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Introduction

As a biological tissue, muscle adapts to the demands of usage. One traditional way of assessing the extent of this adaptation has been to examine the effects of an altered-activity protocol on the physiological properties of muscles. However, in order to accurately interpret the changes associated with an activity pattern, it is necessary to employ an appropriate control model. There exists a substantial literature which reports altered-use effects by comparing experimental observations with those from animals raised in small laboratory cages (e.g., Steffen and Musacchia, 1984; Templeton, Padalino, Manton, Glasberg, Silver, Silver, DeMartino, Le Coney, Klug, Hagler, and Sutko, 1984). Some evidence suggests that small-cage-reared animals actually represent a model of reduced use. For example, laboratory animals subjected to limited physical activity have shown resistance to insulin-induced glucose uptake which can be altered by exercise training (Mondon, Dolkas, and Reaven, 1983).

This project concerned the basic mechanisms underlying muscle atrophy. Specifically, the project addressed the issue of the appropriateness of rats raised in conventional-sized cages as experimental models to examine this phenomenon. The project hypothesis was that rats raised in small cages are inappropriate models for the study of muscle atrophy. The experimental protocol involved: (1) raising two populations of rats, one group in conventional (small)-sized cages and the other group in a much larger (133x) cage, from weanling age (21 days) through to young adulthood (125 days); (2) comparison of size- and force-related characteristics of selected test muscles in an acute terminal paradigm.
**Methods**

**Housing**

Male and female Sprague-Dawley rats were raised from weanling age (21 days) for 89-156 days ($X \pm = 141 \pm 17$) in either of two sized cages. The small-cage animals were raised in conventional small laboratory cages (20 cm x 47 cm x 25 cm, 3-5/cage). The large cage provided approximately 133x more usable surface area (floor and 4 cage walls) for the animals and included climbing structures and exercise wheels. The activity of neither group of animals was monitored. The rats were provided with food and water ad libitum.

Two batches of animals were studied. In the first group (Batch 1), the males were raised and studied first; the females were procured subsequently. In the second group (Batch 2), the males and females were obtained at the same time and raised concurrently although in separate cages. By chance, but of subsequent relevance, the two batches were raised in two different buildings. All other details of the experiments were identical for the two groups.

**Preparation**

Terminal experiments were performed with the rats under halothane anesthesia. The anesthesia was delivered via a face mask, initially at a concentration of 4% and flow rate of 5 L/min. Following intubation of the trachea, anesthesia was maintained at a pain-free level for the extent of the experiment at halothane concentration of 1-2% as needed in a 50% N\textsubscript{2}O-50% O\textsubscript{2} mixture with a total flow of 0.6 L/min. Respiration rate (90-120 breaths/min), percent expired CO\textsubscript{2}, and rectal temperature (35-37°C) were monitored and adjusted to maintain homeostasis throughout the experiment. The left soleus and extensor digitorum longus muscles were surgically isolated by removing overlying and adjacent musculature. All branches of the sciatic nerve were transected near the hip, and those branches innervating soleus (soleus muscle nerve) and extensor digitorum longus (lateral peroneal nerve) were isolated for subsequent stimulation.
The animal was secured in a rigid frame, and the left hindlimb was clamped with the knee at an angle of about 120 degrees. The test muscles were maintained in vivo in a paraffin oil-filled bath at a temperature of 36 ± 1°C.

**Recording Procedures**

The test muscles were activated with a monopolar, stainless-steel, hooked wire electrode (referred to a similar electrode in an adjacent denervated muscle) by supramaximal cathodal stimulation (3-5x threshold, 0.1 ms square waves) of their nerves. The force elicited by such activation was measured by a force transducer that was attached to the tendon of the muscle by a low-compliance (<0.03 μm/N/cm) dacron line (X ± SD length = 15.6 ± 1.3 cm). The whole-muscle electromyogram (e.m.g.) was measured with a spring-mounted, flexible, stainless-steel, intramuscular electrode (18 mm long, 120 μm thick, 40 μm tip) that was thrust into the distal one-third of the muscle, perpendicular to the long axis of the muscle. The intramuscular electrode was referred to a similar electrode placed in an adjacent denervated muscle.

Preliminary experiments indicated that the optimum muscle length for peak twitch force, for both soleus and extensor digitorum longus, was achieved when the muscle was stretched to exert a passive force of 0.15 N. Throughout the test, the length of the test muscle was altered to sustain a passive force of 0.15 N. No attempt was made to determine length-tension relationships during the present experiments for fear of fatiguing the muscle.

**Experimental Protocol**

Each muscle was activated separately with the following 5-step protocol: (1) 32 twitches at 0.5 Hz were elicited by stimulation of the nerve to both evaluate and circumscribe the effects of treppe; (2) a single tetanus of 100 Hz for 500 ms to determine tetanic force (this stimulation elicited approximate peak force in soleus, but stimulation rates of 250 Hz are required to elicit peak force in extensor digitorum longus (Close, 1964); (3) a 6-min application of a standardized stimulus regimen (Burke, Levine, Tsairis,
and Zajac, 1973) which involved repetitive intermittent stimulation (40 Hz) of the nerve with trains of 13 stimuli, each train lasting 330 ms and occurring once each second to evaluate fatigability; (4) 8 twitches at 0.5 Hz to evaluate the effects of the fatigue test on twitch force; and (5) two 100 Hz tetani, 10 s apart to evaluate the effects of the fatigue test on tetanic force. Approximately five seconds elapsed between consecutive phases of the protocol. The stimulus protocol was controlled with a small laboratory computer. Data were recorded on-line with the computer and on FM magnetic tape for a more detailed off-line analysis.

