Neuromuscular Adaptation to Microgravity Environment

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Abstract: Morphological and/or functional characteristics of skeletal muscles have a greater adaptability in response to changes in environmental stimuli. For example, an atrophy associated with a shift of fiber characteristics toward fast-twitch type is a common adaptation of anti-gravity muscle to a microgravity environment. Neuromuscular responses and possible mechanisms of both neural and muscular adaptations to a microgravity environment are discussed in

this article. Responses of morphological, metabolic, and contractile properties, as well as fiber phenotype, of muscles are briefly reviewed. Discussion is further extended to the patterns of electromyogram and tension development of muscle, responses of postural stability and locomotion, and/or motoneurons in order to study the mechanism for muscular adaptation to microgravity. [Japanese Journal of Physiology, 50, 303–314, 2000]

Key words: atrophy, fiber-type transformation, contractile properties, neural responses, microgravity.

The pattern of muscle activity influences the morphological, metabolic, and contractile properties of skeletal muscles. For example, the metabolic capacity of muscles is affected specifically by the type of exercise training [1–3]. Increased activity or over-loading by removing the synergists causes a compensatory hypertrophy [4–11]. Hypertrophy is also induced by the stretching of matured muscles *in vivo* [12, 13] and cultured myotubes and fibroblasts [14–17]. Exercise-induced metabolic adaptation of muscles is lost when exercise training is stopped [18]. Further, muscle atrophy is induced by denervation [10, 19], spinal cord transection [20], joint immobilization [21–23], tenotomy [24, 25], spaceflight [26–29], and/or hindlimb suspension [29–32]. Most of these models are generally presumed to be models of reduced neuromuscular activity. But the level of use may not be the only factor involved in the atrophic process.

There is a close association in the physiological, biochemical, and morphological properties between a motoneuron and the muscle fibers it innervates [1]. Studies to support this view are those in which chronic electrical stimulation at low frequency (1–10 Hz) changes the properties of fast-twitch muscles toward those of slow-twitch muscles [33–38].

Some of the characteristics of muscle fibers are also altered following cross-innervation [39–42]. Buller *et al.* [40] suggested that the neural influence on muscle could be due to neurotrophic effect as well as via nerve impulses.

Muscular Responses to Gravitational Unloading

Morphological properties

Gravitational unloading by exposure to weightlessness [26–29, 43–47] and/or its simulation model, hindlimb suspension [29–32, 48–56], causes rapid atrophy in muscles, such as soleus and adductor longus, that are composed predominantly of slow fibers. The cross-sectional areas (CSAs) of both slow- and fast-twitch fibers of rats were less after 14 d of spaceflight and hindlimb suspension than those in the age-matched controls [29]. But the degree of atrophy was greater in slow- than fast-twitch fibers [28, 29]. Therefore, muscles composed of predominantly slow fibers are more susceptible to atrophy [26, 28, 47]. The weights of fast-twitch ankle dorsi-flexors, such as the tibialis anterior (TA) and extensor digitorum longus (EDL), are also generally less after unloading than those of age-matched controls [31, 57]. But these were

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not different from the pre-unloading levels, suggesting that these muscles do not undergo atrophy during unloading, although their growth is inhibited.

Our data indicated that various skeletal muscles undergo atrophy regardless of the fiber type composition if either tenotomy, denervation, or both of them are combined with unloading [58, 59]. Further, soleus muscle (Fig. 1, Table 1) or muscle fibers (Fig. 2) do not undergo atrophy following hindlimb suspension if the muscle is stretched by the dorsi-flexion of the

ankle joints [60, 61]. It is suggested that muscle atrophy may be closely related to the muscle length and/or activity levels.

Metabolic properties

In young adult rats, the specific activities of mitochondrial enzymes measured in whole homogenates are generally lower in unloaded muscles than normal

Fig. 1. Absolute (mg) and relative wet weight (to body weight) of rat soleus muscle. Mean $\pm$ SEM. See Table 1 for abbreviations. *, [†], [§] p <0.05 vs. cage control, Susp-Free, and Susp-DF, respectively. Cited from Ohira *et al.* [60].

