Endurance exercise modulates neuromuscular junction of C57BL/6NNia aging mice

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Fahim, Mohamed A. Endurance exercise modulates neuromuscular junction of C57BL/6NNia aging mice. J. Appl. Physiol. 83(1): 59–66, 1997.—The effect of age and endurance exercise on the physiology and morphology of neuromuscular junctions (NMJ) of gluteus maximus muscle was studied in C57BL/6NNia mice. Mice were exercised, starting at 7 or 25 mo of age, at 28 m/min for 60 min/day, 5 days/wk for 12 wk, on a rodent treadmill. Intracellular recordings of spontaneous miniature endplate potentials (MEPP) and the quantal content of endplate potentials (EPP) were recorded from NMJ of 10- and 28-mo-old control and exercised mice. Endurance exercise resulted in significant increases in MEPP amplitudes (23%), quantal content, and safety margin, and a significant decrease in MEPP frequency of young mice, with no change in resting membrane potential or membrane capacitance. Three months of endurance exercise resulted in an increase in MEPP frequency (41%) and decreases in MEPP amplitudes (15%), quantal content, and safety margin of old mice. Endurance exercise resulted in significantly larger nerve terminals (24%) in young animals, suggesting functional adaptation. Nerve terminals in exercised 28-mo-old mice were smaller than in the corresponding control mice, an indication that exercise minimized age-related nerve terminal elaboration. It is concluded that the different physiological responses of young and old gluteus maximus muscles to endurance exercise parallel their morphological responses. This suggests that the mouse NMJ undergoes a process of physiological and morphological remodeling during aging, and such plasticity could be modulated differently by endurance exercise.

transmitter release; safety margin; morphology; synaptic plasticity; physiology

REMODELING OF NEUROMUSCULAR JUNCTIONS (NMJ) appears to be a lifelong process, as indicated by ongoing outgrowth, retraction, constriction, and expansion. This morphological turnover of NMJ may be modulated by activity (2, 5–8, 16, 21), seasonal differences, and hormonal status, and may also be dependent on age and muscle fiber type (3, 4, 9, 10, 19, 23). During aging, dynamic structural and functional changes occur at the mouse NMJ, and questions have been raised (23) as to whether these changes result from reduced physical activity or aging per se.

Few studies have addressed the effects of physical activity on the morphology and the physiology of the NMJ, because most exercise studies have focused primarily on whole muscle changes. Depending on the muscle type and the nature of the exercise, different studies have reported increase in oxidative capacity, increases in the number and size of mitochondria, increases in muscle weight, the appearance of split muscle fibers, and an altered distribution of muscle fiber types (I, IIA, IIB) (14, 15, 17, 24, 30). As for NMJ changes induced by exercise, it has been reported (17) that exercise does not affect either the number and distribution of acetylcholine receptors (AChR) or the specific activities of choline acetyltransferase (CAT) and acetylcholinesterase (AChE). However, we have previously reported NMJ structural changes in C57BL/6NNia mice that were exercised for 2 mo (2). In 12-mo-old animals, the extensor digitorum longus (EDL) nerve terminal area was larger in exercised animals than in controls. Additionally, in the same study, exercise was reported to counteract morphological age-related elaboration in EDL, but soleus nerve terminal area was unaffected by exercise (2).

Apart from these observations, a substantial quantitative analysis of physiological NMJ parameters after exercise has not yet been presented. More information is required to determine whether endurance exercise for 12 wk causes structural changes of the NMJ and how these exercise-induced changes relate to age-associated changes and their possible functional consequences in the structure and function of the NMJ.

Therefore, we designed the present experiments to assess the effects of exercise during aging on NMJ structure and function of gluteus maximus (GM) muscle. This muscle was selected because of its importance in extending, laterally rotating, and helping in abduction of the femur. It was reported that, with increased speed during treadmill locomotion, the mean electromyographic activity per step was elevated in the GM muscle while the hip joint angles remained relatively constant (22). This would indicate that this extensor muscle has to contract over a longer distance in a shorter time period during running. Also, GM muscle forms a flat sheet that is amenable to experimental investigation, whereas other limb muscles are cylindrical and more difficult to investigate.

