Effects of Spaceflight on Rhesus Quadrupedal Locomotion After Return to 1G

MATTHEW R. RECKTENWALD, JOHN A. HODGSON, ROLAND R. ROY, SERGEY RIAZANSKI, GARY E. MCCALL, INESSA KOZLOVSKAYA, DAVID A. WASHBURN, JOHN W. FANTON, and V. REGGIE EDGERTON. Effects of spaceflight on Rhesus quadrupedal locomotion after return to 1G. J. Neurophysiol. 81: 2451–2463, 1999. Locomotor performance, activation patterns of the soleus (Sol), medial gastrocnemius (MG), vastus lateralis (VL), and tibialis anterior (TA) and MG tendon force during quadrupedal stepping were studied in adult Rhesus before and after 14 days of either spaceflight (n = 2) or flight simulation at 1G (n = 3). Flight simulation involved duplication of the spaceflight conditions and experimental protocol in a 1G environment. Postflight, but not postsimulation, electromyographic (EMG) recordings revealed clonus-like activity in all muscles. Compared with preflight, the cycle period and burst durations of the primary extensors (Sol, MG, and VL) tended to decrease postflight. These decreases were associated with shorter steps. The flexor (TA) EMG burst duration postflight was similar to preflight, whereas the burst amplitude was elevated. Consequently, the Sol:TA and MG:TA EMG amplitude ratios were lower following flight, reflecting a “flexor bias.” Together, these alterations in mean EMG amplitudes reflect differential adaptations in motor-unit recruitment patterns of flexors and extensors as well as fast and slow motor pools. Shorter cycle period and burst durations persisted throughout the 20-day postflight testing period, whereas mean EMG returned to preflight levels by 17 days postflight. Compared with presimulation, the simulation group showed slight increases in the cycle period and burst durations of all muscles. Mean EMG amplitude decreased in the Sol, increased in the MG and VL, and was unchanged in the TA. Thus adaptations observed postsimulation were different from those observed postflight, indicating that there was a response unique to the microgravity environment, i.e., the modulations in the nervous system controlling locomotion cannot merely be attributed to restriction of movement but appear to be the result of changes in the interpretation of load-related proprioceptive feedback to the nervous system. Peak MG tendon force amplitudes were approximately two times greater post- compared with preflight or presimulation. Adaptations in tendon force and EMG amplitude ratios indicate that the nervous system undergoes a reorganization of the recruitment patterns biased toward an increased recruitment of fast versus slow motor units and flexor versus extensor muscles. Combined, these data indicate that some details of the control of motor pools during locomotion are dependent on the persistence of Earth’s gravitational environment.

INTRODUCTION

The extent that the differential patterns of skeletal muscle activation may reflect the neuromotor properties that develop normally in a 1G environment or the physiology resulting from the evolution of these neural networks in a 1G environment has not been examined extensively. We designed an experiment to determine whether the patterns of activation of motor pools involved in locomotion in the normal adult Rhesus can be modulated by exposure to a microgravity environment for 2 wk. Activation patterns of selected motoneuronal pools that differ in their gravitational dependence were compared. For example, the chronic load-bearing effect imposed at 1G during locomotion requires that those muscles generally characterized as extensors must sustain a standing and locomotor position to support some proportion of the total weight of the body. During standing there is less of a requirement for flexor muscle activity, but during the swing phase of locomotion the lower limbs must be moved against the force of gravity. In a non-loading posture at 1G, or when exposed to 0G, the functional demands of these extensor and flexor motor pools clearly are different. Further, even among extensor muscles the relative function of slow and fast muscles at 1G differ substantially, with the slow muscles often referred to as “antigravity muscles.” Because of the theoretical differences in how these motor pools function at 1G compared with 0G, the activation patterns of “flexor” versus “extensor” muscles and of fast versus slow motor pools in response to chronic exposure to microgravity were studied.

Although normal quadrupedal nonhuman primate locomotion has been studied in a 1G environment (Hurov 1985; Reynolds 1985; Vilensky 1980, 1983), no studies have focused on the ability to control locomotion when returning from microgravity to a 1G environment. In the present study, the recruitment patterns of primary extensor and flexor muscles in the legs during quadrupedal treadmill locomotion were studied before and after 2 wk of spaceflight (BION 11). Locomotor ability was studied before and after a similar period of flight simulation (ground-based experiments during which the Rhesus were maintained in flight chairs for the same period of time as the flight) to distinguish the effects of microgravity from the lack of weight bearing associated with sitting in the flight chair. These data address the issue of whether particular patterns of activation of selected motor pools generally associated with posture and locomotion at 1G reflect innate basic design prop-
erties of the locomotor system versus the properties of a locomotor system that adapted postnatally to the Earth’s gravitational vector.

**Methods**

**Experimental design**

The BION 11 flight mission was one of a series originally formulated by the Russian Space Program to study the role of gravitational factors on a variety of physiological systems. The present studies of locomotor ability represented one of several experiments focusing on issues of motor control. Studies of the physiology of nonhuman primates provided a number of advantages, some of which were the ability to record a large number of physiological variables using chronically implantable devices under more controlled and complex paradigms than is feasible in humans or rats; the monkeys could be trained to perform complex coordination tasks involving the hands, eyes, and head as well as the legs; and the evolutionary proximity of nonhuman primates with humans.

The experiments were part of an extensive study of the effects of a 14-day spaceflight on a number of physiological functions in two Rhesus. The success of initial behavioral training was used as a criterion to select 12 animals as potential flight candidates to be implanted with physiological sensors. These experiments followed the Principles of Laboratory Animal Care (National Institutes of Health Publication 85-23, revised 1985) and were approved by the Institutional Animal Care and Use Committee (IACUC). During the year of testing, body weights in the 12 implanted animals increased from 2.94 ± 0.08 to 4.54 ± 0.16 (SE) kg.