Fig. 2. Cross-sectional area of rat soleus muscle fibers. Mean $\pm$ SEM. The number of fibers analyzed for each group is shown in parentheses. See Table 1 for abbreviations. *, [†], [§] p <0.05 vs. cage control, Susp-Free, and Susp-DF, respectively. Cited from Ohira *et al.* [60].

Table 1. Responses of body weight, wet weight and contractile properties of the soleus muscle of rats to hindlimb suspension with or without ankle joint immobilization.

	Cage control	Susp-Free	Susp-DF	Susp-PF
BW (g)	295 $\pm$ 6	287 $\pm$ 4	289 $\pm$ 7	285 $\pm$ 8
MW (mg)	127 $\pm$ 5	104 $\pm$ 1*	123 $\pm$ 7	106 $\pm$ 8
MW (%BW $\times 10^{-4}$)	4.30 $\pm$ 0.04	3.62 $\pm$ 0.04*	4.30 $\pm$ 0.08 [†]	3.72 $\pm$ 0.10 ^{*§}
Pt (g)	32.5 $\pm$ 1.5	19.2 $\pm$ 1.1*	34.0 $\pm$ 2.0 [†]	23.0 $\pm$ 1.1
Pt/MW (g/g)	256 $\pm$ 11	185 $\pm$ 6	276 $\pm$ 11 [†]	217 $\pm$ 14
TPT (ms)	41.3 $\pm$ 3.7	35.4 $\pm$ 2.5	52.8 $\pm$ 5.6 [†]	37.5 $\pm$ 1.9 [§]
1/2 RT (ms)	45.3 $\pm$ 3.7	38.4 $\pm$ 2.5*	58.8 $\pm$ 2.6 [†]	40.3 $\pm$ 3.1
dp/dt (g/s)	1,727 $\pm$ 109	1,196 $\pm$ 64*	1,212 $\pm$ 210	1,245 $\pm$ 100
dp/dt/Pt (g/s/g)	96 $\pm$ 2	115 $\pm$ 3*	70 $\pm$ 2 ^{*†}	94 $\pm$ 4 ^{†§}
Po (g)	155 $\pm$ 13	65 $\pm$ 2*	157 $\pm$ 6 [†]	103 $\pm$ 3 ^{*§}
Po/MW (g/g)	1,221 $\pm$ 94	629 $\pm$ 35*	1,276 $\pm$ 106 [†]	967 $\pm$ 88
Pt/Po (%)	21.0 $\pm$ 0.02	29.4 $\pm$ 0.01	21.7 $\pm$ 0.02	22.4 $\pm$ 0.04

Mean $\pm$ SEM. n =6 in each group, except in Susp-Free (n =12). Susp-Free, suspended with free ankle; Susp-DF, suspended with ankle immobilization at dorsi-flexed position; Susp-PF, suspended with ankle immobilization at plantar-flexed position; BW, body weight; MW, muscle weight; Pt, maximum twitch tension; TPT, time-to-peak tension; 1/2 RT, one-half relaxation time; dp/dt, rate of tension development in twitch; and Po, maximum tetanic tension. *, [†], [§] p <0.05 vs. cage control, Susp-Free, and Susp-DF, respectively. Cited from Ohira *et al.* [61].

levels [31, 48, 62–64]. However, the responses of enzyme activities to unloading measured in single muscle fibers suggest that succinate dehydrogenase (SDH) activity is often maintained or even elevated in atrophied muscle fibers [28, 29, 45, 55] (Table 2). These different observations and phenomena may be caused by the greater decrease in muscle fiber size than the remaining constituent of muscle such as connective tissues or interstitial space and by relatively parallel decreases in muscle fiber size and mitochondrial enzyme levels [62, 65–68].