METHODS

Animals

Male C57BL/6NNia mice, obtained from the National Institute of Aging colony maintained by Charles River Laboratories, were housed in groups of two in standard mouse cages (28 × 14 × 12 cm) and provided with litter and drinking bottles. This strain of mice was used in the present study because it stays relatively free of organ pathology as well as stays physically active into advanced age. The animals were maintained in a well-ventilated vivarium facility on a 12:12-h light-dark cycle at 25°C and were given food (Wayne Lab Blocks) and water ad libitum.

All of the animals from each age group were familiarized with the treadmill and then randomly assigned to control or exercise groups. Five animals were excluded because they did not adapt easily to the exercise program and were injured.

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When the muscle tissue was obtained from the animals, a routine visual necropsy was performed. Animals with tumors, abscesses, advanced splenomegaly, or ankylosed hindlimb joints (a total of 3 animals) were also excluded (n = 8 animals per group after the exclusions). Virtually all of the 28-mo-old animals did, however, have enlarged, distended, or discolored seminal vesicles; this is considered normal for this strain (10).

Routine Pathology

Samples of adrenal, pituitary, spleen, kidney, liver, heart, and lung tissue were taken from young and old control and exercised animals and were fixed in formalin and then embedded in paraffin. Thick sections were made and stained with eosin and hematoxylin for inspection with light microscopy.

Exercise

Animals were divided into young mature and older age groups which, when killed, were 10 and 28 mo of age, respectively. The mice trained 5 days/wk for a period of 12 wk on a rodent treadmill (Quinton Instruments). Initially, the exercise began with a moderate walk-jog pace of 15–20 m/min for ~30 min. As the mice became increasingly familiar with the treadmill, the velocity and duration of the exercise were gradually increased until they could run between 25 and 28 m/min for 60 min/day. The animals were exercised at this intensity for the final 8 wk of their training.

Traditionally, exercise studies using treadmill running employ mild, escapable electric stimulation as a form of encouragement. However, the stress caused by electrical shock may constitute a confounding variable with respect to nerve terminal morphology. For this reason, only short bursts of compressed air were used for motivation. This kind of motivation did not cause any changes in the structure and function of NMJ (Fahim, unpublished results). Using the same procedure described previously (2), we estimated 25–28 m/min to be 75–80% maximum O₂ consumption. Random daily monitoring indicated no difference in cage activity between sedentary and trained animals. Young and old control animals were placed on a stationary treadmill for 10 min/day, with short bursts of compressed air to rule out any possible effects due to different handling of animal groups.

Surgical Procedure and Preparations

Animals were anesthetized with methoxyflurane (Pitman-Moore) until slow regular animal breathing was achieved. The GM muscles were excised and pinned in a Lucite chamber containing standard Krebs solution (pH 7.2) at 23–25°C for electrophysiological experiments. The Krebs solution consisted of (in mM) 112 NaCl, 4.5 KCl, 2.5 Ca gluconate, 1 MgSO₄, 1 Na₂HPO₄, 15 NaHCO₃, and 11 glucose. The solution was oxygenated (95% O₂-5% CO₂) by a gas-lifting device, which circulates freshly oxygenated solution at a rate of 10–15 ml/min without agitating the recording chamber (3).

Electrophysiological Recording

Glass capillary microelectrodes filled with 3 M KCl and drawn to a tip yielding 8–15 MΩ resistance (measured in Krebs solution) were inserted into muscle fibers at the endplate region to record miniature endplate potentials (MEPP) and endplate potentials (EPP). A combination of oblique and transillumination from a Leitz-Wild microscope was used to locate endplate regions on surface muscle fibers. In addition, a MEPP rise time (time from 10 to 90% of peak amplitude) of <1 ms was used as a criterion for endplate regions. For EPP recording, a partial presynaptic block was administered to prevent muscle contraction. A low-Ca²⁺ (0.5 mM)/high-Mg²⁺ (2.75 mM) solution was used to suppress evoked release so that a regenerative action potential did not occur, and, therefore, no muscle contraction occurred. The nerve supplying the muscle was stimulated via a suction electrode by using a Grass S-44 stimulator and stimulus isolation unit.