**EMG and tendon force transducer implantation procedures**

Twelve clinically normal, juvenile, male Rhesus monkeys (*Macaca mulatta*) were implanted surgically with bipolar intramuscular EMG electrodes, tendon force transducers and other recording devices 6–7 mo before launch. The monkeys were housed individually in standard 4.3 ft² stainless steel cages at the Institute of Biomedical Problems, Moscow (IMBP). Before any surgical procedures, the monkeys were trained to wear a specially designed jacket that would protect exteriorized instrumentation. EMG electrode and tendon force transducer configurations, similar to those described by Sherif et al. (1983), were purchased from a commercial source (Konigsberg Instruments, Pasadena, CA). EMG implants were manufactured from teflon-coated multistrand stainless steel wire (32 gauge; Cooner Wire, Chatsworth, CA). The tendon force transducer was an oval-shaped titanium buckle, 17 mm long, 7.5 mm wide and 3 mm thick with a separate cross piece. Silicon strain gauge sensors were located in a hermetically sealed cavity in the buckle. Wires from the tendon force transducer and those for the EMG implants were embedded in silicon rubber in 1.5 mm silicon rubber tubes terminating in small multipin connectors, which were capped when not being used for recording.

All surgical procedures were performed at the IMBP and used the following anesthesia regimen. Preoperative management consisted of food and water restriction for 14 h. Induction of anesthesia was with ketamine HCl (10 mg/kg im). Atropine sulfate (0.04 mg/kg iv) was administered during induction to prevent intraoperative bradycardia. Anesthesia was maintained with isoflurane gas (1.25%) in 100% oxygen delivered via a cuffed orotracheal tube. Intraoperatively, a dedicated nurse anesthetist monitored heart rate, core body temperature, respiratory rate, respiratory pressure, and tidal volume. Lactated Ringer solution (10 ml·kg⁻¹·h⁻¹) was administered at a continuous rate infusion for the duration of anesthesia. A 4-cm incision was made at the midline of the upper back on line with the caudal border of the scapula. Skin incisions (2–4 cm) were made over the bellies of the vastus lateralis (VL), the triceps surae and the tibialis anterior (TA). The bellies of the VL, medial gastrocnemius (MG), soleus (Sol), and TA in the left leg were exposed as clearly as possible. The distal tendon of the MG was separated from the remainder of the Achilles tendon. The EMG and tendon force transducer cables were passed through the skin on the left midback via small incisions made using a sharp scalpel and fine forceps. Using a smooth, Teflon rod, the tendon force transducer and the EMG wires then were routed subcutaneously from the back to the appropriate locations in the hindlimb. Bipolar intramuscular EMG electrodes were inserted into the mid-belly of the Sol, the medial, deep region of the MG, the mid-belly of the TA, and the lateral, deep region of the VL as described previously in rats (Roy et al. 1991b) and cats (Pierotti et al. 1989). A suture was looped around the MG tendon and used to pull the tendon through the buckle. A small hemostat then was used to grip the cross piece and insert it through the loop in the tendon. Tension in the tendon held the cross piece in place. The tendon force transducer cable and the EMG wires were coiled near the implant site to provide stress relief. To provide further stress relief, the tendon force transducer and EMG cables were coiled in the back region and anchored with sutures to the deep fascia. All incision areas were irrigated liberally with 0.1% povidone iodine in saline and closed in layers, i.e., investing fascia and then the skin. The cables then were anchored firmly exteriorly using mattress stitches. All closed incision sites were cleansed thoroughly with the betadine and saline solution.

After wound closure, a canvas jacket was placed on the monkey to prevent access to the external cable. Postoperative care consisted of intensive monitoring until the monkeys regained their equilibrium and were able to sit upright. Analgesia was provided by butorphanol HCl (0.5–1.0 mg/kg im). The monkeys were monitored closely for caloric intake and were supplemented liberally with fresh fruit on a daily basis. Antibiotic therapy with cephapirin sodium (10 mg/kg im) was initiated preoperatively and continued for 3 days. Wound healing was monitored closely at 48-h intervals. Initial care immediately after surgery consisted of monitoring the transected muscle exit sites for erythema or exudation. If deterioration of the exit sites was noted, topical therapy with dilute povidone iodine (0.1%) in saline was instituted. Systemic antibiotic therapy with cephapirin sodium (10 mg/kg im) or enrofloxacin (5 mg/kg im) was used if exit sites were considered to be infected.

**Treadmill training protocol**

The Rhesus were trained on a motorized treadmill with a plexiglass cover to maintain the animal in position while allowing video recording of the movements. The initial training session was used to acclimatize the animals to the treadmill environment without the belt moving. Subsequent sessions were used to train the animals to locomote consistently, and each animal was trained for approximately six sessions before any locomotor data were collected. All animals were trained to walk quadrupedally at speeds of 0.45, 0.89, 1.34, and 1.79 m/s. All pre- and postflight locomotion comparisons were based on quadrupedal locomotion, i.e., their preferred form of locomotion (Ishida et al. 1985; Iwamoto 1985; Kimura 1985). Each training session consisted of eight locomotor trials (2 repetitions of each speed) with at least a 2-min rest interval between each trial. Each session lasted ~20 min. Additional training sessions were performed on occasion to maintain the performance and comfort of the Rhesus on the treadmill. Food pellets, fruit, or nuts were used as rewards after each locomotor trial.

**Selection of flight subjects**

Each investigator participating in the BION 11 experiment prioritized the animals as flight candidates according to their own requirements. Animal selection then was made based on maximizing the scientific return, and six animals were identified as subjects that would provide a high probability of success for most investigators. These animals were transported to the launch facility by train, where further
preflight testing was performed. Based on these tests, two Rhesus from this group (357 and 484) were selected for flight. Approximately 3 days before launch, the monkeys were placed into form-fitting flight chairs and lightly secured with a chest harness attached to the back of the flight chair to limit leaning forward at the waist. The chair limited lateral movement of the legs in the transverse plane. Additionally, a lap-plate was positioned over the thighs of the monkeys which limited movement of the leg at the hip joint in the sagittal plane. The lower legs were unsecured. The monkeys then were installed in the satellite and the rocket was assembled and prepared for launch.

