Japanese Journal of Comprehensive Rehabilitation Science (2015)

Original Article

Improvement of gait and coordinated movement by forced gait training in cerebellar ataxic B6-wob/t mice

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ABSTRACT
Beppu H, Takayanagi N, Tomita Y, Mizutani K, Orand A, Tamai I, Takahashi H, Sonoda S. Improvement of gait and coordinated movement by forced gait training in cerebellar ataxic B6-wob/t mice. Jpn J Compr Rehabil Sci 2015; 6: 64−70.
Objective: The effect of forced gait training was investigated in cerebellar ataxic mice (B6-wob/t) using gait analysis and a rota-rod test.
Methods: B6 and B6-wob/t mice were divided into non-exercise (NEx) and exercise (Ex) groups. The Ex group received forced gait training with a running wheel at 2 m/min for 50 minutes, three times a day, six days a week, for 12 weeks. The rota-rod test was performed every 4 weeks during this period, and gait was evaluated using our pelvic axis-based gait analysis method (pelvic axis method) when a significant difference was noted between the NEx and Ex groups. The multiple comparison test was used for statistical analysis.
Results: After gait training for 12 weeks, the time until falling in the rota-rod test was significantly extended to 115 seconds in the Ex group compared to that (90 seconds) in the NEx group, and the hindlimb step width measured using the pelvic axis method was significantly narrower in the EX than NEx group.
Conclusion: Forced gait training may be effective for ataxic symptoms of B6-wob/t. The combination of the pelvic axis-based gait analysis and rota-rod test was useful to evaluate the improvement of ataxia.
Key words: cerebellar ataxia, B6-wob/t mouse, forced running wheel exercise, pelvic axis-based gait analysis, rota-rod test

Introduction

We maintain a novel strain of C57BL/6J(B6)-wob/t mice (B6-wob/t) that develop ataxic gait and truncal ataxia [1]. The cause of the pathology of these mice is degeneration and loss of cerebellar Purkinje cells, and the disease manifests about 20 days after birth [2]. They walk with short and wide steps with toe-out hindlimbs to counter trunk sway [3]. This is similar to the gait with a wide base observed in cerebellar ataxia patients [4−6], suggesting that B6-wob/t can assist studies on the treatment of ataxia.

Repeating motion is employed in rehabilitation for ataxia [7−9]. We subjected B6-wob/t to forced gait training using a running wheel, and investigated its effect by gait analysis [3] and the measurement of coordinated movement using the rota-rod test [1, 10, 11].

In studies involving ataxic patients, markers and sensors are attached to the pelvis and spine for analysis [12−14]. Since quadrupedal B6-wob/t mice walk forward by rotating and laterally bending the trunk in the direction of movement [3], a unique analytical system and gait parameters are necessary to evaluate gait objectively, for which we have developed a pelvic axis-based gait analysis method setting the baseline to the pelvic axis [3]. We utilized this method in the present study.

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Accepted: March 16, 2015
Conflict of interest: This study did not receive financial support from any commercial company or for-profit organization.
This study was supported by a Grant-in-Aid for Scientific Research/Challenging Exploratory Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan [KAKENHI2011-12] (No. 22650132).

Animals and Methods

1. Animals

Pregnant C57 BL/6J Jms Slc (B6) (4 weeks old; Japan SLC Inc., Hamamatsu, Japan) and B6-wob/t mice (4 weeks old; Kyudo Co., Ltd., Tosu, Japan) were purchased. These mice were maintained in the Fujita Memorial Nanakuri Institute animal room. They were housed in plastic cages with Mouse Igloo (hives with a diameter of 10 cm; Animec, Tokyo) and wood-chip bedding and reared at a room temperature of 24 ± 2°C and 60 ± 5% relative humidity under lighting from 7:00 to 19:00. All mice were given free access to Oriental MF pellets (Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water. The rearing environment and handling of animals were provided and performed according to the 'Guidelines for the Management of Laboratory Animals in Fujita Health University, Fujita Memorial Nanakuri Institute'. The experimental protocols were approved by the Institutional Animal Care and Use Committee of Fujita Health University (Approval number: N-01-11). This study was classified into Scientists Center for Animal Welfare (SCAW) CategoryD, inducing no distress in the animals. Since B6-wob/t is a spontaneous disease model, underweight and inactive animals were excluded before the experiment.