Supramaximal stimuli of 0.5-ms duration were delivered at a frequency of 0.5 Hz. No facilitation or depression of the EPP was seen at this stimulus frequency, MEPP and EPP were recorded after a total of 40 min in the low-Ca²⁺/high-Mg²⁺ solution. To facilitate penetration of the new solution, it was continuously squirted onto the muscle surface with a pipette. In each muscle, 5–10 muscle fibers were studied. This was considered to be an adequate number, because they are considered replicates rather than independent samples (see Statistical Analysis for more details). From each muscle fiber, 100–200 MEPP and 50–100 EPP were recorded. MEPP and EPP were digitized on-line via an analog-to-digital conversion board (10-kHz sampling rate) in a Northstar Horizon microcomputer that provided values for frequency and amplitude. Recordings were obtained from muscle fibers with resting membrane potentials (Eₘ) more negative than ~70 mV. Data were discarded if the Eₘ fell by >5 mV during the recording period. “Giant” MEPP (those of an amplitude more than twice the SD) were not included when calculating the mean MEPP amplitude or frequency.

Both MEPP and EPP amplitudes were corrected to a standard Eₘ of ~80 mV. Quantal content was calculated by the direct method (corrected mean EPP amplitude/corrected mean MEPP amplitude).

To determine muscle fiber input resistance (Rᵢₘ), two microelectrodes for current injection and potential recording were inserted into the same muscle fiber with ~50 µm of each other. A 10–20 nA hyperpolarizing current (150-ms duration) was injected through one electrode, while the voltage response was recorded from the other. Rᵢₘ was calculated from the ratio of the steady-state membrane potential to the injected current. The membrane capacitance was computed from the time constant (1).

Safety Margin of Synaptic Transmission

The isometric twitch tension evoked by a nerve stimulus (indirect twitch) was measured in two different solutions, a standard Krebs solution (2.5 mM Ca²⁺, 1.0 mM Mg²⁺) and a reduced-Ca²⁺ solution (1.0 mM Ca²⁺, 1.0 mM Mg²⁺). The ratio of the tension in the low-Ca²⁺ solution to the tension in the standard Krebs solution was used as an estimate of summed safety margin of all NMJ in the muscle while Ca²⁺ concentration is reduced. When Ca²⁺ concentration is reduced, the twitch produced by direct muscle stimulation is not affected (3). However, the indirect twitch is depressed, because some fibers will not twitch as transmitter release at their junctions becomes subthreshold for generation of muscle fiber action potential.

The nerve-muscle preparation was placed in the recording chamber, with oxygenated Krebs solution, at room temperature (23–25°C). The proximal tendon was pinned into Syilgard at ~1.1 x the resting length of the muscle. The tendinous insertion was attached to a Grass force-displacement transducer (FT-03C), and the output was differentially amplified and displayed on a storage oscilloscope. Once every 5 min, the nerve was stimulated supramaximally with a suction electrode (0.5-ms duration) to produce maximum indirect twitch tension, and five twitches were measured. For Krebs solution changes, the procedure was the same as that used for transmitter release experiments.
Histology

On completion of the electrophysiology protocol, the muscles were pinned in a Sylgard-lined petri dish at slightly greater than resting length to minimize effects of shrinkage if any occurred during staining. The zinc iodide-osmium (ZIO) technique was used to stain the nerve terminals. This technique produces stained nerve terminals that are morphologically very similar to postsynaptic arborizations obtained through scanning electron microscopy (9). After the 6-12 h of staining, the muscles were teased into bundles of between one and five fibers, mounted on microscope slides in Aquamount, and covered with a standard coverslip. Under magnification with a x100 Olympus SPlan objective, camera lucida drawings were made of 10–30 nerve terminals per muscle from each animal. For a nerve terminal to be acceptable, more than ~95% of its area must have been clearly visible.

Once the nerve terminals were drawn, the images were superimposed onto a computer monitor through a Dage television camera equipped with a Nikon Nikkor 55-mm flat-field macro lens. The drawings were then traced on the screen with a Houston Instruments Hipad, and the morphological measurements determined with the planar morphometry package of Southern Micro Instruments (Atlanta, GA). The morphological parameters evaluated were a subset of those previously described (23) and included nerve terminal perimeter (the continuous length of the complete outline of all stained components of the nerve terminal; nerve terminal area (the integrated area within the nerve terminal perimeter); longitudinal extent length (the distance from one end of the nerve terminal to the other along the longitudinal axis of the muscle fiber); number of nerve terminal branches (including nod-like protuberances extending prominently at < 90° from the parent branch); and incidence of sprouts (filaments arising from and less than ~1/4 the width of major branches). Additionally, the diameter of each muscle fiber was ascertained from a region near, but not part of, the nerve terminal region to minimize the effects of the enlargement of the sole plate area. These parameters were selected for the same reasons as described previously (23).