After launch the remaining four Rhesus were returned to the test facility at IMBP where they were used as ground-based simulation controls. Two animals (396 and 447) were placed in a mock-up flight capsule simulating flight conditions, i.e., temperature, etc., and testing conditions were initiated with a 4-day delay relative to the flight capsule. During the 31 days before the flight simulation experiments, monkeys 501 and 534 were treated similarly except that the simulation period began 17 days after the recovery of the flight capsule. During the 31 days before the flight simulation experiments, monkeys 501 and 534 were maintained under normal preflight conditions, i.e., housed in their cages and tested on the treadmill periodically. This longer delay allowed for the flight conditions to be more accurately simulated for these latter two Rhesus.

The flight simulation test was 17 days, simulating the 3-day prelaunch and the 14-day flight experienced by the flight Rhesus. The flight simulation was therefore able to simulate a flight period of 31 days. The monkeys were placed in simulated flight chairs and transported by helicopter to an airfield (1.5–2 h) and put on plane for Moscow (60 cycles/s) were made using a camera oriented perpendicular to the direction of the locomotion (Panasonic System Camera, WV DS100; Panasonic AG1280P; Panasonic, Cypress, CA).

Before each testing session, a calibration device was placed in the treadmill and recorded. Reflective markers were attached to the greater trochanter at the hip, the knee joint, the maleolus at the ankle, the heel and the distal metatarsal of the fifth digit at the foot. A Society of Motion Picture and Television Engineers (SMPTE) time code generator (model F30, Fast Forward Video, Irvine, CA) was used to synchronize video frames with the EMG signals recorded on FM tape.

The video recordings were viewed to select good sequences of quadrupedal locomotion, i.e., successful, consecutive steps when the monkey was maintaining a relatively constant position on the treadmill. Most of the analyses were performed on these selected steps. However, to determine the mean amplitude adaptations regardless of changes in ground speed associated with forward and backward movements on the treadmill, some burst analyses were done on all postflight steps (see Figs. 6 and 7). The EMG and tendon force signals were sampled into an Amiga computer at 2 kHz. The EMG signals were rectified and smoothed using a 25-point moving average, i.e., a 40-Hz low-pass filter. Computer software designed in-house was used to detect and display the start and end of each EMG burst based on a given threshold level above the baseline noise for each channel. The starting and ending points of the EMG bursts were used to determine the relative timing of EMG activity recorded from different muscles. Cycle period was calculated as the time between the starting points of successive bursts of EMG activity in the Sol muscle. Burst duration was calculated as the time between the start and the end of an individual burst. Mean EMG amplitude was calculated as the integrated area of each unsmoothed burst divided by the burst duration. EMG activity corresponding to poor quadrupedal stepping, i.e., the monkey not taking continuous steps, hopping, jumping, or stepping bipedally, was excluded from the analyses. Tendon force data were calculated using the mean peak force level for a sequence of steps. The raw force trace was smoothed using a 25-point average, then the mean values were calculated using the peak force for each step within a step sequence. Converting the force from computer to force (kg) units was accomplished using conversion factors obtained during the tendon force calibration procedures as described in the following text.

Probability density distributions of EMG amplitudes of pairs of muscles in five successful, consecutive, quadrupedal steps were plotted for each muscle pair. A complete description of this analysis procedure can be found elsewhere (Hutchison et al. 1989). Briefly, the EMG signals of two muscles were analyzed simultaneously. Average amplitudes calculated every 40 ms for the two EMGs were used to target x and y coordinates of a bin in a two-dimensional grid, which then was incremented. The relative number of counts in each bin defined the probability of particular relationships occurring between pairs of muscles. The distribution of counts then was plotted as a three-dimensional landscape where the highest elevations (vertical axis) represented the most commonly observed relationships and therefore the highest probabilities.

Data collection and analysis

A telemeter (Konigsberg Instruments) was attached to the EMG and force transducer cables and placed in a large pouch on the back of the monkey’s jacket. The telemeters weighed ±150 g and did not appear to interfere with the performance of the locomotor task. EMG band-pass was 30 Hz to 1 kHz, and the force transducer band-pass was DC-20 Hz. Output from the telemeter receiver was recorded on FM tape (TEAC Model XR-510, TEAC, Montebello, CA). Video recordings (60 cycles/s) were made using a camera oriented perpendicular to the direction of the locomotion (Panasonic System Camera, WV DS100; Panasonic AG1280P; Panasonic, Cypress, CA).

The recovery team reached the landing site of the flight capsule within 30 min of its return to Earth. Within 1 h, a heated inflatable tent with engineering and surgery rooms was erected to house the monkeys during their physical examinations. While the tent was being constructed, engineers were preparing to remove the monkeys from the capsule with the monkeys in their flight chairs. Within ~2 h of landing the first monkey was removed from its chair and transferred to an examination room. After the initial health examination, the monkeys were placed in simulated flight chairs and transported by helicopter to an airfield (1.5–2 h) and put on plane for Moscow (~6 h flight). Ground transportation at the Moscow airport then transported the monkeys to the IMBP where the monkeys remained in their simulation chairs for the remainder of the night (~14 h). Locomotor tests were performed in the morning within 1 h of their release from the chairs and 24 h after having returned to Earth.

Testing protocol

All flight and simulation monkeys were tested preflight or presimulation and at various times postflight or postsimulation. Recording days are referred to as days after recovery e.g., R+13 indicates the 13th day after the capsule returned to Earth. On the first day of postflight testing (R+1), the tendon force transducer of one of the flight animals (357) could not be adjusted within range and thus no postflight force data were available from this monkey.