Some observations on fibers of young adult rats following 14 d of spaceflight suggest that subsarcolemmal mitochondria may be preferentially altered [47, 69, 70]. Bell *et al.* [69] have shown that the relative loss of SDH activity in the subsarcolemmal vs. intermyofibril region following spaceflight is fiber-type dependent. In all fibers, the distribution of SDH activity was significantly higher in the subsarcolemmal than intermyofibrillar region. After spaceflight, the entire regional distribution of SDH activity was significantly altered in the slow-twitch oxidative fibers. The fast-twitch oxidative-glycolytic fibers of the spaceflight muscles exhibited significantly lower SDH activity only in their subsarcolemmal region.

These data suggest that it is important to consider the location of the enzyme throughout the cross section of a fiber when the influence of spaceflight on oxidative enzymes in muscle fibers is investigated. Furthermore, the functional properties of the soleus that depend on the metabolic support of mitochondria in the subsarcolemmal region may be primarily affected by exposure to microgravity. It has become evident that the adaptive response of skeletal muscle to spaceflight is different depending upon the types of muscles, muscle fibers, or proteins in a fiber. These findings also suggest that, when considering the influence of spaceflight on oxidative enzymes, it is important to consider how and where those enzymes are distributed within a fiber.

The density of β -adrenoceptors in skeletal muscle is three-fold greater in slow- than fast-twitch fibers [71]. Therefore, it is positively correlated with mitochondrial enzyme activities [72–74]. The dominant subtype of β -adrenoceptors was β_2 [75], and the maximum binding capacity ($B_{\max}$) in soleus muscle was decreased following unloading (Fig. 3). Since the affinity was not influenced, such decrease in $B_{\max}$ was due to decreased receptor number (density). A similar decrease in $B_{\max}$ was induced by loading creatine, which inhibits the ATP turnover rate. Depletion of creatine by supplying creatine analogue β -guanidinopropionic acid, on the contrary, caused an increase in

Fig. 3. Maximum binding capacity of β_2 -adrenoceptors of rat soleus muscle. Mean $\pm$ SEM. * p <0.05 and ** p <0.01 vs. ground control, and † p <0.05 and †† p <0.01 vs. control diet group. Cited from Ohira *et al.* [75].

$B_{\max}$ and ATP turnover rate. These data suggest that the unloading-related decrease in $B_{\max}$ may be associated with an inhibited ATP turnover rate.

Fiber phenotype

Hindlimb unloading causes a progressive decrease in the percent distribution of slow (type I) fibers in the soleus [28, 29, 68], but not in fast muscles such as the medial portion of the gastrocnemius and TA [49, 53, 55]. The results of qualitative histochemical staining of myosin adenosine triphosphatase (ATPase) were compared with the results of immunohistochemical analyses for myosin heavy chain (MHC) expression [29, 53]. The slow and fast fibers were confirmed by rhodamine- and fluorescein-labeled MHC antibody staining, respectively. As is shown in Fig. 4, an increased distribution of the intermediately dark fibers in cross-sections stained for myosin ATPase with alkaline pre-incubation (pH 8.75) was seen in approximately 9–14% of the soleus muscles following 2 weeks of spaceflight and hindlimb suspension [29]. These fibers, for example, No. 3 in flight and hindlimb-suspended muscle in Fig. 4, were also stained dark by acid pre-incubation (pH 4.35). Further, these fibers reacted positively with both fast and slow MHC antibodies.

The decrease in the percent distribution of fibers expressing pure type I MHC following spaceflight was associated with a *de novo* appearance of fibers co-expressing type I MHC and/or various type II MHC (Fig. 5) [76]. The results indicated that the percent distribution of fibers which expressed only slow MHC apparently decreased, and some of the slow fibers began to co-express some fast MHC as well. The ex-

Fig. 4. Muscle fiber types of rat soleus muscle. Myosin adenosine triphosphatase (ATPase) and myosin heavy chain (MHC) antibody staining of one control (left), one spaceflight (middle), and one hindlimb-suspended (right) rat soleus. **a:** Myosin ATPase, alkaline pre-incubation (pH 8.75) staining. **b:** Myosin ATPase, acid pre-incubation (pH 4.35) staining. **c:** Fluorescein-labeled fast MHC antibody staining. **d:** Rhodamine-labeled slow MHC antibody staining. Fiber labeled No. 1 reacted exclusively with slow antibody, and fiber labeled No. 2 reacted exclusively with fast antibody. Some fibers cross-reacted with both fast and slow antibodies, e.g., fiber labeled No. 3. Bar, 100 μ m. Cited from Ohira *et al.* [29].