The animal was then terminated with an intracardiac injection of magnesium sulfate. The two test muscles were removed, frozen in isopentane cooled to its melting point, and stored at -70°C for subsequent histochemical analysis.

Data Analysis

Age and body weight were recorded for each rat at the time of the physiological experiment.

Physiological. The issues addressed in the first full-length paper (see Results) concern the affects of cage-size and gender on the force production capacity of the two test muscles as revealed by the above stimulation protocol. Each twitch was characterized by determining the peak force (N), time-to-peak force (ms), and half-relaxation time (ms). The peak force (N) was determined for each of the three 100 Hz tetani. For the 360 trains of the fatigue test, peak force, time-to-peak force, and two relaxation times (i.e., time for decay of force from 90% to 50% and from 50% to 20%) were determined. The Fatigue Index was defined as the peak force of the 360th train of the fatigue test expressed as a percent of the peak force of the initial train.

The e.m.g., which comprised the action potentials associated with each train of stimuli, was represented by the averaged waveform, and quantification was based on this average waveform. Measurements on the average waveform included four measures of amplitude, four of duration, and area, each of which reveals different features of muscle
action-potential generation, and propagation. The e.m.g. measurements are defined in the following manner: (1) absolute peak-to-peak amplitude, the magnitude of the difference (mV) between the absolute maximum and minimum; (2) first peak-to-peak amplitude, and magnitude of the difference (mV) between the maximum of the initial positive phase and the minimum of the subsequent negative phase; (3) mean amplitude, the ratio (mV) of area to waveform duration; (4) "mean" amplitude, the ratio (mV) of area to peak-to-peak duration; (5) baseline-to-baseline duration, the time (ms) from the beginning to the end of the waveform where the beginning and end are defined as a 5% deviation of the waveform from the preceding and succeeding baselines; (6) peak-to-peak duration, the time (ms) between the maximum of the initial positive phase and the minimum of the subsequent negative phase; (7) peak-to-peak rate, the average rate (mV/ms) of change between the initial positive-phase maximum and the negative-phase minimum; (8) normalized peak-to-peak rate, the reciprocal (1/ms) of peak-to-peak duration; (9) area, the integral (mV·ms) of the e.m.g. waveform where the beginning and end are defined for the measurement of baseline-to-baseline duration.

**Histochemical.** Serial frozen sections (10 μm in thickness) from the middle one-third of the muscle belly were cut, with alternate sections stained for succinate dehydrogenase (SDH) according to a modification of the methods of Pette and colleagues (Pette, Wasmund, and Wimmer, 1981; Pette, 1981) or myosin ATPase (pH 9.3 and 4.3) according to the methods of Guth and Samaha (1972). The fiber-type composition of the muscles was determined by classifying approximately 300 fibers per muscle (range = 265-368) located in the more central region of the cross section. Fibers were classified according to the nomenclature of Peter, Barnard, Edgerton, Gillespie, and Stempel (1972): SO (slow, oxidative) fibers stained light for myosin ATPase (using the alkaline preincubation) and intermediate to dark for SDH; FOG (fast-contracting, oxidative, and glycolytic) fibers stained dark for both ATPase and SDH; and FG (fast-contracting, glycolytic) fibers stained dark for ATPase but light for SDH. The relative percent
contribution of each fiber type was determined by dividing the number of fibers of a given type by the total number of fibers counted in that muscle and then multiplying by 100. These values represent the average of results from two independent observers. In addition, a subset of these fibers (70-100 fibers/muscle) were characterized in more detail through the use of a computer-assisted image processing system (made available by Dr. V.R. Edgerton, Department of Kinesiology, UCLA). This involved a microphotometric analysis of SDH activity according to the methods of Castleman, Chui, Martin, and Edgerton (1984) and determination of cross-sectional area.

To test for significant effects due to cage-size, gender, or batch, a multiple analysis of variance was used. A Newmann-Keuls comparison was used for post-hoc analysis with the critical value for significance of $p < 0.05$. Statistical differences in fatigability were assessed with single and multiple linear regression analyses and with an analysis of variances.
Results

The experimental results accumulated during this three-year project have been reported to the scientific community in two formats: (1) as ten abstracts presented at meetings of national and international societies; and (2) as five full-length papers. For this report, the abstract citations are listed, and the abstracts are attached at the end of the report; and summary outlines of the full-length papers are provided. Complete manuscripts can be provided if it is deemed desirable.

Abstracts


-8-


Papers

The results and conclusions of the project are described fully in the following five papers. Drafts of these manuscripts are available upon request.