Statistical Analysis

Data analysis that does not distinguish between observations from the same individual and observations from different individuals is misleading. Statistical analyses in the present study pertain to the distribution in the whole population. Accordingly, the sample size was the number of different individuals (mice) and not the total number of observations. Thus, observations of a given property from a single mouse were treated as replicates or measures rather than independent samples. The mean was obtained from each mouse (experimental unit), then the standard deviation (SD) and error (SE) of the group mean were calculated by using means from different mice in that group. Tabular data are presented as means ± SD in Figures 1-4. Statistical analysis of the data was based on repeated-measures univariate analysis of variance and multivariate analysis of variance. These techniques account for variance among animals in each group and the variance within each animal. This reduces the associated statistical error and the number of animals required. The means were calculated by using the least-squares techniques (expected means for a balanced design). This technique accounts for differences in group sizes, thereby permitting direct comparison between groups.

RESULTS

Body Weight and Muscle Diameter

Mean body weight did not change significantly either with aging [34.8 ± 0.5 vs. 35.0 ± 0.6 g (mean ± SD), young vs. old sedentary mice, respectively] or with aging and exercise (33.9 ± 0.7 vs. 34.7 ± 0.2 g, young vs. old exercised mice, respectively). Also, muscle fiber diameters were not significantly changed with either aging or exercise (Table 1). Thus the load of animal weight on the muscles did not change with either aging or exercise.

Pathology

In both control and exercised young and old mice, routine histological examination of adrenal, pituitary, spleen, kidney, liver, heart, and lung revealed no major changes. However, occasional gross liver or lung tumors and skin abscesses were noticed in the old mice, and these mice were not used in the study (total of 3 animals).

Electrophysiology

MEPP amplitude. At most endplates of 10- and 28-mo-old animals, the distribution of MEPP amplitudes was unimodal but positively skewed. The mean MEPP amplitude increased 19% with age (Table 2). There was no indication that the increased amplitude was caused by a decrease in cholinesterase activity, because MEPP rise times and one-half decay times were the same in both age groups (Table 2). Apparently the increase in mean MEPP amplitude in 28-mo-old animals resulted from a shift in the entire population of recorded NMJ. Moreover, the small number of “giant” MEPP (<2% of the population) was unchanged between 10 and 28 mo of age in both control and exercised animals. Exercise did increase the MEPP amplitude in

| Table 1. Gluteus maximus NMJ morphometry of control and exercised animals |
|-----------------------------|-----------------------------|
| Parameter                   | Young                       | Old                        |
|                             | Control (80)                | Exercised (80)             | Control (75)       | Exercised (72)       |
| NT area, µm²                | 506±24                      | 627±18                     | 614±35            | 511±14               |
| NT perimeter, µm            | 512±28                      | 609±15                     | 597±14            | 524±13               |
| NT extension length, µm     | 64±3                        | 75±2                       | 76±1              | 67±1                 |
| Fiber diameter, µm          | 41±1                        |                           | 42±2             |                     |

Values are means ± SD derived from muscle fibers (nos. in parentheses) studied in 8 animals. NMJ, neuromuscle junction. NT, nerve terminal. *With the exception of muscle fiber diameter, each pair of results is significantly different (exercise from control, old from young), P < 0.05.
Synapse effectiveness was used to obtain a relative measure of old for generation of muscle fiber action potentials. This transmitter release at their junctions became subthreshold; that is, some muscle fibers did not twitch and the nerve-evoked twitch tension was decreased and the 10-mo-old animals (23%), whereas it decreased (15%) the MEPP amplitude in 28-mo-old animals (Table 2).