Fig. 5. Percent distribution of fibers expressing particular myosin heavy chain (MHC) isoforms in rat soleus muscle. Fibers co-expressing various MHC including neonatal (neo) MHC, which were not observed in the control muscle, were noted in the muscles of the spaceflight group. * $p < 0.05$, ** $p < 0.005$, and † $p < 0.001$ vs. control group. Cited from Allen *et al.* [76].

pression of IId MHC (most likely analogous to IIx) has also been observed in the rat soleus of 21- and 28-d suspended rats [67]. A similar tendency in fiber type transformation was also seen in the human vastus lateralis muscle after 11 d of spaceflight [77, 78] and in the soleus after 4 months of bedrest associated with fiber atrophy [79, 80].

The activity (mean $\pm$ SEM) of myosin ATPase in the dark (fast) fibers of control rats ($29.8 \pm 2.9 \times 10^{-3}$, Δ OD/min) measured quantitatively was significantly greater than in the light (slow) fibers ($15.8 \pm 1.4 \times 10^{-3}$, $p < 0.01$, Table 2) [29]. However, that in the intermediate fibers ($16.7 \pm 1.7 \times 10^{-3}$) was similar to the level of slow fibers. The activities (Δ OD/min) of SDH and α -glycerophosphate dehydrogenase (GPD) also tended to be greater in dark than light fibers. Further, the SDH and GPD activities of the fibers that expressed both slow and fast MHC tended to be intermediate. These data also suggest that the shift in MHC expression resulted from a relative increase of fast characteristics due to the loss of slow MHC expression, although the absolute level of fast MHC expression may not have been affected dramatically. Although 9–14% of the fibers expressed both slow and fast MHC after unloading, the remaining slow fibers

Table 2. Enzyme activities and size of soleus muscle fibers in control and 14-d spaceflight (Flight) and hindlimb-suspended (Suspension) rats.