1. "Effects of cage size and gender on the properties of developing rat hindlimb muscle."

   a. Male and female Sprague-Dawley rats were raised in separate groups from weanling age in either of two cage sizes. In terminal experiments, cage-size and gender effects were assessed with measures of size, force, contractile speed, fatigability, muscle fiber-type composition, and oxidative-enzyme activity for two test muscles, soleus and extensor digitorum longus.

   b. In general, cage size did not affect any of these measures.

   c. Similarly, apart from well-documented gender differences (e.g., muscle : body mass, twitch : tetanus force) the effect of cage size was similar for both male and female rats. There was, however, a greater variability (coefficient of variation) in many of the test measures exhibited by the female rats.
d. One group of large-cage male animals did demonstrate a significant effect of cage size relative to control animals. However, this observation probably reflected an environment effect due to the physical characteristics of the environment in which that particular cage was located.

e. It appears that rats of either gender raised in conventional laboratory cages represent an appropriate experimental model for altered-use paradigms.

2. "Fatigability of rat hindlimb muscle: E.m.g.-force relationship."

a. An experimental protocol designed to assess fatigability in motor units (Burke, Levine, Tsairis, and Zajac, 1973) has been applied to the whole-muscles of anesthetized adult rats and the electromyogram (e.m.g.)-force relationship monitored over the course of the test.

b. The animals were raised in either a small or a large cage. As a result, both test muscles (soleus and extensor digitorum longus) exhibited a wide range of fatigability, which was defined as the decline in isometric peak force at 6 min, such that the data for each test muscle were separated into groups of high, intermediate, and low fatigability.

c. The e.m.g. was quantified with four measures of amplitude, four duration variables, and one interaction term (area). Correlation analyses indicated that the e.m.g. was adequately represented by one measure of amplitude (absolute amplitude), one of duration (peak-to-peak duration), and area.

d. The e.m.g.-force relationships for soleus varied markedly between the three fatigability groups. In contrast, all three extensor digitorum longus groups displayed qualitatively similar e.m.g.-force relationships over the course of the test.

e. Multiple regression analyses indicated that the e.m.g. parameters were able to predict peak force better for extensor digitorum longus than for soleus.
Furthermore, for both test muscles the prediction was best for the high fatigability group.

f. The associations between e.m.g. and force appeared to depend upon the measure used to quantify the e.m.g., the fiber-type composition of the test muscle, and the degree of fatigability exhibited by the muscle.

3. "Fatigability of rat hindlimb muscle: Coexistence of potentiation and force decline."

a. An experimental protocol designed to assess fatigability in motor units (Burke, Levine, Tsairis, and Zajac, 1973) has been applied to whole muscle of anesthetized adult rats in order to study the effects of the test on the magnitude of twitch potentiation.

b. The animals were raised in either a small or large cage. As a result, both test muscles (soleus and extensor digitorum longus) exhibited a wide range of fatigability, defined as the decline in isometric force observed at 6 min, and twitch potentiation, defined as the increase in twitch force observed after, compared to before, the fatigue test.

c. In the soleus, 57% (42/74) of the muscles exhibited fatigue (i.e., force at 6 min less than 100% of initial force); of these, 14% (6/42) also exhibited potentiated postfatigue-test twitch force. In contrast, all (68/68) extensor digitorum longus muscles exhibited fatigue, and of these, 46% (31/68) also showed twitch potentiation.

d. In both test muscles, an inverse linear relationship was found between the degree of twitch potentiation and the magnitude of fatigue exhibited, although the relationship in soleus was different from that in extensor digitorum longus.

e. Some recovery of the whole-muscle e.m.g. was evident between the end of the fatigue test and the first posttest twitch. However, there was no correlation between the degree of twitch force potentiation and any of the e.m.g. parameters studied.
f. The simultaneous occurrence of whole-muscle fatigue and twitch potentiation in some muscles suggests that the mechanisms underlying these two phenomena affect different sites within the muscle, but the linear relationship suggests that they are not totally independent of one another.

4. "A comparison of qualitative and quantitative histochemical classification of muscle fiber types."
   a. The metabolic heterogeneity seen within a given muscle fiber type, first described for fibers classified on the basis of myosin ATPase staining, has been confirmed for fibers classified according to the SO-FOG-FG scheme of Peter, Barnard, Edgerton, Gillespie, and Stempel (1972). All 3 fiber types exhibited a broad range of succinate dehydrogenase activities as determined by quantitative histochemical techniques.
   b. In soleus, mean SDH activity for FOG fibers was significantly greater than that for SO fibers; whereas in extensor digitorum longus, mean values for SO and FOG fibers were identical to each other but significantly greater than that for FG fibers.
   c. There was considerable overlap of SDH activities for the 3 fiber types precluding differentiation of fiber type based strictly upon oxidative potential. This, combined with the similarity of values for soleus SO fibers and extensor digitorum longus FG fibers, suggests that the differences in fatigability which characterize these 3 fiber types are not due solely to the differences in their oxidative capacity.

5. "The effects of disuse on skeletal muscle."