2. Gender distribution of the mice experiment group

After weaning, B6-wob/t mice were checked for abnormal gait at 4 weeks after birth [1], and their sexes and weights were determined. Because of the shortage of selected male and female mice, male and female mice were mixed for use in the experiment, as previously reported [3]. The experimental groups were divided into an exercise (Ex) group consisting of 8 animals (4 males and 4 females) and a non-exercise (NEx) group consisting of 8 animals (2 males and 6 females). The Ex group was forced to exercise for 50 minutes on a running wheel (Fig. 1a, circumference: 50 cm, rotation distance: 2 m/min), followed by a 10-minute rest, which was repeated three times. This exercise was performed between 15:00 and 18:00, six days per week, and continued until a significant difference between the Ex and NEx groups was observed on the rota-rod test described in the following. B6 mice (5 males and 3 females) performed no exercise (B6 group). The body weights of all mice were measured every 4 weeks after the start of the study.

3. Rotation basket for forced gait training

The Ex group of B6-wob/t received forced gait training for 50 minutes using a rotation basket (Fig. 1a, circumference: 50 cm, rotation distance: 2 m/min). After a 10-minute break, the training was repeated twice. The training was repeated every day between 15:00 and 18:00 for six days a week until a significant difference was noted between the Ex and NEx groups in the rota-rod test.

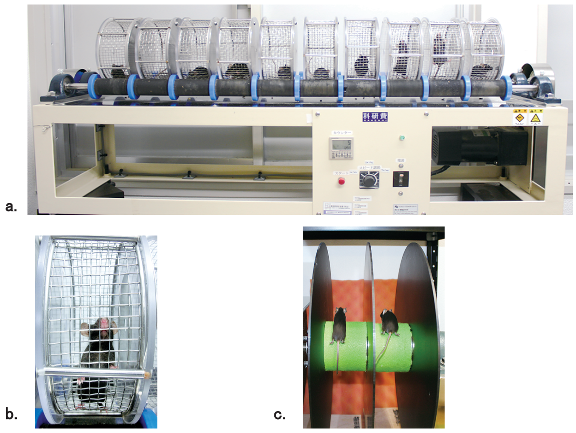

Figure 1. Overall view of the running wheel for forced gait training (a), a B6-wob/t mouse during gait training (b), a view of the rota-rod test (c).

4. Rota-rod test

On the day before initiation of the forced exercise, mice were placed on the rota-rod (diameter: 90 mm, KN-75, Natsume Co., Ltd., Tokyo, Japan) rotating at 7 rpm (Fig. 1c), and the time until falling off it was measured. This test was repeated three times, setting the maximum duration of measurement to 180 seconds, and the mean was adopted as the measured value [10, 15]. The rota-rod test was performed every 4 weeks, and differences among the B6, NEx, and Ex groups were investigated.

5. Gait analysis

Gait analysis was performed using pelvic axis-based gait analysis [3] when a significant difference was noted between the NEx and Ex groups on the rota-rod test. For the gait parameters, the total of the perpendicular lines drawn from the bilateral 3rd metatarsal heads of the hindlimbs to the pelvic axis was evaluated as the hindlimb step width, and the angle formed by a line connecting the 3rd metatarsal head of the hindlimb and heel and the pelvic axis was evaluated as the hindlimb angle. The distance between the initial contact of one hindlimb and that of the opposite limb was measured as the hindlimb step length, and the time taken for this was evaluated as the hindlimb step cycle. In addition, these four gait parameters were normalized by subtracting the mean from the measured value and then dividing it by the standard deviation, and scatter plots of the normalized variables were prepared in all two-variable combinations in the B6, NEx, and Ex groups.