The flight monkeys were tested during a 1- to 2-npp period 3 to 4 mo before launch, at R+1, and periodically throughout R+20. At R+1, both monkeys were tested for 10 steps only at 0.45 m/s to prevent muscle fiber injury and to preclude any readaptation to 1G that may have impacted other experiments resulting from longer locomotor tests and/or more strenuous locomotion observed at higher speeds. Flight monkey 357 died as a result of complications from anesthesia near the end of R+1, and thus no data beyond R+1 are available for this animal. The simulation monkeys were tested at R+1 and periodically throughout R+15.

Recovery of flight animals

The recovery team reached the landing site of the flight capsule and periodically throughout R+15. Both monkeys were tested for 10 steps only at 0.45 m/s to prevent continuous movement of the leg at the hip joint in the sagittal plane. The lower legs were unsecured. The monkeys then were installed in the satellite and the rocket was assembled and prepared for launch.

After launch the remaining four Rhesus were returned to the test facility at IMBP where they were used as ground-based simulation controls. Two animals (396 and 447) were placed in a mock-up flight capsule simulating flight conditions, i.e., temperature, etc., and testing conditions were initiated with a 4-day delay relative to the flight capsule. Two other monkeys (501 and 534) were treated similarly except that the simulation period began 17 days after the recovery of the flight capsule. During the 31 days before the flight simulation experiments, monkeys 501 and 534 were maintained under normal preflight conditions, i.e., housed in their cages and tested on the treadmill periodically. This longer delay allowed for the flight conditions to be more accurately simulated for these latter two Rhesus.

The flight simulation test was 17 days, simulating the 3-day prelaunch and the 14-day flight experienced by the flight Rhesus. Because of technical problems with the electronics for one of the monkeys (396), data were available from only three of the simulation monkeys.

The conditions under which the monkeys lived during the BION 11 mission have been detailed previously for the BION 10 mission (Cosmos 2229) (Korolkov et al. 1996). The animals in the simulation group were treated identically as the flight monkeys. Preliminary analysis of EMG data recorded for brief periods during flight and flight simulation suggests that the legs were much less active than under control recording conditions (unpublished observations).

Locomotor tests and/or more strenuous locomotion observed at higher

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Kinematic analyses were performed on the same step cycles for which EMG activity was analyzed. The x and y coordinates of all bony landmarks were digitized on an Amiga 2500 computer using a genlock to overlay video and computer images. A reference point was digitized to assure accurate registration of successive frames. The amount of forward and backward hindlimb movement was measured as the displacement of the x coordinate of the foot marker (head of the 5th metatarsal) relative to the x coordinate of the hip marker (greater trochanter) during touchdown and toe-off. Hip height during stance was measured as the mean displacement of the y coordinate of the hip relative to the y coordinate of the foot marker throughout the duration of the stance phase. Muscle activation relative to swing and stance was calculated by synchronizing kinematic and EMG data using the time code generator. Initiation and termination of muscle activity relative to touchdown and toe-off then were measured in terms of computer units and converted to milliseconds via conversion factors taking into account the sample rate at which EMG data were collected and the number of frames of video per second. Swing was defined as the period starting from the forward movement of the foot (toe-off) and ending with, but not including, foot contact (touchdown). Stance was defined as the period beginning with foot contact and ending at toe-off, i.e., just before the lifting of the foot off the treadmill during the initiation of the swing phase.

**Force buckle calibration procedures**

Two methods of calibration of the tendon force transducer were used. First, a static calibration was performed by the manufacturer for each transducer before implantation. The second method was an adaptation of the calibration described by Komi (Komi et al. 1987) for force transducers implanted on the human Achilles tendon. Each Rhesus was anesthetized deeply with a short-acting dissociative agent (ketamine HCl, 10 mg/kg) and placed in a standard Primate Products Restraint Chair (model R001, Primate Products, Redwood City, CA). The leg implanted with the force transducer and EMG electrodes was secured with the foot held against a hinged plate secured to the chair (Fig. 1). The foot was positioned so that the pivot point of the plate coincided with the center of rotation of the ankle joint. A calibrated force transducer attached to the plate recorded the external force generated by passive and active plantarflexor torques. The legs were flaccid during the calibration procedure and monitoring of the force baseline indicated no disproportionate changes in force relative to stimulation intensity. Stimulation of the MG muscle via the intramuscular EMG electrodes elicited a contraction, which was registered at both the tendon force transducer and the foot plate force transducer. The torque at the ankle was calculated from the foot plate data and the force on the MG tendon was computed from the ankle torque and the Achilles tendon moment arm. This in vivo calibration was determined around the time of treadmill locomotion recordings, ~4 mo before launch and ~1 mo after recovery of the capsule.

**Statistical procedures**

Statistical analysis was done using a 2-arm (spaceflight group and simulation group) repeated measure study. The outcome variables are the EMG cycle period, burst duration, and mean amplitude for four muscles. For each subject, measurements were obtained from several consecutive steps during treadmill locomotion before and after the experiment, respectively. A random effect model was developed to examine whether spaceflight affected these outcome variables. In the model, GROUP (spaceflight or simulation) and TIME (pre- or postexperiment) and their interaction are considered as fixed effects. The random effect in this model is the sequence of STEPS. The covariance structure for the random effect is unspecified. Data were collected during treadmill locomotion at 0.45 m/s.

When investigating the change of the mean EMG amplitude, controlling for cycle period, data collected in the precondition at 0.45, 0.89, 1.34 and 1.79 m/s and in the postcondition at 0.45 m/s are used for the analysis. Because the cycle period did not have significant interactions with GROUP and TIME, only CYCLE PERIOD, GROUP, TIME, and GROUP*TIME were included in the model as fixed effects. The sequence of STEPS is the random effect.