This final manuscript is a review which is organized in the following manner:

Effects of Reductions in Muscle Usage

Models of Reduced Use
Neural Alterations
Muscular Alterations
Effects on Muscle Properties
Morphological Changes
  Macroscopic
  Ultrastructural
Histochemical and Biochemical Changes
  Metabolic Capacity
  Myofibrillar Components
  Additional Biochemical Alterations
Fiber-Type-Distribution
Physiological Changes
  Membrane Properties
  Contraction Time
  Force
  Fatigability
Effects on Motoneuron Properties
Potential Relevance of Overuse Models
Effects of Environment
  Central Nervous System
  Muscle
References


-14-
WEIGHTLESSNESS HYPOKINESIA: SIGNIFICANCE OF MOTOR UNIT STUDIES

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ABSTRACT

In preliminary experiments, we found that medial gastrocnemius (MG) motor units in cage-reared rats were unusually sensitive to fatigue, a test for which is required to distinguish between FF- and FR-unit types. This observation prompted an analysis of whole-muscle fatigue. It revealed an impairment of the electrochemical process of neuronal excitation-contraction coupling at one or several sites between motor axons and the contractile machinery. (Supported by NASA Grant NAGW-338).

INTRODUCTION

Following preliminary experiments (see Abstract), we undertook an analysis of whole-muscle fatigue in the MG of cage-reared rats, together with an estimation of the fatigability characteristics of MG in a theoretical "noncaged-reared" rat model, assuming the theoretical muscle would have unit properties like those found in hindlimb muscles of noncage-reared cats (3,5,6).

METHODS

We used 500 g male and female 100-day old Sprague-Dawley rats, who had spent their life in 46-49 x 25-28 x 20 cm cages (4-7/cage). Such confinement is typical for commercially bought rats. Under pentobarbital sodium anesthesia (30-70 mg/Kg, I.P.), MG was prepared for force and EMG measurements (3,5,6).

RESULTS

Experiments. Fig. 1 shows force and EMG responses of MG (N = 7). Note three points:
1. Before the fatigue test, the disparity between force output for direct and nerve stimulation was greater than considered normal (2,4).
2. The fatigue test produced more substantial reductions in force and EMG than anticipated (3,5). Unfortunately, there are no data comparable to Fig. 1 for noncage-reared rats. However, Table 1 (lines 8, 11) suggests that EMG (force) reduction would be 23% (62%) in the theoretical MG as compared to 66% (79%) in our test MG (Fig. 1).
3. Intermittent and abrupt changes in EMG amplitude were a feature of each stimulus train of the fatigue test, even before force declined.

Estimations. Three assumptions in the Table 1 estimations require explanation:
1. Values for the relative distribution of the different fiber types (Table 1, line 1) are only available for cage-reared rats. They should not differ markedly in noncage-reared rats (3).
2. The assumption that the amplitude of the EMG co-varies with muscle-fiber diameter to the power of 3/2 is based on measurements made on "in-continuity" mammalian nerve axons (8).
3. At first glance, the assumption might seem far-fetched that relative (inter-unit) features of motor-unit anatomy (Table 1, lines 1-4), force development (line 5) and fatigability (lines 7, 10) are similar for rat MG and cat tibialis posterior (TP; footnotes 3-4). However, the specific tension (force/cross-section) of muscle tissue is quite similar across species (6), as are presumably the EMG:force relationships.

DISCUSSION

Orderly motor-unit recruitment (3) should prevail in cage-reared rats with cumulative activation (recruitment) of progressively more forceful units for progressively stronger contractions. As a result, it is likely that the FF units of cage-reared rats are used to less extent than the less forceful FR and S units (3,6). On this basis, we propose that the impairment in neuronal excitation-contraction coupling observed in these experiments is more pronounced in FF than FR and S units. We also anticipate that the observed impairments would be less in the soleus (SOL) muscle because, for the motor activities possible in a confined environment, SOL is presumably used to a greater extent than MG (3,6). Conversely, the observed deficits should be similar in another commonly studied muscle, extensor digitorum longus (EDL), because, like MG, its usage is limited during less demanding contractions (unpublished observations).

To address these issues, we intend to examine the dependence of the observed impairments on the fatigability of muscle by comparing the fatigue profiles of MG, SOL and EDL in exercise-deprived, exercised-exposed and wild rats. The existence of such a relationship would underscore the desirability of assuring an appropriate level of functional integrity of test muscles prior to the application of a disuse protocol to simulate weightlessness.
Fig. 1. Reduction in EMG and force output of the whole MG muscle of a cage-reared rat. Upper force profiles: averages of 8 supramaximal twitches (1 Hz). Lower force and EMG traces: single-sweep records of subfused tetani (40 Hz). Stimulus duration: 2 ms for direct intramuscular stimulation (A, A¹), 0.1 ms for muscle nerve (B, B¹). Arrows (mins): stimulus sequence (interstimulus intervals). 'Fatigue test: 330 ms-duration 40 Hz stimulus trains at 1 Hz for two min. Records indicate 1st (B), 31st, 61st and 121st (B¹) trains. Amplitudes of EMG (mean) and force (peak) expressed as reduction from "control" values (OZ). Insert: sites at which fatigue might occur during muscle-nerve stimulation (7).

