM	6	7	38	73	51	26	18	10	5	6	0	0	0	0	0	10.78	1.82

*Pooled zero week animals.

Table C-3. Nucleolus diameter frequencies for treatments within durations

Treatment	Nucleolus Diameter (μ) 1	2	3	4	5	6	Mean	S.D.
Duration 0* weeks								
CON	0	14	334	927	399	6	4.03	.70
VOL								
SHT								
MED								
LON								
ESC								
SWM								
Duration 4 weeks								
CON	0	1	42	108	78	11	4.23	.81
VOL	0	4	42	123	67	4	4.10	.76
SHT	0	3	57	106	74	0	4.05	.77
MED	0	4	45	78	84	29	4.37	.98
LON	0	5	52	87	88	8	4.17	.88
ESC	0	0	72	92	76	0	4.02	.79
SWM	0	2	66	100	65	7	4.04	.84
Duration 8 weeks								
CON	0	0	30	127	83	0	4.22	.65
VOL	0	0	39	103	97	1	4.25	.69
SHT	0	10	63	108	59	0	3.90	.82
MED	0	0	55	117	60	8	4.09	.78
LON	0	1	36	106	91	6	4.27	.76
ESC	0	8	50	141	41	0	3.90	.71
SWM	0	2	36	125	72	5	4.17	.73
Duration 12 weeks								
CON	0	6	43	112	58	21	4.19	.92
VOL	0	3	55	100	74	8	4.12	.84
SHT	0	3	50	120	66	1	4.05	.74
MED	0	5	55	126	54	0	3.95	.73
LON	0	7	62	101	65	5	4.00	.86
ESC	0	1	45	109	84	1	4.16	.74
SWM	0	4	45	135	56	0	4.01	.70

*Pooled zero week animals.

Table C-4. Sin^{-1} Nissl substance concentration frequencies for treatments within durations

Treatment	Sin^{-1} Nissl Substance (Percent Light Absorbed)																					
	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	Mean	S.D.
Duration 0* weeks																						
CON	56	22	20	32	44	52	48	83	111	193	187	234	234	162	115	57	22	8	0	0	55.76	3.64
VOL																						
SHT																						
MED																						
LON																						
ESC																						
SWM																						
Duration 4 weeks																						
CON	18	6	5	1	3	3	7	9	15	33	36	34	42	16	10	1	0	0	1	0	55.11	3.84
VOL	7	2	3	1	4	2	5	17	20	42	39	33	28	20	12	4	1	0	0	0	55.65	3.09
SHT	11	7	3	4	2	8	6	13	4	19	30	37	31	25	22	13	4	1	0	0	55.96	4.00
MED	7	6	3	8	4	13	8	14	21	33	47	36	26	8	3	3	0	0	0	0	54.74	3.27
LON	4	4	5	3	4	4	11	10	20	24	34	28	32	22	17	15	2	1	0	0	56.00	3.50
ESC	5	1	7	4	3	10	12	24	28	24	42	32	30	14	3	1	0	0	0	0	54.91	3.02
SWM	4	9	6	6	8	14	19	14	47	25	37	18	10	7	6	6	1	2	0	1	54.23	3.50
Duration 8 weeks																						
CON	8	8	4	1	8	7	8	16	20	37	27	28	23	18	17	8	1	1	0	0	55.24	3.72
VOL	8	5	10	9	6	7	11	8	14	14	34	27	21	28	26	6	5	1	0	0	55.48	4.14
SHT	6	4	2	7	12	17	22	17	25	36	27	23	15	8	6	5	8	0	0	0	54.06	3.54
MED	53	16	13	4	8	9	15	15	19	20	11	7	10	14	15	6	3	2	0	0	52.37	5.10
LON	20	3	8	8	5	5	10	9	18	10	28	40	32	22	12	7	3	0	0	0	54.98	4.28
ESC	37	11	18	21	18	17	20	17	14	13	6	11	4	6	10	9	4	2	2	0	52.05	4.78
SWM	20	18	16	29	28	35	26	18	10	14	8	6	3	5	3	1	0	0	0	0	51.07	4.37

Table C-4 (cont'd.)

Treatment	Sin^{-1} Nissl Substance (Percent Light Absorbed)																					
	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	Mean	S.D.
Duration 12 weeks																						
CON	47	6	10	21	19	20	12	7	7	6	8	16	16	15	17	6	4	2	0	1	52.60	5.22
VOL	6	3	0	4	6	13	9	17	29	28	23	31	27	25	12	6	1	0	0	0	55.40	3.35
SHT	11	2	2	5	3	8	17	30	46	42	33	16	13	8	3	1	0	0	0	0	54.14	2.95
MED	10	3	2	2	8	5	9	7	10	20	28	49	29	29	16	11	2	0	0	0	55.99	3.65
LON	24	7	7	6	7	7	19	15	24	32	28	19	7	13	9	7	4	3	1	0	53.94	4.42
ESC	41	4	7	12	5	7	13	17	18	24	16	29	10	16	11	5	3	1	1	0	53.44	4.77
SWM	17	3	4	6	6	10	16	16	35	40	40	23	8	10	2	3	1	0	0	0	54.00	3.46

*Pooled zero week animals.