6. Statistical analysis

The gait parameters were analyzed among the B6, NEx, and Ex groups, using one-way ANOVA, followed by Bonferroni/Dunn post hoc multiple comparison.

The data are presented as the mean ± standard deviation, and $p < 0.05$ was regarded as significant.

Results

Ataxic gait was observed at 4 weeks after birth in all B6-wob/t mice. No death or weakening due to exercise was noted throughout the 12-week period in any of the 8 animals allocated to the Ex group. The gait training cage was a running wheel made of mesh, so that even B6-wob/t mice with ataxic gait could move forward corresponding to the rotation speed without losing their footing (Fig. 1b), and continuous gait training was possible. Body weight changes determined for male and female mice together or separately at 4 weeks before birth (pre-test) and at 4 weeks (8 weeks old), 8 weeks (12 weeks old), and 12 weeks (16 weeks old) after the start of training are shown in Table 1. In the present study, the results from rota-rod and gait analysis tests in the male and female mice of the NEx and Ex groups throughout the study period were examined (Table 1). The results showed no significant difference in body weight between the NEx and the Ex groups and among all of the groups throughout the study period.

1. Rota-rod test

Figure 2 shows the rota-rod test results of the B6 and B6-wob/t (NEx and Ex) mice. The bar graphs represent the durations of staying on the rotating rod at 4 and 12 weeks after initiation of the forced running wheel exercise, designating that before initiation of the exercise as Pre. B6 achieved 180 seconds at all time-points, and the time was significantly longer than those in the NEx and Ex groups ($p < 0.01$). At 12 weeks, the time was significantly longer in the Ex (115 ± 16.1 seconds) than in the NEx group (90 ± 24.5 seconds) ($p < 0.05$). The relationship between the body weight and staying time on the rotating rod in all of the mice that received 12-week gait training is shown as scatter plots (Figure 3). The results showed no correlation between the body weight and staying time in the rota-rod test, suggesting no influence on the numbers of mice and body weight in the NEx and

Table 1. Body weight changes during the study period.

Group	Male or female	N	Pre (4w[3])	4W[1] (8W[3])	8W (12W[3])	12w[2] (16W[3])
B6	male and female	8	15.0 ± 1.72	20.50 ± 1.82	22.6 ± 2.26	25.9 ± 4.02
	female	3	13.7 ± 1.23	18.31 ± 0.71	20.5 ± 1.27	22.4 ± 0.90
	male	5	15.7 ± 1.57	21.04 ± 1.43	23.9 ± 2.83	28.1 ± 3.53
B6-wob/t NEx	male and female	8	12.9 ± 1.77	18.14 ± 1.79	20.1 ± 2.24	22.9 ± 1.36
	female	6	12.4 ± 1.69	17.47 ± 1.44	19.1 ± 1.69	22.3 ± 0.92
	male	2	14.3 ± 1.41	20.14 ± 1.25	22.7 ± 1.08	24.6 ± 0.45
			ns	ns	ns	ns
B6-wob/t Ex	male and female	8	12.8 ± 1.78	17.93 ± 1.03	19.9 ± 1.41	22.8 ± 1.83
	female	4	11.6 ± 1.20	17.38 ± 1.05	19.1 ± 1.30	21.3 ± 1.25
	male	4	14.1 ± 1.00	19.31 ± 1.42	20.8 ± 1.03	24.2 ± 0.85

Forced running wheel exercise experiment[1)] start[2)] end stage. (Age[3)]).
Mean ± Standard deviation;
ns, not significant in Bonferroni/Dunn multiple comparison test.