CYCLE PERIOD, the quadratic term of CYCLE PERIOD and TIME are in the model as a fixed effect and STEP as a random effect when studying the differences in the medial gastrocnemius tendon forces during locomotion for monkey 464 preflight, immediately postflight (R+1) and 20 days postflight (R+20). There was no significant CYCLE PERIOD with TIME interaction, therefore, the interaction term is not included in the model.

The main interests of this study are in the changes of each outcome variable from precondition (baseline) to the postcondition within each study group. Therefore each outcome variable pre- versus post- within each study group was compared. For each outcome variable, the difference between the two study groups, i.e., spaceflight or simulation, pre- and postexperiment, respectively was also tested. For each group, each time point and each muscle, the generalized least-squares means and standard errors of the three outcome variables also are estimated.

The statistical analysis was carried out by using statistical software package SAS (Littell et al. 1996).

**RESULTS**

**Behavioral adaptations**

After the 14-day spaceflight, the two flight monkeys demonstrated overt behavioral changes suggesting that they were having difficulty readapting to the 1G environment. For example, before the flight both animals showed considerable activity in the treadmill box, i.e., walking and jumping and usually sitting only when eating. In contrast, immediately postflight both monkeys were much more tentative under the same conditions, i.e., their movements appeared to be slower and more deliberate, and they generally preferred to sit in one place while in the treadmill box. In addition, they had some difficulties in maintaining their equilibrium even when adjusting their posture while sitting. Some visible tremor (clonus) occurred occasionally in the hindlimbs when the monkeys were sitting. This was apparent in the EMG recordings from the hindlimb muscles (Fig. 2A).

During the locomotor tests at R+1, despite their tentativeness, the flight animals successfully completed quadrupedal stepping at 0.45 m/s. The monkeys took shorter steps after than...
Before flight, with the average cycle period decreasing significantly to 83% of preflight levels immediately after flight (Fig. 3). The mean height of the hip relative to the ground during the stance phase was increased by an average of 0.81 cm after flight, the change being more prominent in 484 (1.25 cm) than in 357 (0.36 cm). After the locomotor tests, the flight monkeys were relatively inactive and reluctant to exit the treadmill box. This behavior was contrary to that observed preflight when they usually remained quite active and immediately exited the treadmill on completion of the tests. The postflight recovery pattern was determined in only one monkey (484) after R+1. The average cycle period remained ~80% of preflight levels for the 20 postflight days during which locomotion was tested.

In contrast to the flight animals, the behavior of the simulation animals in the treadmill box before and after the locomotor tests was similar pre- and postsimulation. In addition, no tremor was observed in the hindlimbs of the simulation animals, no equilibrium problems were evident, and during the locomotion test, they were easily capable of successfully completing quadrupedal steps. The average cycle period of the simulation group was unaffected at R+1. Further, the average cycle period in two simulation monkeys was relatively unaffected throughout the experimental period. The mean height of the hip throughout the stance phase decreased significantly in the two simulation animals (average of 2.41 cm; 3.3 and 1.51 cm in 501 and 447, respectively). No kinematic data were available for simulation monkey 534.

Alterations in EMG burst characteristics

Although the general shapes of the EMG bursts for all muscles studied were similar pre- and postflight, there was clonic-like activity during some steps post- but not preflight, particularly in 357 (Fig. 2B). Similar to the flight animals, the general shapes of the EMG bursts were similar pre- and postsimulation. However, no clonic-like activity was observed postsimulation. The differences in EMG burst durations between pre- and postflight and pre- and postsimulation were consistent with the changes in cycle period (Fig. 3). For example, all three extensor muscles studied tended to have shorter EMG burst durations post- compared with preflight,
whereas there was a tendency for the extensors to have longer EMG burst durations post-compared with presimulation. The burst duration of the TA was unaffected by either flight or simulation. Similarly, there were no consistent changes in the Sol:TA, MG:TA, or Sol:MG burst duration ratios as the result of flight or simulation (Table 1).

During the 20-day postflight recovery period, the EMG burst durations of the extensors in the one flight monkey (484) remained below preflight levels (Fig. 4A). In contrast, burst durations studied over a 15-day flight-simulation period in three monkeys were elevated for one wk and then returned to approximately the same level as preflight by 15 days postsimulation (Fig. 4B). These differences in the pre-post flight compared with pre-post simulation are important because they suggest that the consistently reduced extensor burst durations seen postflight reflected the effects of spaceflight rather than being due to gradual changes in stepping patterns that could be associated with repeated testing on the treadmill.

The mean EMG burst amplitude of the Sol was significantly lower on day R+1 in both the flight and simulation groups (Fig. 5). The amplitudes of the two fast extensor muscles (MG and VL), however, were unchanged postflight and significantly higher postsimulation. The amplitude of the TA was elevated in the flight group and unchanged in the simulation group. The data in Fig. 5 represent only five steps when the animal maintained a relatively constant position (i.e., speed) on the treadmill. A comparison of EMG amplitude and cycle duration (Figs. 6 and 7) enabled us to include postflight data from additional steps where the animal moved forward or backward on the treadmill (i.e., steps at different cycle periods). These

### Table 1. Burst duration ratios pre- and postflight and pre- and postsimulation on day R+1 during treadmill stepping at 0.45 m/s

<table>
<thead>
<tr>
<th>Muscle Ratios</th>
<th>Pre</th>
<th>Post</th>
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<tr>
<td>Sol:TA</td>
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<td></td>
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<tr>
<td>Flight</td>
<td>2.6</td>
<td>2.6</td>
</tr>
<tr>
<td>Simulation</td>
<td>2.8 ± 0.2</td>
<td>2.4 ± 0.2</td>
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<tr>
<td>Mean ± SE</td>
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<td>MG:TA</td>
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<td>Flight</td>
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<td>2.9</td>
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<tr>
<td>Simulation</td>
<td>3.0</td>
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</tr>
<tr>
<td>Mean ± SE</td>
<td>3.0</td>
<td>2.6</td>
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<tr>
<td>Sol:MG</td>
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</tr>
<tr>
<td>Flight</td>
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<tr>
<td>Simulation</td>
<td>0.9 ± 0.0</td>
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<tr>
<td>Mean ± SE</td>
<td>0.9</td>
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Values represent the ratio for each of the combinations of muscles listed. There were no significant differences between any ratios.