Fiber type	Control	Flight	% Δ	Suspension	% Δ
All fibers					
<i>n</i>	450	471		479	
SDH	38.5 $\pm$ 6.8	28.0 $\pm$ 4.8	-27	37.0 $\pm$ 4.5	-4
GPD	0.6 $\pm$ 0.3	0.6 $\pm$ 0.3	0	1.5 $\pm$ 0.6	+150
ATPase	18.6 $\pm$ 3.1	18.6 $\pm$ 3.7	0	19.2 $\pm$ 3.9	+3
CSA	3,261 $\pm$ 398	2,261 $\pm$ 350*	-31	1,848 $\pm$ 293	-43
GPD/SDH	13 $\pm$ 6	18 $\pm$ 9	+38	43 $\pm$ 17	+230
GPD/ATPase	28 $\pm$ 14	28 $\pm$ 14	0	74 $\pm$ 21*,†	+164
ATPase/SDH	507 $\pm$ 61	690 $\pm$ 117	+36	548 $\pm$ 122	+8
% Slow fibers	79.8	66.2	-17	69.5	-15
<i>n</i>	359	312		333	
SDH	33.8 $\pm$ 4.8	25.1 $\pm$ 3.6	-26	34.0 $\pm$ 3.8	+1
GPD	0.4 $\pm$ 0.2	0.4 $\pm$ 0.2	0	0.9 $\pm$ 0.3	+125
ATPase	15.8 $\pm$ 1.4	16.6 $\pm$ 3.1	+5	15.1 $\pm$ 1.8	-4
CSA	3,442 $\pm$ 382	2,407 $\pm$ 327*	-30	1,933 $\pm$ 309*	-44
GPD/SDH	10 $\pm$ 5	14 $\pm$ 8	+40	31 $\pm$ 11	+210
GPD/ATPase	24 $\pm$ 12	21 $\pm$ 11	-13	62 $\pm$ 20*,†	+158
ATPase/SDH	499 $\pm$ 62	686 $\pm$ 118	+37	485 $\pm$ 88	-3
% Fast fibers	19.8	19.7	-1	23.2	+17
<i>n</i>	89	93		111	
SDH	57.0 $\pm$ 6.9	37.7 $\pm$ 5.9	-34	45.3 $\pm$ 4.6	-21
GPD	1.4 $\pm$ 0.5	1.1 $\pm$ 0.3	-21	3.1 $\pm$ 0.7†	+121
ATPase	29.8 $\pm$ 2.9	27.8 $\pm$ 3.5	-7	31.9 $\pm$ 3.6	+7
CSA	2,558 $\pm$ 289	2,156 $\pm$ 326	-16	1,616 $\pm$ 247*	-37
GPD/SDH	24 $\pm$ 7	28 $\pm$ 8	+17	77 $\pm$ 24	+220
GPD/ATPase	46 $\pm$ 13	38 $\pm$ 12	-17	99 $\pm$ 20*,†	+115
ATPase/SDH	541 $\pm$ 51	785 $\pm$ 99	+45	760 $\pm$ 135	+40
% S-F fibers‡	0.4	14.1	+3425	7.3	+1,725
<i>n</i>	2	66		35	
SDH	54.0 $\pm$ 12.6	28.3 $\pm$ 4.1	-47	38.8 $\pm$ 4.3	-28
GPD	0.9 $\pm$ 0.3	0.6 $\pm$ 0.3	-33	1.8 $\pm$ 0.4†	+100
ATPase	16.7 $\pm$ 1.7	15.0 $\pm$ 2.1	-10	17.6 $\pm$ 1.4	+5
CSA	2,315 $\pm$ 108	1,722 $\pm$ 328	-24	1,769 $\pm$ 177	-24
GPD/SDH	14 $\pm$ 3	22 $\pm$ 13	+57	52 $\pm$ 13	+271
GPD/ATPase	47 $\pm$ 15	43 $\pm$ 21	-9	104 $\pm$ 15†	+121
ATPase/SDH	337 $\pm$ 47	574 $\pm$ 49	+70	478 $\pm$ 61	+42

Mean $\pm$ SEM. *n*, number of fibers analyzed. CSA, cross-sectional area, μm^2 ; SDH, succinate dehydrogenase activity, changes (Δ) in optical density (OD)/min $\times 10^{-3}$; GPD, α -glycerophosphate dehydrogenase activity, $\Delta\text{OD}/\text{min}\times 10^{-3}$; ATPase, adenosine triphosphatase activity, $\Delta\text{OD}/\text{min}\times 10^{-3}$; GPD/SDH, $\times 10^{-3}$; GPD/ATPase, $\times 10^{-3}$; ATPase/SDH, $\times 10^{-3}$. * $p < 0.05$ vs. control, † $p < 0.05$ between flight and suspended rats. ‡Fibers that expressed both slow and fast myosin heavy chain. Cited from Ohira *et al.* [29].

were unchanged even though they also atrophied. It is not clear why some slow fibers respond differently. Some possibilities are that the slow fibers that remained unchanged do not possess the fast MHC expression genetically, or that slow MHC expression in these fibers do not respond to unloading.

Contractile properties

Following the induction of atrophy, the magnitude of decrease in the maximum tetanic tension is generally greater than that in muscle mass [32, 64, 81, 82]. Thus, the specific tension per unit weight or CSA is lowered. Such phenomena may be due to the greater decrease in the concentration of myofibrillar protein [56, 83] and the relative increase in the non-contractile tissue [62, 65, 66, 68] and interstitial volume [84]. The decrease in the specific tension of single fibers is only half of that in whole muscle [32, 65, 81, 85]. The re-

sults reported by Stevens *et al.* [86] showed that the specific tension of skinned soleus fibers in rats suspended for 15 d was similar to cage control. The specific tension in predominantly fast muscles such as medial gastrocnemius [32, 65, 81], TA [32], and EDL [85] are not influenced by suspension and may even be increased [87].