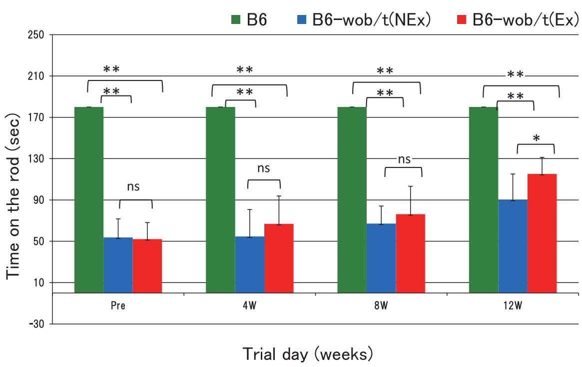

Figure 2. Rota-rod test of 16-week-old B6, B6-wob/t (NEx), and B6-wob/t (Ex) mice.
Mean ± SD, Bonferroni/Dunn.
*: $p < 0.05$; **: $p < 0.01$; ns: not significant.

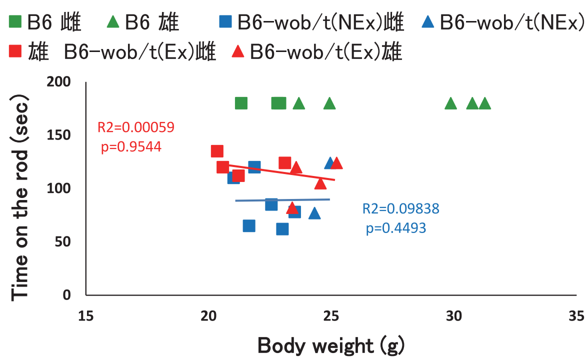

Figure 3. Scatter plots and correlation coefficients of body weight and staying time in the rota-rod test.

Ex groups.

2. Gait analysis

The four gait parameters of pelvic axis-based gait analysis are compared among the three groups in Fig. 4. The hindlimb step width was significantly wider in the NEx (43.1 ± 2.3 mm) and Ex groups (39.0 ± 2.6 mm) than in the B6 group (30.3 ± 1.6 mm) ($p < 0.01$). A significant difference was also noted between the NEx and Ex groups, and it was significantly narrower in the Ex group (Fig. 4a). The hindlimb angle was significantly wider in the NEx (40.1 ± 3.8 degrees) and Ex groups (32.3 ± 1.9 degrees) than in the B6 group (21.9 ± 3.7 degrees) ($p < 0.01$). A significant difference was also noted between the NEx and Ex groups, and it was significantly narrower in the Ex group (Fig. 4b). The hindlimb step length was significantly shorter in the NEx (44.1 ± 4.6 mm) and Ex groups (45.2 ± 3.6 mm) than in the B6 group (55.9 ± 4.2 mm) ($p < 0.01$), but no significant difference was noted between the NEx and Ex groups (Fig. 4c). The hindlimb step cycle was significantly longer in the NEx (0.50 ± 0.07 sec) and Ex groups (0.42 ± 0.05 sec) than in the B6 group (0.31 ± 0.05 sec) ($p < 0.01$). A significant difference was also noted between the NEx and Ex groups ($p < 0.05$), and it was significantly shorter in the Ex group (Fig. 4d).

Scatter plots of the normalized hindlimb step width and angle are shown in Fig. 5a. It was possible to draw dividing lines with a −1 slope to separate the data among the three groups completely. The scatter plots of the normalized hindlimb step width and length, normalized hindlimb step width and cycle, normalized hindlimb angle and step length, normalized hindlimb angle and step cycle, and normalized hindlimb step length and cycle are shown in Fig. 5b−5f, respectively. B6 and B6-wob/t could be separated in all plots of Fig. 5b-5f, but the plots of the Ex and NEx groups overlapped and could not be separated.

Discussion

Repetitive gait training has been suggested to be effective to improve ataxic gait and walking speed in cerebellar ataxic patients [16]. We forced cerebellar ataxic mice to perform gait training, and ataxic gait and coordinated movement were improved after exercise for 12 weeks.

