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Research report

Intermittent application of hypergravity by centrifugation attenuates disruption of rat gait induced by 2 weeks of simulated microgravity

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HIGHLIGHTS

- We examined the effect of hypergravity on locomotion in rats.
- Rats that their hindlimb unloaded exhibited hyperextension in their knee and ankle during stance phase.
- Altered motions persisted after the termination of unloading.
- Intermittent exposure to normal gravity (1G) did not inhibit the gait alteration.
- Intermittent exposure to twice of gravity (2G) inhibited the gait alteration.

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ABSTRACT

The effects of intermittent hypergravity on gait alterations and hindlimb muscle atrophy in rats induced by 2 weeks of simulated microgravity were investigated. Rats were submitted to hindlimb unloading for 2 weeks (unloading period), followed by 2 weeks of reloading (recovery period). During the unloading period, animals were subjected to the following treatments: (1) free in cages (Control); (2) continuous unloading (UL); (3) released from unloading for 1 hour per day (UL + 1G); (4) hypergravity for 1 h per day using a centrifuge for small animals (UL + 2G). The relative weights of muscles to the whole body weight and kinematics properties of hindlimbs during gait were evaluated. UL rats walked with their hindlimbs overextended, and the oscillation of their limb motion had become narrowed and forward-shifted after the unloading period, and this persisted for at least 2 weeks after the termination of unloading. However, these locomotor alterations were attenuated in rats subjected to UL + 2G centrifugation despite minor systematic changes in muscle recovery. These findings indicate hypergravity application could counteract the adverse effects of simulated or actual microgravity environments.

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1. Introduction

Exposure to microgravity environments induces multiple alterations in sensorimotor apparatuses, necessitating an adaptation to such unfamiliar environments. Although such alterations involve muscular and skeletal properties [1,2], recent studies show that exposure to microgravity environments alters the nervous system [3,4], behavior [5,6], and kinematics properties as well [7–9]. Regarding locomotion, rats exposed to microgravity exhibit altered motion characterized by hyperextension of the knee and ankle joints during stance phase, which is described as "walking on its toes" [8], hypodynamia, and forward-shifted motion of the hindlimbs (i.e., less extension backward) [10–12] (Supplementary

Abbreviations: ASt, ankle angle at mid stance; CO, center of oscillation; KSt, knee angle at mid stance; MG, medial gastrocnemius; MTP, 5th metatarsophalangeal joint; RO, range of oscillation; Sol, soleus; UL, unloaded; UL + 1G, unloaded +1 gravity application (normal gravity); UL + 2G, unloaded +2 gravity application (twice normal gravity [hypergravity]).

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Fig. 1). Furthermore, exposure to altered gravity might irreversibly transform gait characteristics after termination of the perturbation [8,11,13,14]. Several countermeasures such as strength training, treadmill exercises, and lower-body negative pressure (LBPP) have been employed to reduce the adverse effects on musculoskeletal and kinematics properties induced by microgravity [15].

One such countermeasure is artificial gravity produced by a centrifuge, which could provide an effective solution, because most of the disturbances due to microgravity can be attenuated by a sufficient gravity load. However, to date, most of these studies investigating advantages of artificial gravity have been focused on musculoskeletal aspects [2,16,17], and little is known about the motion deficits. Further, regarding practical applications, the time that one can spare for daily countermeasures is limited [2,18]. Nevertheless, the effectiveness of intermittent centrifugation as a countermeasure against microgravity-induced deficiencies are not well understood not only in musculoskeletal issues but also in motor alteration although Bouet et al. have investigated effects of chronic hypergravity on locomotion in rats by using artificial gravity (i.e., 2× gravitational force: 2G) [14].

Therefore, in this study, we investigated the following: (1) whether intermittent application of hypergravity using centrifugation prevents the disruption of gait induced by 2 weeks of hindlimb unloading in rats; (2) whether the recovery of atrophy in hindlimb extensor muscles is associated with the gait alterations.

2. Materials and methods

We used 4 groups of 8-week-old male Wistar rats ($N = 72$) following 1 week of training for treadmill walking. All animals were kept in the same temperature- and light-controlled room. The study protocol was approved by the Animal Experimentation Committee of the Graduate School of Medicine, Kyoto University. All experiments were conducted in accordance with the National Institutes of Health's Guidelines for the Care and Use of Laboratory Animals.