TABLE 1. Estimation of Mean Reductions in EMG and Force Output of MG Motor Units of Theoretical "Noncage-reared" Rats During a Fatigue Test

<table>
<thead>
<tr>
<th>Unit (Fiber) Type</th>
<th>FF(FG)</th>
<th>FR(FOG)</th>
<th>S(SO)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Muscle fiber distribution (%)²</td>
<td>38</td>
<td>58</td>
<td>4</td>
</tr>
<tr>
<td>(2) Relative muscle-fiber diameter³,4</td>
<td>1.26</td>
<td>0.95</td>
<td>0.79</td>
</tr>
<tr>
<td>(3) Relative motor-unit innervation ratio³,4,5</td>
<td>1.26</td>
<td>0.84</td>
<td>0.90</td>
</tr>
<tr>
<td>(4) Motor-unit distribution (%) (100-(1)·(3)²-1·(1)²-1·(3)⁻¹)</td>
<td>29</td>
<td>67</td>
<td>4</td>
</tr>
<tr>
<td>(5) Relative force ratio³,4,6</td>
<td>2.20</td>
<td>0.61</td>
<td>0.19</td>
</tr>
<tr>
<td>(6) Contribution to whole-muscle EMG (Z) (100-(1)·(2)³/2·(1)²-(1)⁻³/2)</td>
<td>49</td>
<td>49</td>
<td>2</td>
</tr>
<tr>
<td>(7) EMG reduction after 2 min fatigue test (Z)³,8</td>
<td>40</td>
<td>5</td>
<td>14</td>
</tr>
<tr>
<td>(8) Contribution to % reduction in whole-muscle EMG ((6)-(7)·100⁻¹)</td>
<td>20</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>(9) Contribution to whole-muscle force (Z)</td>
<td>60</td>
<td>39</td>
<td>1</td>
</tr>
<tr>
<td>(10) Force reduction after 2 min fatigue test (Z)³,8</td>
<td>96</td>
<td>11</td>
<td>1</td>
</tr>
<tr>
<td>(11) Contribution to % reductions in whole-muscle force ((9)-(10)·100⁻¹)</td>
<td>58</td>
<td>4</td>
<td>0</td>
</tr>
</tbody>
</table>

1 Test described in Fig. 1.  
2 Cage-reared rat MG data (1).  
3 Noncage-reared cat TP data (4,5).  
4 Ratio of mean value to mean value for all unit (fiber) types.  
5 Mean number of muscle fibers in motor unit.  
6 Based on fused (100-200 Hz) tetanus value.  
7 Amplitude of extracellularly recorded EMG assumed to co-vary with fiber diameter³/2 (7).  
8 Reduction in mean value(s) for 121st stimulus train with respect to 1st train.

REFERENCES


NEURAL FACTORS IN WHOLE-MUSCLE FATIGUE OF RAT SOLEUS AND MEDIAL GASTROCNERIUS MUSCLES. MJ Joyner,* RM Enoka,* LL Rankin,* KA Volz,* and DG Stuart* (intr. by D Van Wyck), Departments of Physiology and Physical Education, University of Arizona, Tucson, Arizona.

Inasmuch as fatigue can be defined as "... a failure to maintain the required or expected force" (Edwards, 1982), there are several sites between the command (supraspinal) and effector (muscle) centers at which this failure can occur. Among these are axonal-branch-point and neuromuscular-junction transmission failure. These aspects of fatigue were examined by comparing the force elicited in the soleus (n = 5) and medial gastrocnemius (n = 3) muscles of Sprague-Dawley rats (3.1-4.8 N) by nerve and direct-muscle stimulation before and after a fatigue test (6 min of 1 Hz trains with each train lasting 330 ms and including 13 stimuli; Burke et al., 1971). There was no difference in the mean peak twitch force obtained by nerve and direct-muscle stimulation before the fatigue test; the nerve-elicited values were 97.9 ± 5.9% of those obtained by direct-muscle stimulation. In contrast, this ratio was 77.9 ± 19.9% after the fatigue test. This post-fatigue test difference in the peak twitch force produced by nerve and direct-muscle stimulation was not muscle-dependent (soleus 16.5 ± 11.9%, medial gastrocnemius 31.3 ± 29.8%) but rather was correlated significantly (r² = 0.89, p < .05) with the amount of fatigue (i.e., force reduction) exhibited during the fatigue test. These data underscore a neural contribution to whole-muscle fatigue in animals without neuromuscular lesions.


A quantitative approach is being used to assess the relationship between neuromuscular muscle physiology and biochemical properties of individual motor units. Following collection of physiological data on single motor units of the tibialis posterior muscles of adult cats and glycogen depletion of the motor unit identified histologically were dissected from the lyophilized sections and analyzed biochemically with micromethods.

Biochemical and physiological data were, thus obtained on the same 4 fast-contracting-fatiguing motor units; Table 1. "Motor Unit Activity" in the Unit Table.