Several parameters were also examined in sedentary and exercised animals. As shown in Table 2, there were no significant changes in $E_m$ with either aging or exercise, and, therefore, $E_m$ would not affect MEPP amplitude significantly. There was a significant increase (40%) in $R_{in}$ between 10 and 28 mo of age in control animals. Exercise did not affect 10-mo-old animals but decreased (28%) the $R_{in}$ in 28-mo-old animals. Finally, there was no change in membrane capacitance with either sedentary or exercised animals, whether young or old animals.

MEPP frequency. The distribution of MEPP frequencies was unimodal but often negatively skewed. The mean MEPP frequency decreased (32%) with sedentary aging (Table 2). Endurance exercise has a different effect dependent on age. Although exercise decreased (31%) MEPP frequencies of 10-mo-old mice, it increased (41%) MEPP frequencies of 28-mo-old animals (Table 2). Fiber diameters did not significantly change with either aging or exercise (Table 1).

EPP amplitude. There was a pronounced increase in EPP amplitude with age (Table 2). This increase was due to a shift in the whole population. Calculated quantal content in low-Ca$^{2+}$/high-Mg$^{2+}$ Krebs solution was increased by 45% in 28-mo-old animals compared with 10-mo-old animals. With exercise, quantal content increased 25% in young animals whereas it decreased 28% in old trained animals (Fig. 1).

Safety Margin of Synaptic Transmission

To estimate synaptic transmitter output averaged over the entire population of junctions in 10- and 28-mo-old control and exercised mice, the indirect twitch was measured under conditions of reduced Ca$^{2+}$. Under these conditions, transmitter release was reduced and the nerve-evoked twitch tension was depressed; that is, some muscle fibers did not twitch as transmitter release at their junctions became subthreshold for generation of muscle fiber action potentials. This phenomenon was used to obtain a relative measure of synaptic effectiveness (safety margin). Low-Ca$^{2+}$ Krebs solution caused a more pronounced decline in twitch tension in young control than in old control muscles. After 3 mo of exercise, GM twitch tension was significantly higher (25%) in young than in control mice, whereas the muscle twitch tension in GM from 24-mo-old trained mice was 24% lower than in nontrained mice (Fig. 2).

Nerve Terminal Morphology

Effects of age. Morphological measurements of ZIO-stained nerve terminals showed an increase in junctional area in 28-mo-old unexercised mice compared with 10-mo-old controls. In aged motor nerve terminals, the dispersion of terminal branches into a series of islets or regions with fine axonal interconnections was striking and consistent with indications of increased remodeling. Also, each of the morphological parameters measured in aging nerve terminals increased with age in the control groups (Table 1). These changes were generally represented by a shift of the entire popula-

Table 2. Electrophysiological properties of NMJ of gluteus maximus muscles of exercised and control animals

<table>
<thead>
<tr>
<th>Property</th>
<th>Control Young</th>
<th>Control Old</th>
<th>Exercised Young</th>
<th>Exercised Old</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting membrane potential, mV</td>
<td>82.40 ± 0.50</td>
<td>82.71 ± 0.62</td>
<td>78.91 ± 0.70</td>
<td>78.50 ± 0.42</td>
</tr>
<tr>
<td>MEPP amplitude, mV</td>
<td>0.61 ± 0.02</td>
<td>0.75 ± 0.10</td>
<td>0.72 ± 0.01</td>
<td>0.62 ± 0.02</td>
</tr>
<tr>
<td>MEPP rise time, ms</td>
<td>0.47 ± 0.01</td>
<td>0.38 ± 0.02</td>
<td>0.51 ± 0.06</td>
<td>0.46 ± 0.05</td>
</tr>
<tr>
<td>MEPP 1/2 decay time, ms</td>
<td>0.82 ± 0.10</td>
<td>0.85 ± 0.10</td>
<td>0.83 ± 0.04</td>
<td>0.80 ± 0.07</td>
</tr>
<tr>
<td>MEPP frequency, Hz</td>
<td>2.97 ± 0.10</td>
<td>2.02 ± 0.04</td>
<td>2.00 ± 0.11</td>
<td>2.82 ± 0.12</td>
</tr>
<tr>
<td>EPP amplitude, mV</td>
<td>0.75 ± 0.02</td>
<td>1.60 ± 0.06</td>
<td>1.30 ± 0.12</td>
<td>0.79 ± 0.14</td>
</tr>
<tr>
<td>Input resistance, MΩ</td>
<td>0.30 ± 0.01</td>
<td>0.32 ± 0.11</td>
<td>0.42 ± 0.03</td>
<td>0.30 ± 0.10</td>
</tr>
<tr>
<td>Membrane capacitance, nF</td>
<td>2.21 ± 0.21</td>
<td>2.40 ± 0.30</td>
<td>2.12 ± 0.30</td>
<td>2.31 ± 0.40</td>
</tr>
</tbody>
</table>