The speed-related properties are shifted toward fast-type in slow soleus, although fast muscles are unaffected by unloading [32, 65, 68, 81, 85, 88]. The time-to-peak tension and one-half relaxation time are decreased (Table 1) [61], possibly due to changes in sarcoplasmic reticulum kinetics. The maximum shortening velocity of whole muscle [32, 65, 81, 85] and single fiber [89, 90] is increased following unloading. The level of myosin ATPase activity is greater in fast than slow fibers [29, 91]. However, the change in myosin ATPase activity is relatively minor even in

muscle with increased shortening velocity [29, 56, 88].

The fatigue resistance remains remarkably high after chronic unloading even in the soleus muscle [32, 65, 81, 92], although it seems to be affected more after a prolonged fatigue test [93]. The fatigability of fast muscles is moderately affected in response to suspension [32, 65, 81, 92]. The maintenance of fatigue resistance in atrophied muscles may be attributable to, in part, (1) lowered absolute tension production, (2) relatively stable oxidative enzyme levels in fibers, and/or (3) shorter diffusion distance to the center of fibers due to decreased CSA.

Muscle Activity during Gravitational Unloading

Electromyogram

The electromyogram (EMG) of the soleus and medial gastrocnemius disappears immediately in response to hindlimb suspension of rats [94, 95]. Furthermore, the total amount of daily EMG activity in these muscles is significantly reduced on the day of suspension. However, the EMG activity is gradually increased and is similar to the control level after 7–10 d of suspension. It is maintained near-normal thereafter, even though muscle atrophy is induced [32]. These studies suggest that the cause of the atrophy can not be attributed simply to disuse of the muscle. The daily EMG activity of ankle flexor TA, which is not subjected to atrophy, was above normal during the suspension.

As is indicated above, muscle shortening caused an immediate disappearance of EMG, and the EMG activity gradually recovered even though a shortened muscle length was maintained [95]. On the contrary,

EMG activity was maintained during hindlimb suspension if the muscle was stretched. No suspension-caused atrophy was induced in the stretched soleus (Figs. 1 and 2, Table 1). These results suggest that one of the causes of unloading-related atrophy of skeletal muscle is reduced muscle length, but not EMG activity. The length of TA even increased slightly during hindlimb suspension.

The tonic EMG activity in human soleus, which accounts for about 80% of plantar-flexor torque, is reduced during spaceflight, whereas that in TA, dorsiflexor, is increased during a postural test such as standing erect [96–98]. Such reversal in the roles of ankle extensors and flexors has also been seen during parabolic flight in humans [96] and monkeys after 14 d of space flight [99]. Although more motor units in the soleus than gastrocnemius are recruited in human when plantar-flexion is performed in upright position in 1-*g* environment, the degree of recruitment was reversed when the same task was performed in the head-down position [100]. The shift of EMG activity in rat soleus from “tonic to phasic” was also seen following hindlimb suspension [101]. These studies suggest that changes in gravitational loading have significant effects on the neuromuscular system.

Tension development

Another contributing factor in the regulation of muscle mass seems to be the chronic tension that it produces or is imposed on it. During hindlimb suspension, the ankle joints of rats are often extended [95, 101] so that the length of the ankle extensor is passively shortened. For example, the length of soleus muscle of a rat having a body weight of 318 g, excluding the tendons, was approximately 28 and 20 mm

Fig. 6. Isometric force production and electromyogram (EMG) in rat plantaris muscle with various ankle joint angles. I EMG, integrated EMG. Cited from Ohira *et al.* [95] and Ohira and Edgerton [102].

when the angle of the ankle joint was fixed at $\sim 50^\circ$ and $\sim 160^\circ$, respectively. Tension production was measured by placing a buckle force transducer on the distal tendon of the plantaris and the EMG recorded [95, 102]. When the plantaris muscle was stretched keeping the anterior angle of the ankle joint at $\sim 30^\circ$, a greater tension was produced when the EMG was present (Fig. 6). However, no tension was developed when the muscle was passively shortened by fixing the joint angle at $\sim 160^\circ$, which is approximately equivalent to the angle of the freely suspended ankle. Tension was produced when the ankle joint was fixed at $\sim 90^\circ$.