2.1. Treadmill acclimatization and grouping

One week prior to the experiments, all animals were trained to walk on a treadmill as previously described [8,19–22]. The rats were made to walk for 20 min at 20 cm s^{-1} [20] every other day during the acclimatization period. Subsequently, the rats were randomly distributed into 4 groups: (1) control (Control), (2) unloaded (UL), (3) unloaded +1G (UL + 1G [normal gravity]), and (4) unloaded +2G (UL + 2G [twice normal gravity]). As the Control group, rats were reared in regular cages and were allowed to move freely throughout the 4-week experimental period. The other groups were submitted to hindlimb unloading for the first 2 weeks, followed by another 2 weeks of free movement (see sections below for details).

2.2. Hindlimb unloading

In the first 2 weeks of the experiment, the rats in the UL, UL + 1G, and UL + 2G groups were unloaded by their tail, and allowed to move freely on their forelimbs (unloading period). The hindlimb unloading was performed according to a modified procedure borrowed from Wronski and Morey-Holton [23,24]. The rats in the UL group were kept unloaded throughout the unloading period. Meanwhile, in the UL + 1G group, the animals were relieved from unloading and placed on the ground 1 hour per day 6 times per week. In the UL + 2G group, the animals were relieved from unloading and submitted to centrifugation at the same timing [2,18]. After the initial 2 week unloading period, the UL + 1G and UL + 2G groups were re-loaded and allowed to move freely for another 2 weeks (recovery period) until the final evaluation. Evaluations were performed every 2 weeks. Six animals were extracted at each time point of interest (0 week [beginning of the unloading period], 2 weeks [termination of the unloading period], 4 weeks [end of the recovery period]) and subjected to evaluation (Supplementary Fig. 2 for details).

2.3. Intermittent centrifugation

We used a centrifuge customized for small animals [25,26], with a 0.5-m-radius arm (Uchida Electron, Tokyo, Japan). Rotating the arm at 56 rpm generates 2G artificial gravity in the resultant force line between the centrifugal force and vertical gravity. Animals in the UL + 2G group were centrifuged while relieved from unloading. During centrifugation, they were placed in individual cages, each equipped with a small video camera to monitor their behavior and posture (Supplementary Video 1).

2.4. Kinematic analysis

At each time point of interest, 6 animals from each group were randomly selected and the kinematics properties of hindlimbs during ambulation on a treadmill moving at 20 cm s^{-1} were assessed. The motion was captured at 120 Hz using a 3-dimensional (3-D) motion capture apparatus (Kinema Tracer System, Kissei Comtec, Nagano, Japan). This system consists of four CCD (charged coupled device) cameras (two of which are placed in line on both the right and left side of the treadmill), and of an image processor that allows reconstruction of 3-D movements from the captured movies (Supplementary Video 2). Before each capture session, colored hemispherical plastic markers (diameter: 0.3 cm), which correspond to 5 landmarks employed in order to detect joint displacements, were attached onto shaved skin while the animal was under light anaesthesia induced using isoflurane (Supplementary Fig. 3). The landmarks were as follows: the anterior superior iliac spine, trochanter major (i.e., hip), knee joint (knee), lateral malleolus (ankle), and the 5th metatarsophalangeal joint (MTP). Then, each rat walked on a treadmill moving at 20 cm s^{-1}. Although each recording session involved several trials until the animal performed successive gait, each bout lasted <10 s, and breaks for the subject were introduced to avoid fatigue. For subsequent analysis, a total of 10 steps for each animal were obtained from portions of sequences in which the animal walked at an uniform velocity for at least 5 consecutive steps [22]. To ensure data accuracy, the precise coordinates were calibrated by recording a cube of known size ($5 \times 20 \times 10$ cm [$x \times y \times z$]) before each session. The coordination of the 3-D directions for the x-, y-, z-axes were lateral, anterior, and vertical, respectively (i.e., the right-hand rule: Supplementary Video 2, right panel).

After tracing the markers, joint displacements, which represent the kinematics properties, were automatically calculated by the system. The parameters were defined as follows: (1), the knee angle and (2), the ankle angle at stance phase (KSt and ASt, respectively): the angle of knee and ankle joint when the MTP marker was vertical with the hip marker in the y-z plane during the stance phase; (3), limb angle: the angle between the y-axis and the line connecting the hip and the ankle marker; (4), range of oscillation (RO): the difference in the limb angle between the paw contact and lift off; (5), center of oscillation (CO): the mid-point limb angle over the RO [8,27,28]. For instance, when the limb angle of the paw contact and lift off is 70° and 130° respectively, the CO is 100°. The smaller value of KSt and ASt represent a more flexed joint. The smaller RO represents a narrower range, and the smaller CO represents forward shift of the limb angle. Forward-shifted CO implies less push off at the end of stance phase (Supplementary Fig. 1).

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