**Fig. 4.** Mean EMG burst durations for the Sol, MG, VL, and TA during a sequence of steps at 0.45 m/s preflight (A) or presimulation (B) and at various days postflight or postsimulation (R+1). Values are shown for flight monkey 484 and for the mean values of simulation monkeys 447, 501, and 534. Bars in B are SE.

**Fig. 5.** Mean EMG burst amplitudes for the Sol, MG, VL, and TA for the flight (A) and simulation (B) groups during a sequence of stepping at 0.45 m/s on day R+1. Bars are SE. Muscle abbreviations are the same as in Fig. 2. *significant pre-post difference (P ≤ 0.05).
data were compared with control data from trials using several treadmill speeds to cover the same range of cycle periods. Using this analysis, it is evident that the Sol and VL amplitudes postflight were reduced consistently and significantly compared with preflight in both monkeys (Fig. 6, A and C). There were no consistent changes in the MG or TA EMG burst amplitudes after flight (Fig. 6, B and D). In the simulation group, the EMG amplitude relative to cycle period was decreased in the Sol (Fig. 7A) and increased in the VL (Fig. 7C). No consistent effect of simulation was observed in the EMG burst amplitudes of either the MG or TA, although there was tendency for an increase in the MG in two of the three monkeys.

Comparisons of mean EMG amplitudes of the four muscles during a 20-day postflight recovery period demonstrated the consistency of the recordings during repeated test conditions in one animal (Fig. 8A). The data suggest that the reduced Sol EMG amplitude seen on R+1 was of a relatively short duration, perhaps having recovered to preflight levels by R+7 and at least by R+17. Similarly, the increase in TA amplitude observed at R+1 seemed to have recovered to near preflight levels by R+7. The reduced Sol amplitude in the simulated monkeys also had recovered to presimulation levels by R+7, whereas the increased amplitude of the MG and VL had recovered by 15 days (Fig. 8B).

Analysis of the burst integrals supported all postflight and postsimulation trends observed in the burst duration and mean burst amplitudes. The mean burst integral of the Sol, MG, and VL in the flight group decreased 35, 15, and 23%, respectively, postflight, whereas in the simulation group the Sol decreased 19% and the MG and VL increased 29 and 52% postsimulation, respectively. The flexor (TA) mean burst integral increased by 60% postflight and 34% postsimulation.

In addition to the changes observed in the bursts themselves, the onset and offset of extensor activity immediately postflight were delayed with the effect being much more apparent in 484 (Fig. 9A). The only exception was in the Sol of 357, which showed an earlier activation before touchdown after than before flight. No consistent changes were observed for the activation and deactivation of the TA. Within the simulation animals, the activation times were affected to a lesser degree and the changes were more variable in the simulation compared with the flight animals (compare Fig. 9, A and B). The only clear pattern was a delay in the deactivation times of all four muscles of simulation monkey 447. Based on kinematic

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**Fig. 6.** Relationship between mean EMG amplitude and cycle period for individual steps during preflight (○) stepping at 0.45, 0.89, 1.34, and 1.79 m/s and during postflight (R + 1) (●) stepping at 0.45 m/s. Lines represent a linear best fit curve through the preflight data. Muscle abbreviations are the same as in Fig. 2. There were significant mean pre-post decreases for the Sol and VL (P < 0.05).

**Fig. 7.** Relationship between mean EMG amplitude and cycle period for individual steps during presimulation (○) stepping at 0.45, 0.89, 1.34, and 1.79 m/s and during postsimulation (R + 1) (●) stepping at 0.45 m/s. Lines represent a linear best fit curve through the presimulation data. Muscle abbreviations are the same as in Fig. 2. There was a significant pre-post decrease in the Sol and increase in the VL (P < 0.05).

**Fig. 8.** Comparison of mean EMG amplitudes of the four muscles during a 20-day postflight recovery period demonstrated the consistency of the recordings during repeated test conditions in one animal (Fig. 8A). The data suggest that the reduced Sol EMG amplitude seen on R + 1 was of a relatively short duration, perhaps having recovered to preflight levels by R + 7 and at least by R + 17. Similarly, the increase in TA amplitude observed at R + 1 seemed to have recovered to near preflight levels by R + 7. The reduced Sol amplitude in the simulated monkeys also had recovered to presimulation levels by R + 7, whereas the increased amplitude of the MG and VL had recovered by 15 days (Fig. 8B).

**Fig. 9.** Analysis of the burst integrals supported all postflight and postsimulation trends observed in the burst duration and mean burst amplitudes. The mean burst integral of the Sol, MG, and VL in the flight group decreased 35, 15, and 23%, respectively, postflight, whereas in the simulation group the Sol decreased 19% and the MG and VL increased 29 and 52% postsimulation, respectively. The flexor (TA) mean burst integral increased by 60% postflight and 34% postsimulation.