Table C-5. Soma diameter frequencies for durations within treatments

Duration	Soma Diameter (μ) 16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	Mean	S.D.
CON																																	
0*	2	4	12	34	34	36	30	76	96	76	108	90	156	131	136	110	101	120	58	36	52	46	28	16	22	22	12	16	10	4	6	29.44	5.53
4	2	5	8	2	1	4	0	5	15	14	13	8	25	20	14	11	13	15	8	9	6	5	5	3	0	0	3	2	3	4	17	30.56	7.29
8	0	0	1	1	6	8	4	13	8	15	15	10	29	17	23	20	14	14	12	7	9	4	5	1	1	2	0	1	0	0	0	29.10	4.73
12	0	0	2	4	1	2	4	6	3	11	6	12	17	12	20	19	14	19	18	5	8	5	4	4	2	5	7	4	1	3	22	32.60	6.90
VOL																																	
0																																	
4	0	1	2	5	8	5	7	7	2	6	8	3	13	17	14	18	17	21	18	14	10	10	8	2	10	3	2	6	0	2	1	31.26	6.11
8	2	0	5	8	1	4	6	7	8	6	14	11	16	14	16	11	12	20	12	11	11	10	6	8	10	3	2	4	1	0	1	30.67	6.26
12	0	3	1	3	8	7	10	9	12	12	16	15	22	18	15	15	10	16	9	6	3	4	5	4	3	2	0	6	1	1	4	29.25	6.14
SHT																																	
0																																	
4	3	1	6	7	7	8	4	4	6	7	9	18	18	4	16	22	14	13	13	15	10	9	7	4	1	1	3	5	2	2	1	30.08	6.46
8	0	2	8	10	11	13	10	12	26	11	21	10	12	14	6	11	7	14	6	9	10	3	7	4	0	1	1	0	1	0	0	27.33	5.89
12	1	3	4	7	6	7	4	14	18	14	14	11	18	12	15	11	14	20	7	7	5	9	8	2	3	1	0	1	2	1	1	28.77	5.99
MED																																	
0																																	
4	2	0	3	1	3	4	3	6	3	10	8	9	11	7	8	9	14	29	11	10	12	14	2	12	6	7	7	5	5	7	12	33.34	7.05
8	1	1	4	1	2	6	6	6	13	11	10	9	15	15	16	6	12	17	14	8	12	7	4	3	4	4	3	4	7	0	19	31.82	7.33
12	1	2	4	4	4	8	3	18	10	7	9	13	14	15	19	15	16	20	20	4	10	5	5	6	0	6	0	0	1	0	1	29.52	5.71
LON																																	
0																																	
4	0	3	6	2	7	5	5	13	14	13	16	6	12	12	9	16	7	13	7	14	13	9	6	6	4	7	5	2	3	0	5	30.37	6.91
8	1	0	3	3	2	4	3	5	3	8	12	9	11	10	11	14	6	20	16	12	16	6	16	7	5	8	8	10	4	2	5	32.44	6.65
12	4	1	8	9	4	14	5	8	6	5	10	7	11	11	9	20	10	21	16	12	8	5	6	5	4	5	2	3	4	2	5	30.19	7.23

Table C-5 (cont'd.)

Duration	Soma Diameter (μ) 16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	Mean	S.D.
ESC																																	
0																																	
4	2	1	8	9	9	9	7	8	17	10	11	13	16	17	13	13	9	15	12	12	7	6	3	3	3	1	3	2	1	0	0	28.47	6.13
8	2	2	3	4	3	6	11	15	22	26	25	13	12	17	16	14	8	10	10	12	4	0	1	2	0	1	0	1	0	0	0	27.42	4.82
12	2	0	4	2	4	6	0	14	6	14	15	8	10	13	9	12	10	12	10	18	9	4	5	12	5	5	6	6	1	5	13	31.95	7.38
SWM																																	
0																																	
4	2	3	4	6	1	7	5	13	13	9	9	11	19	14	12	23	11	20	12	14	5	8	6	6	3	0	1	1	0	1	1	29.45	5.82
8	2	3	8	1	7	9	14	17	12	20	15	11	17	16	13	9	8	11	4	10	7	2	3	7	3	4	2	2	1	1	1	28.19	6.32
12	1	3	4	6	10	5	6	10	7	15	12	13	29	16	25	20	11	10	15	5	7	3	1	0	1	3	0	1	1	0	0	28.29	5.20

*Pooled zero week animals.