For evaluation of the motor function of mice, the rota-rod test and gait parameter analysis are widely used [10, 11]. In the rota-rod test, mice are placed on a rod rotating at a specific speed, and the time until

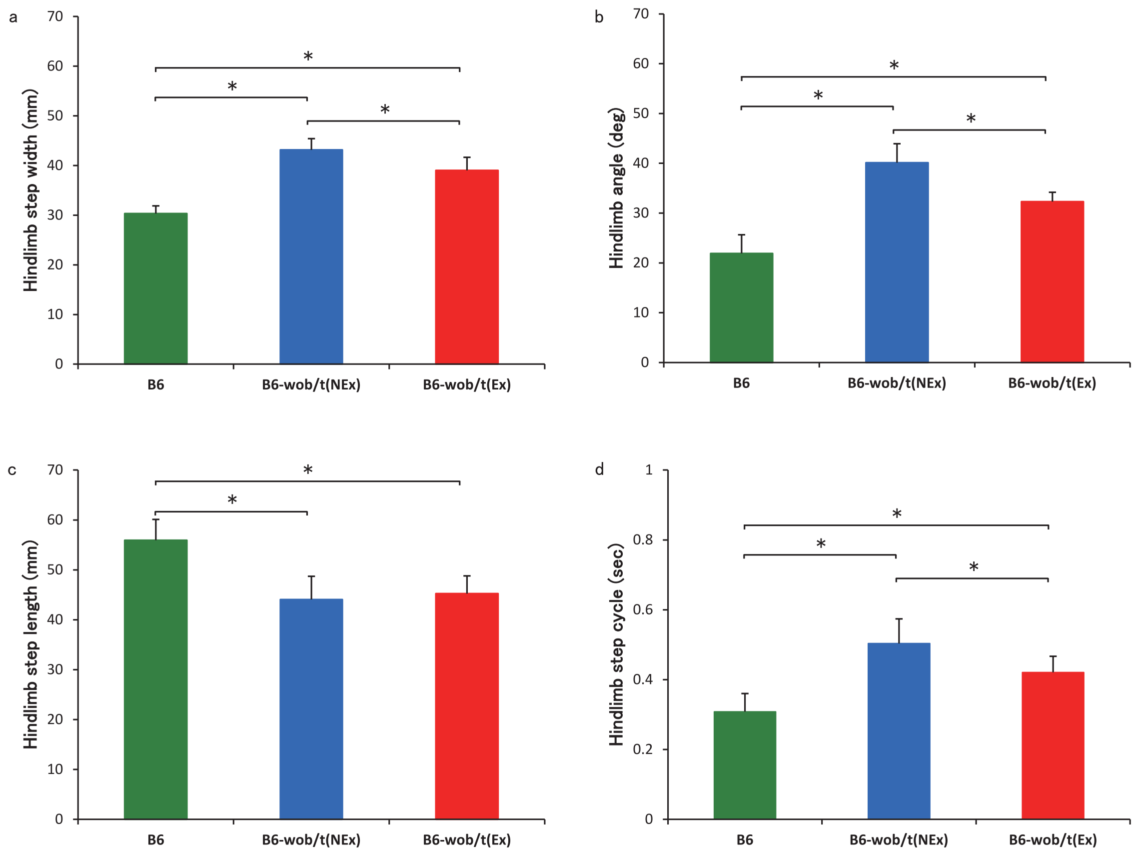

Figure 4. Comparison of the gait parameters among the three groups.
a: Hindlimb step width, b: hindlimb angle, c: hindlimb step length, d: hindlimb step cycle, mean ± SD, Bonferroni/Dunn *: $p < 0.01$.

