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Coordination of Locomotor Activity in Transgenic C57Bl/6 Mice with Hereditary Neuropathy

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Locomotor activity of C57Bl/6 mice with hereditary motor and sensory neuropathy (HMSN; an animal model of Charcot–Marie–Tooth, CMT, disease) was investigated in males and females of two ages (15 and 20 weeks) using the balance beam test (inclined beam); such indices as time of traveling via the beam to the shelter and number of slippings of the hindlimbs from the beam were recorded. It was found that C57Bl/6 mice spent dramatically more time for traveling than control C57Bl mice with no neuropathy, and the number of erroneous movements (slippings of the hindlimbs) during traveling in mice with HMSN was many times greater than that in the controls. The deficiency of control of locomotion in C57Bl/6 animals was found to be sex- and age-dependent. Females of this strain moved significantly slower than males of the same age categories; both 20-week-old males and females with HMSN spent significantly more time for traveling the test distance than 15-week-old animals and demonstrated more motor failures. Thus, symptoms of HMSN are more pronounced in females (probably due to the specificity of the hormonal background in the latter), and the severity of pathology increases with age. The balance beam test appears acceptable for obtaining easily interpretable quantitative characteristics of the quality of locomotion control in experimental animal models of neuropathies.

Keywords: locomotor activity, balance beam test, peripheral neuropathy, transgenic C57Bl/6 mice, Charcot–Marie–Tooth disease

INTRODUCTION

Hereditary motor-sensory neuropathy (HSMN), or Charcot–Marie–Tooth disease of type 1A (CMT1A), accounts for about 80% of all demyelinating hereditary neuropathies [1]. This disease results from the destruction of one or several different but interrelated cellular pathways, each of which is necessary for normal myelination of the nerve fibers [2]. Point mutations in the *PMP22* gene are one of the main reasons for the development of this disease; these mutations induce disorders of the process of myelin assemblage. This gene is expressed in many cells of the organism, but such expression is most intense in Schwann cells [3, 4]. Another pathway for the development of HSMN is DNA duplication at the site of the *PMP22* gene. Such duplication causes a disorder in peripheral myelination, which results in slowing down of the conduction via motor and sensory nerve fibers; this leads to a decrease in muscle strength and to impaired mobility of the upper and lower extremities; subsequently, CMT1A leads to deformation of the limbs and strong disruption of the biomechanics [5, 6].

Genetically modified animals are at present extensively used as models of some diseases in humans. As was found, the C57Bl/6 strain of mice is a most adequate animal model for the studies of CMT1A [7]. The course of such pathology in mice is maximally similar to that of the above disease in humans. Since there is no effective medical treatment for this disease (the treatment can be only symptomatic), the current studies mostly point to physical rehabilitation as the main and necessary tool in the treatment of people suffering from CMT. It is obvious that understanding the pathogenesis of changes in the musculoskeletal system during this disease is a prerequisite for finding the most adequate and effective pharmacological means and methods of physical rehabilitation [8–11].

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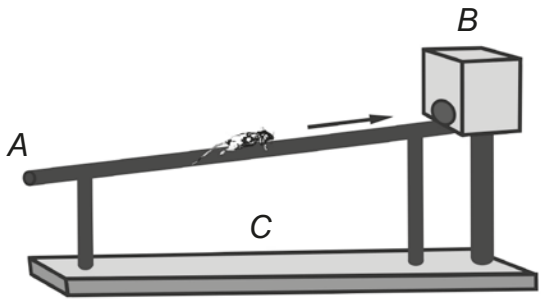
In our previous publication [12], we described changes in the conduction velocity via a peripheral nerve and in the process of neuromuscular transmission in the C57Bl/6 mice compared to C57Bl animals. Considering that information on the specificities of motor activity in C57Bl/6 mice is rather limited we, in the experiments described below, estimated the pattern of locomotor coordination in these animals using a balance beam test. This test allows one to indirectly evaluate the dependence of motor activity in normal and transgenic mice on the level of disorders in the process of myelination of nerve fibers.