In addition to the changes observed in the bursts themselves, the onset and offset of extensor activity immediately postflight were delayed with the effect being much more apparent in 484 (Fig. 9A). The only exception was in the Sol of 357, which showed an earlier activation before touchdown after than before flight. No consistent changes were observed for the activation and deactivation of the TA. Within the simulation animals, the activation times were affected to a lesser degree and the changes were more variable in the simulation compared with the flight animals (compare Fig. 9, A and B). The only clear pattern was a delay in the deactivation times of all four muscles of simulation monkey 447. Based on kinematic
Alterations in the recruitment patterns of ankle joint synergists and antagonists

Probability density distributions of EMG amplitudes of pairs of muscles at R +1 clearly show a shift toward a higher activation of the MG relative to the Sol post- compared with preflight (Fig. 10) and post- to presimulation (Fig. 11). The mean Sol:MG ratio was significantly smaller post- compared with preflight (29%) and post- compared with presimulation (39%) (Table 2).

On the basis of these probability density distributions, there was little coactivation between the Sol and TA during stepping preflight (Fig. 12). After flight, however, there was an increase in the coactivation between these antagonists in both flight monkeys. The coactivation always was observed at the termination of swing and the initiation of stance. Little coactivation of the Sol and TA was observed either pre- or postsimulation (Fig. 13). The Sol:TA mean amplitude ratios significantly decreased in the flight (59%) and simulation (36%) monkeys (Table 2). The MG:TA ratios also were significantly lower (46%) in the flight group, whereas the mean pre-post MG:TA ratio for the simulation group was not significantly different (Table 2).

Alterations in MG tendon forces

Tendon force recordings from the MG were available for one flight (484) (Fig. 14) and one simulation (534) monkey on R +1 and for the same flight monkey through R +20 (Fig. 16). In addition, force data were obtained from simulation monkeys 534 at R +4 and R +5 and 501 at R +4 (Fig. 15). Tendon forces were unavailable from the other monkeys due to recording problems postflight or postsimulation. Compared with preflight, the mean MG force approximately doubled at R +1 in

![Graph](image-url)
the flight animal (Fig. 15). Tendon force data from each individual step preflight, immediately postflight (R\textsubscript{1}) and 20 days postflight (R\textsubscript{20}) demonstrate that there was a significantly higher force output at R\textsubscript{1} and that these forces returned to near preflight levels within 20 days postflight (Fig. 16). The postflight MG forces were highly variable across steps with the values ranging between 62 and 243% of mean preflight levels. Compared with presimulation, the MG tendon forces in 534 approximately doubled at R\textsubscript{1} and returned to presimulation levels within 1 wk (Fig. 15). In simulation monkey 501, the MG tendon forces were increased by \sim 150% at R\textsubscript{4}. Similar to that observed in the flight monkeys, the postsimulation tendon forces across steps were highly variable, being 75–333% of mean presimulation levels.

**DISCUSSION**

Postural and locomotor control in the Rhesus seems to be compromised by exposure to microgravity as has been observed in humans. Less stable standing posture, increases in tremor-like activity, and delays in making postural corrections have been reported in humans (Clement and Lestienne 1988; Edgerton and Roy 1997; Kozlovskaya et al. 1981; Lestienne and Gurfinke 1988). Crew members returning from spaceflight consistently show disturbances in gait (Layne et al. 1997), and a variety of compensatory mechanisms are used to step successfully. For example, taking shorter steps, increased reliance on visual input, and generally being more cautious during the performance of movements are common characteristics (Edgerton and Roy 1996). The present study provides evidence that chronic exposure to microgravity changes the normal neurophysiological responses of the motor system to gravitational or load-related cues in a nonhuman primate. These modified responses are reflected in the altered relative recruitment bias of flexors versus extensors and fast versus slow motor pools.

**Elevated flexor:extensor EMG ratios after spaceflight**

Differences in extensor and flexor motor pool recruitment patterns after flight may be the result of a reduction in the amount of load-related proprioceptive feedback from muscle spindles and Golgi tendon organs (Kozlovskaya et al. 1981, 1988; Roll et al. 1993). After unloading of the hindlimbs via spaceflight or ground-based hindlimb suspension, it has been

**TABLE 2.** Mean amplitude ratios pre- and postflight and pre- and postsimulation on day R\textsubscript{1} during treadmill stepping at 0.45 m/s

<table>
<thead>
<tr>
<th></th>
<th>Sol:TA</th>
<th>MG:TA</th>
<th>Sol:MG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flight</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>357</td>
<td>5.0</td>
<td>1.4</td>
<td>3.9</td>
</tr>
<tr>
<td>484</td>
<td>3.8</td>
<td>0.6</td>
<td>6.4</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>4.4 ± 0.6</td>
<td>1.0 ± 0.4</td>
<td>5.1 ± 1.2</td>
</tr>
<tr>
<td>Simulation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>447</td>
<td>2.6</td>
<td>0.6</td>
<td>4.6</td>
</tr>
<tr>
<td>501</td>
<td>7.9</td>
<td>1.6</td>
<td>5.2</td>
</tr>
<tr>
<td>534</td>
<td>3.5</td>
<td>0.6</td>
<td>5.9</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>4.7 ± 1.7</td>
<td>0.9 ± 0.3</td>
<td>5.2 ± 0.4</td>
</tr>
</tbody>
</table>

Values represent the ratio for each of the combinations of muscles listed. *, significant differences between pre- and postflight or pre- and postsimulation (P ≤ 0.05). There were no significant differences between the flight and simulation groups.
shown consistently that extensor activity decreases, whereas flexor activity increases resulting in a “flexor bias” (Alford et al. 1987; Clement and Lestienne 1988; Edgerton and Roy 1994; Lestienne and Gurfinkel 1988; Roy et al. 1991a). This flexor bias has been attributed to less inhibition of extensor and more facilitation of flexor motor pools immediately following unloading of the hindlimbs in humans and rats (Edgerton and Roy 1994; Roy et al. 1991a). The decrease in the Sol:TA and MG:TA mean amplitude ratios of the flight animals during postflight locomotion in the present study demonstrates that the presence of this flexor bias can be modulated in the normal adult nonhuman primate. Although changes in muscle properties, e.g., atrophy, or alterations in the nervous system or motor pools that control locomotion cannot be ruled out, the present data are consistent with the conclusion that at least some component of the flexor bias is an adaptive state acquired by the nervous system when it is subjected to microgravity. These changes seem rather significant considering that these animals were exposed to microgravity for only 14 days.