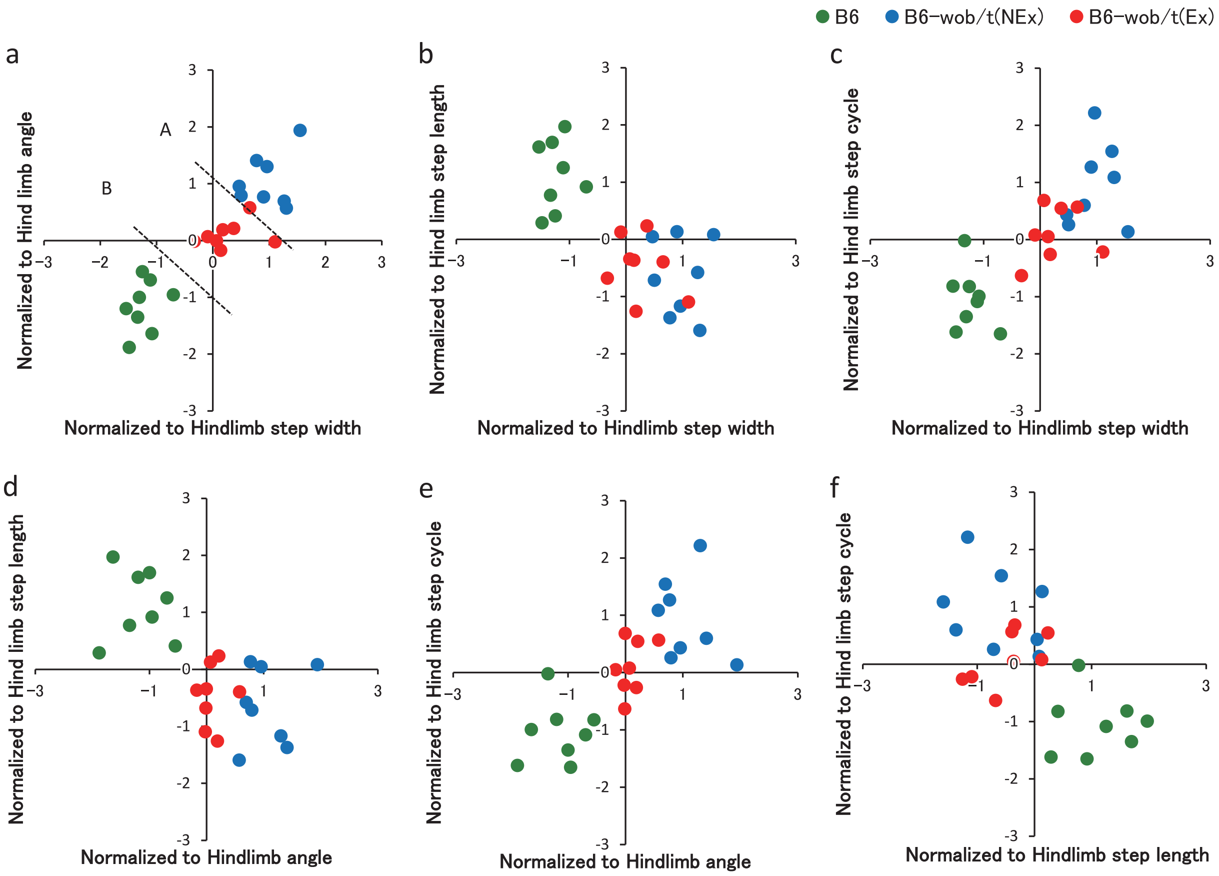

Figure 5. Comparison of gait parameters among the three groups.
Scatter plots of the normalized gait parameters (variables) in the three groups
a: Scatter plot of the normalized hindlimb step width and angle
b: Scatter plot of the normalized hindlimb step width and length
c: Scatter plot of the normalized hindlimb step width and cycle
d: Scatter plot of the normalized hindlimb angle and step length
e: Scatter plot of the normalized hindlimb angle and step cycle
f: Scatter plot of the normalized hindlimb length and step cycle
It was possible to draw dividing lines with a −1 slope to separate the data completely among the three groups in a.

falling off it is compared to investigate coordination of motor function and balance [17]. Cendelín et al. reported that the rota-rod test results were markedly poorer in Lurcher mutant mice as an olivocerebellar degeneration model than in the wild type, but no correlation was noted between the gait parameters and rota-rod test results [18].