The wet weights of ankle flexors, TA and EDL, suspended for 10 d were identical to the pre-suspension controls, but were less than the age-matched controls [103]. This suggested that these muscles were not subjected to atrophy but the growth rate was inhibited by hindlimb suspension. However, atrophy was induced if the ankle joint was maintained in a dorsi-flexed position [103, 104]. Thus, it is suggested that the length or degree of loading of ankle flexors was also reduced by dorsi-flexion. These results indicate an important role of tension development for the maintenance of muscle mass regardless of the type of muscle. Although there have been numerous experiments which have demonstrated the importance of muscle length on the degree of atrophy or hypertrophy, no experiments have recorded the force imposed on a specific muscle as a result of changing the angle of ankle joint.

Neural Responses to Microgravity

Postural stability and locomotion

Microgravity results in significant changes to the physiological and biomechanical characteristics of an organism [105]. Perhaps the most pervasive change is the loss of sensory activities from the otolith organs and sensory receptors responding to loading produced by body weight under normal gravitational conditions. Such changes can be expected to contribute to modifications of motor behavior during weightlessness which result in altered patterns of muscle activity. For example, there are decreased levels of soleus activity and elevated levels of TA activity [98, 106] and diminished H-reflex excitability after vestibular stimulation in the medial gastrocnemius muscle during spaceflight [107]. Furthermore, there appears to be an adaptation of the H-reflex response to vestibular stimulation throughout the duration of spaceflight, resulting in an increased response on return to normal gravity, which takes many days to return to preflight levels [107].

The results obtained in one spaceflight rhesus mon-

key from the COSMOS 2044 flight indicated a significant reorganization of recruitment patterns of slow and fast ankle extensor muscles following 14 d of spaceflight [99]. Upon return to 1-*g* environment, there appears to be increased activation of the medial gastrocnemius muscle relative to the soleus muscle, suggesting that spaceflight resulted in an adaptation of the motor system which persisted for several days after returning to normal gravity. This adaptation was evident 5 d after flight but the recruitment patterns appeared to return to their normal levels within 2 weeks.

Postural stability of the Sky Lab crew members was found to be particularly compromised after spaceflight when they closed their eyes [108]. Similar phenomena were seen after a 18-d Soyuz-9 mission [109, 110]. Such effects were marked immediately after flight but were normalized after approximately 10 d. After 140 and 185 d of spaceflight, Kozlovskaya *et al.* [105] found that the ratio of EMG amplitude to the perturbation force during standing posture was more than double compared to the pre-flight level. They also reported that the time taken for balance recovery after external disturbances increased, the thresholds of corrective EMG responses decreased, and the EMG amplitudes and duration were longer than before flight.

The recruitment patterns of the ankle dorsi- (TA) and plantar-flexors (soleus and medial gastrocnemius) are analyzed during the specific task in rats [111], cats [112] and humans [113]. Changes in the gravitational environment may alter the recruitment patterns of these muscles. Further, any changes in recruitment patterns during spaceflight may result in readjustments of the motor system which may persist for a few days after flight since significant differences in human proprioceptive reflexes can persist for days even after relatively short spaceflights of 7 to 14 d [114].

Disturbance of locomotion, such as swimming performance, posture balance, air-righting reflex, or treadmill walking, and increased EMG activity in TA are seen in neonatal and adult rats following hindlimb suspension [115–119]. Negative effects of 9 weeks of hindlimb suspension on motor performance in adult rats were irreversible for at least 8 weeks [115]. The most severe effects were induced by suspension 8–13 d after birth. Further, such changes did not recover even after 1 month, suggesting that influence of unloading at a critical age may be irreversible. These results suggest that gravitational loading is essential for the maintenance of neuromuscular performance in adult rats and for normal neural development when new-born rats learn motor skills during the first weeks of life.