Table C-6. Nucleus diameter frequencies for durations within treatments

Duration	Nucleus Diameter (μ) 7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	Mean	S.D.
CON																	
0*	30	66	214	540	335	152	123	64	70	34	10	22	14	6	0	11.08	2.25
4	6	11	37	64	47	18	19	8	6	1	3	3	5	10	2	11.43	3.06
8	1	5	21	82	71	31	16	8	2	1	2	0	0	0	0	10.87	1.47
12	3	1	10	42	56	31	29	10	9	11	6	14	10	4	4	12.76	3.11
VOL																	
0																	
4	6	9	28	68	37	22	21	11	13	15	7	2	1	0	0	11.47	2.51
8	7	9	18	68	43	22	24	13	12	8	7	8	1	0	0	11.60	2.57
12	4	10	28	74	47	19	29	8	5	6	4	3	1	2	0	11.19	2.32
SHT																	
0																	
4	8	12	31	62	61	13	22	13	7	6	3	1	1	0	0	10.98	2.17
8	4	19	51	63	43	16	8	10	9	9	7	1	0	0	0	10.81	2.33
12	3	7	37	79	54	25	17	10	5	2	0	0	1	0	0	10.75	1.71
MED																	
0																	
4	6	12	20	55	44	22	25	12	11	8	5	8	6	4	2	11.95	3.04
8	2	7	14	62	33	19	29	17	19	20	8	6	4	0	0	12.28	2.71
12	7	7	32	75	51	31	13	10	10	1	2	1	0	0	0	10.85	1.89
LON																	
0																	
4	6	16	36	55	26	24	28	15	13	7	0	2	4	6	2	11.55	2.94
8	4	9	14	49	30	18	21	25	20	13	11	14	7	5	0	12.77	3.17
12	7	14	29	52	48	15	24	10	12	14	3	8	3	1	0	11.57	2.78
ESC																	
0																	
4	6	22	38	55	40	25	39	7	5	2	0	0	1	0	0	10.75	1.97
8	3	8	31	70	36	20	23	20	15	9	4	1	0	0	0	11.39	2.77
12	4	10	23	54	40	20	22	16	12	18	6	8	5	2	0	12.06	2.90
SWM																	
0																	
4	5	9	32	54	48	27	30	16	12	3	1	2	0	1	0	11.26	2.15
8	3	13	45	62	51	19	13	3	10	9	5	5	2	0	0	11.07	2.46
12	6	7	38	73	51	26	18	10	5	6	0	0	0	0	0	10.78	1.82

*Pooled zero week animals.

Table C-7. Nucleolus diameter frequencies for durations within treatments

Duration	Nucleolus Diameter (μ) 1	2	3	4	5	6	Mean	S.D.
CON								
0*	0	14	334	927	399	6	4.03	.70
4	0	1	42	108	78	11	4.23	.81
8	0	0	30	127	83	0	4.22	.65
12	0	6	43	112	58	21	4.19	.92
VOL								
0								
4	0	4	42	123	67	4	4.10	.76
8	0	0	39	103	97	1	4.25	.69
12	0	3	55	100	74	8	4.12	.84
SHT								
0								
4	0	3	57	106	74	0	4.05	.77
8	0	10	63	108	59	0	3.90	.82
12	0	3	50	120	66	1	4.05	.74
MED								
0								
4	0	4	45	78	84	29	4.37	.98
8	0	0	55	117	60	8	4.09	.78
12	0	5	55	126	54	0	3.95	.73
LON								
0								
4	0	5	52	87	88	8	4.17	.88
8	0	1	36	106	91	6	4.27	.76
12	0	7	62	101	65	5	4.00	.86
ESC								
0								
4	0	0	72	96	76	0	4.02	.79
8	0	8	50	141	41	0	3.90	.71
12	0	1	45	109	84	1	4.16	.74
SWM								
0								
4	0	2	66	100	65	7	4.04	.84
8	0	2	36	125	72	5	4.17	.73
12	0	4	45	135	56	0	4.01	.70

*Pooled zero week animals.