METHODS

Experimental Groups. In the study, 8 groups of mice weighing 22–29 g were used. Groups 1–4 included control (C) C57Bl animals of both sexes (male, M, and female, F), aged 15 and 20 weeks, respectively (CM15, CM20, CF15, and CF20; $n = 6$, in each group). Groups 5–8 consisted of genetically modified animals of strain C57Bl/6 with peripheral neuropathy (Np), also of both sexes and of the same age, i.e., NpM15, NpM20, NpF15, and NpF20, respectively; all above groups also included 6 mice each. All animals were obtained from The Jackson Laboratory (USA). Mice were kept under standard vivarium conditions, with free access to food and water. Twenty minutes before the start of testing, the cages with mice were housed in a darkened room, which allowed the animals to adapt more quickly to the testing conditions and to reduce the stress level.

Balance Beam Test. To perform this motor test, we used a special setup consisting of a cylindrical wooden beam (diameter 2 cm, length 110 cm), mounted at a small angle (about 10 deg) with respect to the horizontal plane and leading to an entry in a darkened box, which represented a comfortable room (shelter) for the tested mouse (Fig. 1). Motor behavior of an animal on the beam was recorded by a video camera. Using these records, full time of passing through the beam by the animal and motor failures (slippings of the hindlimbs from the beam) could be estimated.

Before each testing, each mouse was trained by placing it on the beam at a small distance from the entry in the box, allowing it to travel the set distance. In the subsequent trials, the distance gradually increased. Trainings were carried out until the mouse became stably able to cover the entire distance without assistance. Under the beam, there was a foam pad preventing injury of the animal in the case of fall. After successful training, the mouse was put on the route beginning; its motor behavior until its entry in the box was observed and video recorded. After this, the entry was closed, and the animal was allowed to rest for 1 min. Then, the testing was repeated (10 successful trials for each animal). Before testing of the next mouse, the set was cleaned with 70% ethanol.



F i g. 1. Scheme of the set for the balance beam test. A) Cylindrical wooden beam, length 110 cm, diameter 2.0 cm; B) darkened room (shelter), and C) basement with a foam pad.

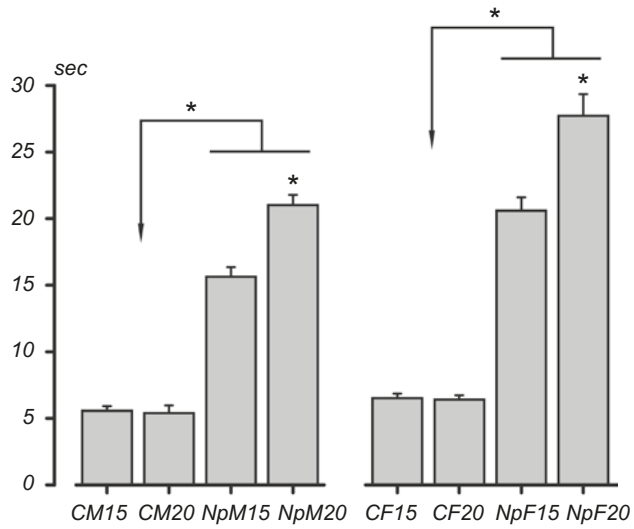
Statistics. Means and s.e.m. values were calculated for the examined groups and compared using a two-way ANOVA. The significance of intergroup differences was assessed using the Student's *t*-test; these differences were considered significant at $P < 0.05$.

RESULTS

The behavioral test used was carried out for estimation of deficiencies in coordination of locomotor activity in male and female mice of different ages; groups of mice suffering from HSMN were compared with sex- and age-matched groups of control animals. As was found, all animals after the respective training were capable of successfully performing the balance beam test; mice of the C57Bl/6 strain required a considerably longer time of training.