The rota-rod test suggests possible differences in body weight between the NEx and Ex groups. Body weight differences may influence the results of the rota-rod test. Body weight changes during the study period are shown in Table 1. No difference was noted between the NEx and Ex groups, and no relationship was noted between the staying time on the rotating rod and body weight (Fig. 3).

Quantitative gait analysis is used to clarify the neurological characteristics of gait disorder [19]. The pelvic axis-based gait analysis method reported by us [3] is capable of quantifying the hindlimb angle of ataxic mice that walk by laterally bending the trunk, which is difficult to measure using the conventional analytical method [3]. On the pelvic axis-based gait analysis, the hindlimb step width and angle were significantly wider, the hindlimb step length was significantly shorter, and the hindlimb step cycle was significantly longer in the NEx than in the B6 group, suggesting that sway of the center of gravity is compensated for by widening the hindlimbs to increase the support base area, forming the ataxic pattern of B6-wob/t. In addition, the gait parameters in the Ex group were intermediate between those of the NEx and B6 groups, suggesting that the forced gait training directly improved ataxia and reduced compensatory movement. The level of this improvement may be quantified based on the distances in the y and x directions on scatter plots of the normalized hindlimb angle and step width [3], for which further investigation is awaited.

In the animal experiment, the numbers of male and female mice differed among the B6 group and the NEx and Ex groups of B6-wob/t. This may have resulted in weight differences among the groups and influenced the results of rota-rod and gait analysis tests. However, we selected body weights and phenotypes before the start of the test [1], as previously reported [3]. As a result, no difference was noted between the NEx and Ex groups during the study period (Table 1). In addition, no relationship was noted between the staying time on the rotating rod and body weight (Figure 3). The step widths and angles of the B6-wob/t NEx group were significantly larger than those of the B group with greater body weight (Fig. 4). These results are as previously reported [3]. After gait training, the step widths and angles of the Ex group became significantly smaller than those of the NEx group, and became closer to, but were still significantly larger than, those of the B6 group. Thus, the gait parameters, step width and angle, were not correlated with the body weight. In the future, the breeding technology of B6-wob/t will be improved in order to examine male and female mice separately.

The total amount of gait training in the 12-week period was 21.6 km and about 518,000 steps (41.7 ± 4.7 mm per step; Figure 4 and [3]) in the B6-wob/t Ex group. This was about 4.7 times the total number of steps in a normal maintenance gauge (about 110,000 steps converted from 1,327 steps/day using a spontaneous momentum measurement system, ACTIMO-100N (Shifactory Co. Ltd., Fukuoka, Japan)). This level of difference in the amount of exercise was necessary to obtain a significant difference between the NEx and Ex groups, supporting the opinion of Kottke et al. [20] that a large number of repetitions is necessary to form an engram in the brain. Larsen et al. analyzed cerebellar Purkinje cells from normal rats at 5−23 months of age after horizontal treadmill forced running exercise at 20 m/min for 20 minutes twice daily, five days a week [21]. In terms of the results, the numbers of Purkinje cells decreased through degeneration in the aged rats of the non-exercise group, but remained the same in those of the exercise group, compared with adult rats. Although the training conditions are different in animal species and exercise amounts between Larsen's and our experiments, it should be noted that the Purkinje cells of B6-wob/t spontaneously degenerated and were lost at 20 days after birth [2]. Exercise with gait training as early as at 4 weeks of age may improve Purkinje fiber degeneration. This will be histopathologically investigated using cerebellar specimens. Regarding the mechanism of improvement, the protection of Purkinje cells by forced exercise is considered, as reported by Larsen et al. [21].

The effect of forced gait training using a running wheel in cerebellar ataxic B6-wob/t mice could be revealed by the combination of pelvic axis-based gait analysis and the rota-rod test. We are planning to apply this system to judge the effects of gait training, drugs, and rehabilitation on ataxic symptoms.

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