Table 1. "Motor Unit Activity" in the Unit Table

| Motor Unit | Lactate | Deoxyglucose | Deoxyglucose
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.29%</td>
<td>0.49%</td>
<td>0.89%</td>
</tr>
<tr>
<td>2</td>
<td>2.35%</td>
<td>0.89%</td>
<td>1.38%</td>
</tr>
<tr>
<td>3</td>
<td>3.72%</td>
<td>1.38%</td>
<td>1.89%</td>
</tr>
<tr>
<td>4</td>
<td>5.16%</td>
<td>2.09%</td>
<td>2.56%</td>
</tr>
</tbody>
</table>


This project addressed the effects of usage on muscle properties with the assumption that muscle weight and size is influenced by the environmental physical environment. Seventy-two rat weanling rats were assigned either to a small FDA-approved laboratory cage (2/cage) or one 474x larger (15/cage). Subsequently, some contractile, electrical, and fatigue characteristics were determined in a muscle from the extensor digitorum longus (EDL) and soleus (SOL) muscles characterized as predominately fast-contracting ("fiber-type" distribution approximately 3% SF, 70% FM, 2% FG, and slow-contracting 2% SO, 16% FG, respectively) (Lamano et al., Histochim. Cytom. 21: 31, 1971). The results included in (T = 12h).


As an assessment of cage-size effects on the electromyogram (EMG), we have monitored the compound muscle action potential (AP) during fatigue in the EDL in which the muscle was activated by supramaximal intermittent stimulation of its nerve. Average measurements of the duration, area, "mean" amplitude, and normalized depolarization rate for the 13 AM activity units were obtained at selected intervals during the fatigue test (Stuart et al., Physiol. Rev. 70, 1980). The tests (n = 6) were for SOL and EDL of small- and large-cage reared rats.

For the large-cage rats developed a muscle weight relative to body weight (mN/N; Force (100 Hz stimulation)/relative total body weight) for EDL (n = 6; p < 0.10) and for SOL (n = 6; p < 0.01). Force of small and large-cage groups were comparable for the two muscles. The changes in force and muscle weight, however, appeared to be muscle specific; the large-cage rats maintained initial "mean" amplitude values longer than the small-cage rats. Supported by grants from NASA (NAGW-338) and NIH (HL07249).
**RELATION BETWEEN WHOLE-MUSCLE EMG AND FORCE DURING A FATIGUE TEST**


In an attempt to identify the mechanisms associated with the decline in force during a standardized fatigue test (Burke et al., J. Physiol. 275:713, 1973), we have monitored, in vivo, the compound muscle action potential (AP) and the isometric force during a 6-min test in which the test muscle was activated by supramaximal intermittent stimulation of its motor nerve. Accordingly, the soleus (SOL) and extensor digitorum longus (EDL) muscles of male and female SD rats (436 and 248 N, respectively; 120-150 days) were subjected to a protocol that included 1 Hz trains of 13 stimuli delivered at a rate of 40 Hz. The APs were recorded with a pair of intramuscular stainless-steel electrodes and were quantified by average measurements of the area and "mean" amplitude (MA) for the 13 APs within each train (Stuart et al., Proc. XXIV Int. U.P.S. XV; 190, 1983).

The fatigability of the muscles was quantified by a measure of fatigue resistance (FR); peak tetanic force at 6 min relative to initial force. By raising the rats in small and large cages, the FR of both test muscles extended over a range of values such that the muscles clustered into significantly different (p < .001) groups of relative high, intermediate, and low FRs: for SOL, these values (X ± SD) were 72 ± 7 (n = 10), 92 ± 6 (n = 7), and 67 ± 19% (n = 5), respectively; while for EDL, the values were 48 ± 5 (n = 8), 33 ± 2 (n = 9), and 10 ± 7% (n = 8); respectively. Despite these differences in FR for EDL, the 3 groups exhibited a qualitatively similar relationship between AP area and levels of the fatigue test. In all 3 groups, the relationship was characterized by an initial potentiation and a subsequent parallel decline in both AP area and force. Quantitative measures of the potentiation of both force and AP area. Somewhat similar relationships were apparent between MA and peak force for EDL, although MA did not potentiate initially as did AP area. The changes in area and MA during the test were similar for EDL (r² = 0.83; p < 0.1). In contrast, the high and intermediate force groups demonstrated a differential relationship between force and AP area: the low FR group in that essentially remained constant while AP area potentiated for the former groups and, conversely, area remained constant while force declined for the low FR group. In addition, the changes in AP area and MA for SOL, while significant (p < 0.01), were less related (r² = 0.33) than for EDL.

It was apparent that under these conditions (albeit Sandercock et al., J. Appl. Physiol. 58:1073, 1985) both AP area and MA changed during the course of the fatigue test. However, we are uncertain at this point as to whether these changes reflect a true fatigue process, a post-tetanic, filter-effect, or some combination of the two mechanisms.

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CHARACTERISTICS, DISTRIBUTION, AND INTERRELATIONSHIPS OF SOMATOATIN, NEUROPEPTIDE Y, AND NADPH DIAPHORASE NEURONS IN HUMAN CAUDATE NUCLEUS.