At birth, the level of differentiation of fiber types in the rat soleus is rather incomplete [120]. After birth, the fiber phenotypes gradually change and the speed-related contractile properties are shifted toward slower type during the first 4 weeks, although the speed of contraction increases again between the 5th and 16th week, or by 1 year [121]. It is reported that the fast-type characteristics of EDL are gained at 21 d of age [122] and the speed of contraction is further slowed down slightly at the age of 1 year [123]. Elder and McComas [124] reported that muscle activity has an important role in the development of slow-twitch muscles. Unloading-related changes of muscle properties, such as fiber type, contractile properties, or atrophy, may also have some effects on motor performance besides neural factors.

Motoneuron

The effects of 14 d of spaceflight and 9 d of recovery on cell body size as assessed by CSA and SDH activity of ventral horn neurons and dorsal root ganglion neurons at the L₅ segmental level of the spinal cord in adult rats were studied [125, 126]. The results were compared to those in age-matched, ground-based control rats. Spinal cord was quick-frozen, and the SDH activity and CSA of a sample of neurons with visible nuclei were determined using a computer-assisted image analysis system. An inverse relationship between CSA and SDH activity of ventral horn neurons was observed in all groups of rats. No change in the mean CSA, mean SDH activity or size distribution of neurons was observed following spaceflight or recovery. However, there was a selective decrease in the SDH activity of neurons with soma CSA between 500 and 800 μm^2 in the spaceflight rats, and this effect persisted for at least 9 d after returning to 1-*g* [125].

In the dorsal root ganglion neurons, the mean SDH activity was significantly lower in spaceflight rats compared to the aged-matched control rats, whereas the mean SDH was similar in the age-matched control and spaceflight-plus-recovery rats [126]. The mean SDH activity of neurons with CSA between 1,000 and 2,000 μm^2 was lower (between 7 and 10%) in both the spaceflight and spaceflight-plus-recovery groups compared to the respective control groups. The reduction in the oxidative capacity of a subpopulation of sensory neurons having relatively large CSA immediately following spaceflight and the sustained depression for 9 d after returning to 1-*g* suggest that the microgravity environment induced significant alterations in proprioceptive function.

Afferent input

Compensatory hypertrophy in plantaris following the tenotomy of gastrocnemius does not occur if deafferentation is performed [127], suggesting an important role of afferent input for the induction of hypertrophy. Further, a similar degree of atrophy was induced in the gastrocnemius by tenotomy and deafferentation. Since our results suggest that afferent input may be reduced if the muscle is shortened during hindlimb suspension [128], tenotomy may be similar functionally to deafferentation by dorsal root transection. Although the plantaris EMG and efferent neurogram were maintained during 3 d of hindlimb suspension, the magnitude of afferent neurogram tended to be reduced. These results also indicated the involvement of afferent input in the regulation of muscle mass.

CONCLUSION

Neuromuscular responses and possible mechanisms for adaptation to microgravity environment were briefly reviewed. Atrophy associated with a shift of muscle fiber characteristics toward fast-twitch type is a well-known phenomena observed in antigravity muscle. A prominent atrophy is induced in slow-twitch fibers, even though the growth of fast-twitch fibers is also inhibited by gravitational unloading. Therefore, unloading-related atrophy is induced mainly in muscle composed predominantly of slow-twitch fibers. Gravitational unloading causes a decrease in the percent distribution of fibers expressing pure type I MHC and an increase in the percent of hybrid fibers co-expressing various types of MHC. Therefore, the speed-related contractile properties are shifted toward the fast-twitch type.

The EMG activity disappears instantaneously in response to unloading. Although the EMG activity gradually recovers during unloading, muscle atrophy is still induced. The EMG remains active and atrophy and fiber transformation can be prevented if the muscle is stretched. Therefore, the adaptation of muscle to unloading may be related to shortened muscle length, which inhibits tension development, not necessarily to EMG-related muscular activity. Gravitational unloading also causes some detrimental effects on motor performance. Decreased SDH activity is observed in some motoneurons of rats after spaceflight. An important role of afferent input on the plasticity of muscle is also suggested. These findings indicate a close association of neural and muscular factors for morphological and functional adaptation to a microgravity environment.

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