Table C-8. Sin^{-1} Nissl substance concentration frequencies for durations within treatments

Duration	Sin^{-1} Nissl Substance (Percent Light Absorbed)																					
	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	Mean	S.D.
CON																						
0*	56	22	20	32	44	52	48	83	111	193	187	234	234	162	115	57	22	8	0	0	55.76	3.64
4	18	6	5	1	3	3	7	9	15	33	36	34	42	16	10	1	0	0	1	0	55.11	3.84
8	8	8	4	1	8	7	8	16	20	37	27	28	23	18	17	8	1	1	0	0	55.24	3.72
12	47	6	10	21	19	20	12	7	7	6	8	16	16	15	17	6	4	2	0	1	52.60	5.22
VOL																						
0																						
4	7	2	3	1	4	2	5	17	20	42	39	33	28	20	12	4	1	0	0	0	55.65	3.09
8	8	5	10	9	6	7	11	8	14	14	34	27	21	28	26	6	5	1	0	0	55.48	4.14
12	6	3	0	4	6	13	9	17	29	28	23	31	27	25	12	6	1	0	0	0	55.40	3.35
SHT																						
0																						
4	11	7	3	4	2	8	6	13	4	19	30	37	31	25	22	13	14	1	0	0	55.96	4.00
8	6	4	2	7	12	17	22	17	25	36	27	23	15	8	6	5	8	0	0	0	54.06	3.54
12	11	2	2	5	3	8	17	30	46	42	33	16	13	8	4	0	0	0	0	0	54.14	2.95
MED																						
0																						
4	7	6	3	8	4	13	8	14	21	33	47	36	26	8	3	3	0	0	0	0	54.74	3.27
8	53	16	13	4	8	9	15	15	19	20	11	7	10	14	15	6	3	2	0	0	52.37	5.10
12	10	3	2	2	8	5	9	7	10	20	28	49	29	29	16	11	2	0	0	0	55.99	3.65
LON																						
0																						
4	4	4	5	3	4	4	11	10	20	24	34	28	32	22	17	15	2	1	0	0	56.00	3.50
8	20	3	8	8	5	5	10	9	18	10	28	40	32	22	12	7	3	0	0	0	54.98	4.28
12	24	7	7	6	7	7	19	15	24	32	28	19	7	13	9	7	4	3	1	0	53.94	4.42

Table C-8 (cont'd.)

Duration	$\sin^{-1}$ Nissl Substance (Percent Light Absorbed)																					
	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	Mean	S.D.
ESC																						
0																						
4	5	1	7	4	3	10	12	24	28	24	42	32	30	14	4	0	0	0	0	0	54.91	3.02
8	37	11	18	21	18	17	20	17	14	13	6	11	4	6	10	9	4	2	2	0	52.05	4.78
12	41	4	7	12	5	7	13	7	18	24	16	29	10	16	11	5	3	1	1	0	53.44	4.77
SWM																						
0																						
4	4	9	6	6	8	14	19	14	47	25	37	18	10	7	6	6	1	2	0	1	54.23	3.50
8	20	18	16	29	28	35	26	18	10	14	8	6	3	5	3	1	0	0	0	0	51.07	4.37
12	17	3	4	6	6	10	16	16	35	40	40	23	8	10	2	3	1	0	0	0	54.0	3.46

*Pooled zero week animals.

APPENDIX D

TISSUE PREPARATION AND STAINING PROCEDURES

APPENDIX D

TISSUE PREPARATION AND STAINING PROCEDURES

Tissue Preparation

1. Spinal cord segments placed in 10% formalin for 24 hr.
2. Wash in tap water overnight.
3. Dehydrate

50% ethyl alcohol	4 hr
70% ethyl alcohol	overnight
80% ethyl alcohol	2 hr
95% ethyl alcohol	3 changes, 1 hr each
100% ethyl alcohol	2 changes, 1 hr each
100% ethyl alcohol:Terpinol (1:1)	overnight in 37°C oven
Pure Terpinol	at least 2 hr at 37°C

4. Paraffin infiltration . . . . . 4 changes, at least 1 hr each (last change use fresh paraffin)
5. Embed tissue.

Staining Procedures

1.	Xylene	3 min
2.	Xylene	3 min
3.	100% alcohol	3 min
4.	95% alcohol	3 min
5.	95% alcohol	3 min
6.	Luxol Fast Blue	16-24 hr at 50°C
7.	95% alcohol	wash
8.	Distilled water	wash
9.	Dilute Li_2CO_3 (fresh)	dip
10.	95% alcohol	differentiate--continue until gray matter and white matter can be distinguished
11.	Distilled water	wash
12.	Dilute Li_2CO_3 (fresh)	rinse
13.	70% alcohol	differentiate until gray matter colorless and white matter green-blue
14.	Distilled water	wash
15.	Cresyl-echt Violet	4 min at 37°C
16.	95% alcohol	3 min
17.	95% alcohol	3 min
18.	100% alcohol	3 min
19.	Xylene	3 min
20.	Xylene	
21.	For film technique cover sections with liquid plastic.	