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In the present study we determined the topography of somatostatin (SS), neuropeptide Y (NPY) and NADPH-diaphorase (NADPH-D) neurons in human caudate and their relationships to zones of low and high acetylcholinesterase (AChE) activity (striosomes). Twelve blocks of striatum (fixed in neutral buffered formalin) at 4 C for 48 hours. Sections were incubated with antisera to SS and NPY followed by amplification and detection using an avidin-biotin peroxidase method or indirect immunofluorescence. Absorption controls were consistently negative. NADPH-D staining was performed employing the avidin-biotin method with Triton X-100 (0.8%) and monosodium malate (125mg%) to the incubation medium. These modifications enhanced staining of both NADPH-D sites and reticular background staining. Serial sections were incubated for 90 min at room temperature for 30 min with 1 nm-5-F-NS in 50 nm tris-acetate-saline buffer, pH 7.4. Adjacent sections for nonspecific binding were incubated with the same concentration of NS. NADPH-D reactions were observed and processed using antigen-antibody complexes.
CAGE-SIZE AND GENDER EFFECTS ON FATIGUE IN RATS

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As a biological tissue, muscle adapts to the demands of usage. One traditional way of assessing the extent of this adaptation has been to examine the effects of an altered-activity protocol on the physiological properties of muscles. However, in order to accurately interpret the changes associated with an activity pattern, it is necessary to employ an appropriate control model. There exists a substantial literature which reports altered-use effects by comparing experimental observations with those from animals raised in small laboratory cages (e.g., 9, 10). Accumulating evidence suggests that small-cage-reared animals actually represent a model of reduced use. For example, laboratory animals subjected to limited physical activity have shown resistance to insulin-induced glucose uptake which can be altered with exercise training (3). Similarly, in male rats such parameters as body mass, muscle mass, fatigue resistance, and muscle-relaxation characteristics have been shown to be affected by cage size (8). Furthermore, during the course of this project it appeared that the cage-size effects were confounded by a gender effect. This report further examines these issues by considering the effects of cage size during rearing and of gender on the physiological properties of adult rat muscle.

METHODS

Adult (100-135 days old) male and female Sprague-Dawley rats were raised in separate groups from weaning age (21 days) in either of two cage sizes. The small cages (47 x 23 x 20 cm) each contained 3-5 rats while there were 6-7 animals, separated by gender, in each of the two large cages (320 x 183 x 99 cm). The large cages provided approximately 133x more surface area (floor + 4 walls). There were 6-7 rats in each of the 4 groups; namely, small-cage males, large-cage males, small-cage females, large-cage females.

The two test muscles were soleus and extensor digitorum longus (EDL). Soleus is a slow-contracting, antigravity, plantarflexor muscle which comprises about 84% type 50 and 16% type 50 muscle fibers (1). In vivo electromyographic (EMG) recordings reveal constant motor-unit recruitment while the animal is awake (6). In contrast, EDL is a fast-contracting, weight-bearing, dorsiflexor muscle with a fiber-type distribution of about 8% SO, 57% FOG, and 37% FG (11). EMG recordings show that EDL is silent when the animal is at rest and activated phasically during locomotion (6).

In terminal experiments, with the rats under deep barbiturate anesthesia, the soleus and EDL muscles were surgically isolated and maintained in a paraffin-filled muscle bath at a temperature of 36°C. The following experimental protocol was used: 32 twitches were elicited by stimulation through the nerve to evaluate treppe; 1 single tetanus at 100 Hz for 500 ms to determine the peak force; a standardized fatigue test (2) which involved intermittent stimulation (40 Hz) of the nerve with trains of 13 stimuli, each train lasting 330 ms and occurring once each second; 8 twitches to evaluate post-tetanic potentiation; and finally, two 100 Hz tetani. Force was measured using a force transducer to which the tendon of the test muscle was attached via a low-compliance dacron line. The compound muscle action potentials generated by the stimuli were recorded with a pair of intramuscular stainless-steel wire electrodes. The 13 action potentials associated with each train of stimuli during the fatigue test were averaged and quantification was based on the average waveform. Measurements on the average waveform were made of area relative to the isoelectric line and of peak-to-peak amplitude. Statistcal differences were assessed with a t-test for two means.

RESULTS

It is well documented that male rats are larger than the females of a similar age (7). This is apparent in Table 1 in the measurements of whole-body and test-muscle mass in that the female values averaged 67% of those for the males. These size differences, however, were not affected by cage size since within each of the 4 animal groups the mass of the two test muscles was not different; the average values for soleus and EDL were 0.23 and 0.25 g for the males and 0.17 g for both muscles in the females.

Qualitatively similar trends were apparent in the absolute force data in that there was no cage-size effect, but there was a partial gender effect. That is, the peak force exerted by EDL in response to a 330-ms train of 40 Hz stimuli was greater in males (X = 2.13 N) than females (X = 1.29 N). When the EDL force measurements were normalized relative to test-muscle mass, however, this difference disappeared. In contrast, the absolute 40 Hz force exerted by soleus was not affected by cage size or gender despite gender differences in soleus mass. However, normalization of soleus force did reveal a cage-size effect in that the force exerted per unit muscle mass of the small-cage males (6.0 N/g) was significantly less than the value for the large-cage males (7.9 N/g).