Values are means ± SD derived from muscle fibers (nos. in parentheses) studied in 8 animals. MEPP, miniature endplate potential; EPP, endplate potential. *All are significantly different (exercise from control, and old from young) P < 0.05. **Significant differences between young and old control, P < 0.05.
tion. The changes in nerve terminal area of the aging GM best exemplified this (Fig. 3). The NMJ were larger, more complex, and highly branched with advancing age (Fig. 4). The nerve terminal area was 21% larger, the perimeter was 17% longer, and the overall extent length was increased by 19%.

Effectsof exercise. Endurance exercise had a differential effect on nerve terminal morphology, depending on age (Table 1). In young animals, the 3-mo training protocol produced a significantly larger (24%) nerve terminal area. The ensuing hypertrophy, similar to that observed during aging, was characterized by an upward shift of the sample population of nerve terminals rather than by the appearance of a new subgroup. Exercised GM nerve terminals in young animals had 19% longer perimeters and 17% longer extension lengths. In old exercised animals, the training protocol produced a markedly different effect. Several morphological measurements were significantly smaller than the corresponding controls, which were undergoing an age-related expansion. The distribution of terminals from old exercised mice was more homogenous than the controls, suggesting that the exercise protocol prevented further age-related expansion of the neuromuscular junction. GM nerve terminals from old exercised mice had 17% smaller areas, 12% shorter perimeters, and 12% shorter extension lengths than those in control old mice.

DISCUSSION

The most significant finding in the present study was the differential effect of endurance exercise in young and old NMJ. Young NMJ undergo a process of hypertrophy in response to exercise. The imposition of a relatively intensive exercise protocol probably recruits a sizeable percentage of the muscle fibers not normally utilized during cage activity. The resulting shift in the basal activity level was sufficient to induce structure remodeling. The hypertrophy of the NMJ resulted in a concomitant increase in transmitter release. A morphological hypertrophy of the NMJ would indeed increase the surface area in contact with the muscle fiber. This could result in more release sites and, hence, more transmitter release. Endurance exercise resulted in significant reductions in the structure and function of old NMJ. The smaller size of NMJ in old exercised mice may represent decreased adaptive capacity and greater nerve terminal withdrawal. The reduction of MEPP and EPP amplitude in old NMJ after exercise may be explained by diminished quantum size. This, added to the reduction of size of NMJ, would result in the observed reduction of quantal content.

MEPP amplitude was found to increase during sedentary aging, and this increase was not associated with any significant change in membrane capacitance or muscle fiber diameter. The $E_m$, MEPP rise time, and one-half decay time remained unchanged, whereas MEPP frequency decreased during sedentary aging. Assuming that AChE activity and AChR density at the NMJ are not altered with aging (3, 29), the present findings suggest that the number of acetylcholine molecules released or the junctional response per quantum may change with aging. However, the increase in MEPP amplitude with age is almost matched by the increase in $R_{in}$, which, therefore, seems to be the most likely explanation for the change.

Decreased MEPP frequency during sedentary aging, reported here, could be explained by a smaller endplate area, because a positive correlation was previously reported between MEPP frequency and endplate area (12). However, the present morphological results rule out such a possibility, because nerve terminal area increased significantly during sedentary aging. Other possible explanations for reduced MEPP frequency would be reduced numbers of available synaptic vesicles or altered level of free intraterminal Ca$^{2+}$ (1).