Elevated fast:slow muscle ratios after spaceflight

The second point of emphasis from the present data is the reorganization in the recruitment patterns of a slow and a fast synergistic extensor muscle of the hindlimb in response to microgravity. The present as well as previous primate studies [Cosmos 2044 (Hodgson et al. 1991) and Cosmos 2229 (Roy et al. 1996b)] suggest that the nervous system adapts to space-
flight by decreasing the recruitment bias of a slow relative to a fast extensor motor pool during locomotion immediately after 2 wk in a microgravity environment. These changes in the recruitment patterns suggest that the neural networks responsible for the net excitation of slow muscles were altered more than similar networks that excite a synergistic fast muscle. This selective effect on the Sol may reflect the much higher density of spindles and tendon organs in the Sol compared with the MG as well a higher proportion of the afferents projecting from the Sol back to the homonymous motor pool compared with the MG (Mendell et al. 1990) both of which normally contribute to a disproportionate input to the slow motor pool. Another contributing factor could have been the greater atrophy in the Sol compared with the MG, which has been observed in rats in response to spaceflight (Edgerton and Roy 1996; Roy et al. 1996a). However, there is not clear evidence that a significant level of muscle fiber atrophy occurred in either the Sol or MG in a previous spaceflight mission in which Rhesus were exposed to ~2 wk of microgravity (Bodine-Fowler et al. 1992) or ground-based simulation (Fitts et al. 1998) nor from the examination of fiber size in pre- and postflight or simulation biopsies obtained in the present study (R. R. Roy, H. Zhong, S. C. Bodine, and V. R. Edgerton, unpublished observations).

Although some changes in the recruitment patterns could be attributed to prolonged chair sitting (simulation conditions), most changes seemed to be directly attributable to microgravity. For example, ground-based simulation alone modified the output of the slow extensor muscle (Sol) by reducing the motoneuronal output in both the flight and simulation groups. However, the fast extensors (MG and VL) increased in output in the simulation group while exhibiting no change in the flight group, and the flexor output increased postflight but did not change postsimulation. Furthermore the differences between the flight and simulation groups provide evidence that the absence of weight-bearing alone cannot account for all of the effects observed after spaceflight (compare Fig. 12 with 13).

The significance of the role of gravity in shaping the properties of the locomotor system in mammals, including nonhuman primates and humans, is becoming increasingly apparent (Edgerton and Roy 1996; Roy et al. 1996a). It is also evident...
that this gravity dependence is present within the neural networks located in the spinal cord as well as in those networks descending from supraspinal centers. For example, the experiments of Sherrington demonstrated the importance of gravitational loading in spinal cats (Sherrington 1905). Several more recent studies have demonstrated the critical influence that the loading levels and patterns have on the hindlimb in organizing the motor output that generates stepping in spinal cats (de Guzman et al. 1991; Edgerton et al. 1992; Lovely et al. 1990; Pearson 1995). Furthermore Harkema et al. (1997) demonstrated considerable load sensitivity in human subjects during locomotion, this being evident in subjects with complete or incomplete thoracic spinal cord lesions as well as in uninjured subjects. Although the papers noted above demonstrate that the neuromotor system is highly responsive to loading and that even chronically load-bearing steps in a completely spinal animal can modulate the neural control properties of the hindlimbs, these studies do not address the issue of how the normal neural control properties are modulated by the chronic absence of gravitational loading as occurs on Earth. It is of interest to note, however, that a select population of both motoneurons and dorsal root ganglia neurons of the lumbar segments of the rat showed reduced succinate dehydrogenase activities (an oxidative marker enzyme) after a 14-day spaceflight (Ishihara et al. 1996, 1997). Further this change persisted for ≥9 days after return to Earth.

In addition to the apparent intrinsic design of the spinal cord to be responsive to gravitational loading, it also appears that the ability to modulate the locomotor motor pools can be affected substantially by changing the chronic levels of loading on these motor pools. For example, cats with complete spinal cord transections can learn to step or learn to stand when trained for that motor task and also can forget how to execute these tasks after the training is terminated (de Leon et al. 1998a,b; Edgerton et al. 1997; Hodgson et al. 1994). These studies leave open the possibility that the changes that occurred in the activation patterns of the motor pools of the lower limbs as a result of spaceflight, and to some extent simply chronic periods of unloading (e.g., chaired), might be attributable in part to neurophysiological plasticity in the spinal circuits that are fully capable of appropriately interpreting the load-associated kinetics of stepping at 1G.

Conclusions and perspective

It was possible for the first time in any animal species to record detailed output properties of motor pools known to differ in function with respect to gravity during a well-controlled locomotor task before and after exposure to microgravity. Unlike numerous studies of humans after having flown in space, we recorded from chronically implanted intramuscular electrodes and a force transducer on the MG tendon that remained stable for months. Therefore pre- and postflight recordings from each subject could be compared readily. Further the activities of the monkeys were much more controlled during flight than is possible with the numerous and continuously varying tasks that humans must perform and that rats and other animals have been able to perform during a spaceflight mission. A limiting feature of the present experiment, however, was the small number of subjects that could be flown in the BION satellite. Although this limits the extent to which the present findings can be generalized to other Rhesus, it does not limit the level of assurance of the effects on locomotor capacity that were found in this study.

These data demonstrate that some features of the control of locomotion in nonhuman primates are acquired as a result of exposure to a 1G environment postnatally, i.e., ontogenetic as well as phylogenetic influences. Further it is important to note that these changes occurred in normal, nonhuman primates; adult animals; and after only 2 wk of spaceflight.

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