In addition to these measures of size and force, the ability of the test muscles to sustain activity was also examined. The 4 measurements used to characterize this capability were fatigue index (FI), relaxation time, area and amplitude of EMG. These 4 parameters are used as indices of contractile fatigue (FI; 2), the energy-state of the muscle (relaxation time; 4), and precontractile fatigue (EMG; 3). The test muscles were subjected to a standardized fatigue test, and the 4 measures were made at selected intervals throughout the test; the final values are reported in Table 1. There were no cage-size effects among any of these parameters for the test muscles of the female rats. In contrast, the males exhibited 3 differences: (1) a cage-size effect for the FI of soleus; (2) a gender effect for the FI of the large-cage rats; (3) a combined (cage-size and gender) effect for the EMG area of small-cage EDL. Taken together, these results suggest that male rats are more sedentary in the small cages (decreased normalized muscle force and FI for

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Table 1. An overview of the effects of cage size during rearing and of gender on size, contractile, and electromyographic (EMG) characteristics of the test muscles.

<table>
<thead>
<tr>
<th></th>
<th>Mass Whole Body (g)</th>
<th>Muscle Mass (g)</th>
<th>Muscle Forcea Absolute (N)</th>
<th>Muscle Forcea Relative to Muscle Mass (N/g)</th>
<th>Fatigue Indexb (%)</th>
<th>Relaxation Timec (ms)</th>
<th>Relaxation Area d (%)</th>
<th>Absolute Force (N)</th>
<th>Amplitude d (%)</th>
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</thead>
<tbody>
<tr>
<td>Small Cage:</td>
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<td></td>
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<tr>
<td>Male:</td>
<td>440±5 (20)</td>
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<tr>
<td>Soleus</td>
<td>0.26±1 (0.06)</td>
<td>1.42 (0.41)</td>
<td>6.06 (1.8)</td>
<td>898 (21)</td>
<td>45 (20)</td>
<td>103 (34)</td>
<td>81 (25)</td>
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<tr>
<td>EDL</td>
<td>0.26±1 (0.03)</td>
<td>2.05±1 (0.17)</td>
<td>8.0 (1.2)</td>
<td>35 (17)</td>
<td>31 (8)</td>
<td>50±6 (19)</td>
<td>30 (25)</td>
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<tr>
<td>Female:</td>
<td>260 (10)</td>
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<td>Soleus</td>
<td>0.170 (0.02)</td>
<td>1.35 (0.16)</td>
<td>8.0 (1.5)</td>
<td>96 (16)</td>
<td>42 (22)</td>
<td>132 (18)</td>
<td>95 (37)</td>
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<tr>
<td>EDL</td>
<td>0.170 (0.01)</td>
<td>1.16 (0.27)</td>
<td>6.9 (1.9)</td>
<td>33 (18)</td>
<td>26 (12)</td>
<td>20 (14)</td>
<td>13 (15)</td>
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<tr>
<td>Large Cage:</td>
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<tr>
<td>Male:</td>
<td>430±5 (30)</td>
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<td>Soleus</td>
<td>0.220±1 (0.01)</td>
<td>1.71 (0.15)</td>
<td>7.9 (1.1)</td>
<td>112±1 (13)</td>
<td>37 (12)</td>
<td>127 (23)</td>
<td>123 (40)</td>
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<tr>
<td>EDL</td>
<td>0.220±1 (0.02)</td>
<td>2.21±1 (0.12)</td>
<td>9.6 (1.1)</td>
<td>27 (16)</td>
<td>39 (11)</td>
<td>26 (18)</td>
<td>9 (6)</td>
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<tr>
<td>Female:</td>
<td>271 (10)</td>
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<tr>
<td>Soleus</td>
<td>0.160 (0.02)</td>
<td>1.40 (0.19)</td>
<td>8.8 (0.7)</td>
<td>87 (18)</td>
<td>45 (21)</td>
<td>135 (34)</td>
<td>90 (34)</td>
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<tr>
<td>EDL</td>
<td>0.170 (0.01)</td>
<td>1.43 (0.27)</td>
<td>8.9 (1.5)</td>
<td>22 (15)</td>
<td>26 (9)</td>
<td>23 (10)</td>
<td>7 (4)</td>
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</table>

- a Peak force at 40 Hz; b Ratio of the peak 40 Hz force at 6 min relative to the initial value. The protocol involved a standard fatigue test (Burke et al., 1973); c Difference in the time for the force to decline to 20% of the peak force at the end of the fatigue test (6 min) relative to the initial tetanus; d Values expressed relative to measurements on the initial average action potential; e Values are X (±SD); f p < 0.05 between large and small cage

soleus) and more active in the large cages (increased soleus FL).

CONCLUSIONS
1. Cage size and gender had minimal effects on changes in the EMG measures.
2. Gender affected whole body mass, test muscle mass, and absolute force.
3. Cage size and gender affected normalized force and fatigability.
4. Since the cage-size effects were only present in the male rats, these results suggest caution in the interpretation of results based on this animal model.

REFERENCES