In Mg$^{2+}$ block, a larger EPP amplitude was observed in old compared with young NMJ. The calculated quantal content confirmed the increased transmitter release capacity of old NMJ. Postsynaptic junctional AChR in mice and rats are not increased in number, despite the increased quantal content during aging (3, 25). Indeed, nerve terminal expansion does account for at least some of the increased quantal content from old nerve terminal (23). However, another possible explanation would be that an increased Ca$^{2+}$ influx on nerve terminal depolarization is responsible for the observed increase of quantal content during aging (1).
Quantal content and MEPP frequency are usually correlated, but a dissociation occurred here, where decreased MEPP frequency was associated with increased evoked transmitter release. One possible explanation would be the expansion of nerveterminal branching that has been reported with aging (9). This could possibly lead to both an increased quantal content and a decrease in MEPP frequency. There is support for the idea that acetylcholine efflux in response to presynaptic action potentials has been reported to increase at aged nerve terminals (26). The increased safety margin in old muscles supports the suggestion that the increase in evoked transmitter release may be attributed to the more extensive nerve terminal configurations.

During sedentary aging, a selective reduction does occur in the number of functional motor units (18, 29), increasing the demand on the remaining ones. If true, with exercise training imposing a routine activity pattern on the muscles, exercise could increase the number of firing motor units and/or their frequency of discharge. With more motor units being actively recruited, there is less variability in the distribution among the recruitment patterns of muscle fibers. Alternatively, aging may cause conversion of motor units from fast to slow, and, consequently, the modulation of recruitment thereof must be considered (18, 30). The resulting change in the variability of physical activity is accompanied by corresponding changes in the structure of the nerve terminals. Smaller NMJ in the old exercised mice may result from the reduced capacity of the older animals to adapt to the functional demands of exercise training and/or greater withdrawal of degenerated nerveterminal. Another explanation for the smaller nerve terminals in the older exercised mice may relate to an activity-dependent switch in fiber type from fast-twitch with large arborization to slow-twitch type with compact and simpler configuration (9). The absence of decreased $E_{m}$, of increased muscle $R_{m}$, and of altered rise and decay times of MEPP that are usually common to denervation supports the idea that increased activity may enhance removal of unfunctioning nerve terminals. Furthermore, intrinsic neuronal factors, such as a change in axonal transport or nerve growth factor secretion, may intervene, causing the increased retraction and consequent reactive sprouting of nerve terminal branches. Indeed, brain-derived neurotrophic factors have been reported to cause an increase in the frequency of spontaneous synaptic currents and in the amplitude of evoked transmitter release (27). In this context, the role of an exercise-dependent presynaptic Ca$^{2+}$-activated neuronal prote-
ase in governing the assembly of cytoskeletal elements and terminal remodeling cannot be ruled out (28).

The other possibility revolves around the role of stress and stress hormones with age. Recent work in our laboratory suggests that chronic glucocorticoid treatment of young rats results in NMJ that are morphologically similar to those of older animals (6). The study postulated that stress may play an etiological role in the premature aging of these NMJ. This may partially explain the continued elaboration of nerve terminals in the absence of significant age-related changes in activity. Additionally, stress might partially explain the regenerative effects of exercise.

Adding endurance exercise to the aging condition prevented the age-related increase of quantal content. The physiological responses of GM muscles to exercise parallel the morphological responses and are in agreement with a previous report (2) for exercised 24-mo-old C57BL/6N-na mice. Factors such as hormones, trophic substances, or blood supply might play a role in the exercise-induced reduction of quantal content. Exercise during aging prevented the increase in safety margin seen in sedentary muscle. The similar safety margins in young control and old exercise muscles in the present results can mainly be explained by their similar quantal content. Perhaps the intensity of endurance exer-
cise leads to complete withdrawal of degenerated nerve terminal. Electrical stimulation of motor neurons has been previously reported to affect endplate structure and function (5). Moreover, elevated natural activity (unforced running on wheels) over several months has been reported to cause increased transmitter release and safety margin of synaptic transmission in trained mice (5).

In summary, the aging NMJ undergoes a continual process of remodeling and expansion during normal sedentary cage activity. The experiments reported here show that when a young animal is challenged by an endurance exercise program, the NMJ undergo a process of hypertrophy as a compensatory response. If the exercise training is introduced during old age, the age-related expansion is minimized and modulated at a lower level compared with sedentary controls that are undergoing an age-related expansion. These results provide a quantitative morphological analysis of physiological NMJ parameters that indicate that endurance exercise affects both the physiology and morphology of the NMJ but does so differently, depending on age.

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