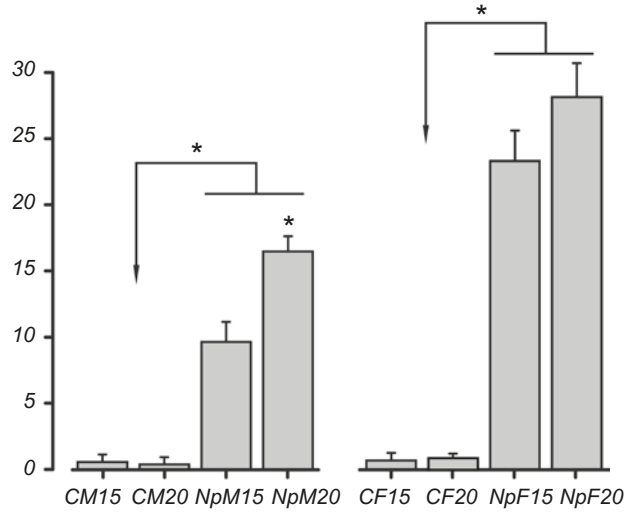
As can be seen in Fig. 2, the average time of passing the test distance by different control animals varied within a 5.4 ± 0.7 to 6.5 ± 0.4 sec range. The differences between these data obtained for control animals of group C57Bl did not reach the significance level ($P > 0.05$ in all comparisons); nonetheless, it can be mentioned that female control mice spent a slightly (insignificantly) longer time for travelling by the beam than control males.

At the same time, mice with HSMN demonstrated dramatically greater durations (3–5 times, on average) of travelling via the beam to the shelter. In these animals, the mean values of this index varied from 15.7 ± 0.7 to 27.7 ± 1.6 sec in both males and females. The differences in travelling times between comparable sex and age groups of C57Bl and C57Bl/6 mice were highly significant ($P < 0.01$). It should also be mentioned that C57Bl/6 female mice covered the test distance noticeably slower (by 5–8 sec, on average) than the age-comparable males with the same pathology ($P < 0.05$). In addition, difficulties in the control of locomotion typical of C57Bl/6 mice demonstrated certain age dependence. In both male and female mice with HSMN, 20-week-old animals showed slightly but significantly longer times for reaching the shelter than 15-week-old mice ($P < 0.05$; Fig. 2).



F i g. 2. Mean values (M ± s.e.m.) of time, sec, spent by experimental animals for traveling to the shelter. Groups of animals are shown below: CM15, CM20, NpM15, and NpM20 are males of the control group (C) and males with neuropathy (Np) aged 15 and 20 weeks, respectively; CF15, CF20, NpF15, and NpF20 are females of the respective groups. Asterisks show the significance of intergroup differences ($P < 0.05$).

During the performance of the beam test, we also calculated the number of motor failures (slippings of the hindlimbs from the beam). Animals of all control groups usually walked the test distance either with no slippings of the hindlimbs or with a small number of such events. Usually, the mean number of slippings per one trial was below 1.0 for both male and female mice of the C57Bl strain (in females, this index was slightly greater; Fig. 3). There were no significant differences between control groups of males and females of different ages from the above aspect. At the same time, the number of such events shown by mice with HSMN (strain C57Bl/6) was dramatically (more than by an order of magnitude) greater. In particular, 15- and 20-week-old males (groups NpM15 and NpM20) showed, on average, 9.9 ± 1.5 and 16.4 ± 1.1 hindlimb slippings per trial, while females of the same age (groups NpF15 and NpF20) had 23.2 ± 2.3 and 28.0 ± 2.6 slippings, respectively (Fig. 3). As can be seen, there was a certain dependence of these indices on sex and age of the animals. Differences in the mean number of slippings between groups of males NpM15 vs. NpM20 and also between groups NpM15 vs. NpF15 and NpM20 vs. NpF20 were significant ($P < 0.05$).



F i g. 3. Mean number of motor failures (slippings of the hindlimbs from the beam) during traveling the test distance by experimental animals. Designations are similar to those in Fig. 2.

DISCUSSION

The development of hereditary neuropathy is accompanied by motor and sensory disorders manifesting as excessive or insufficient motor activity, impairment of motor coordination, and that of somatosensory sensitivity (mostly in distal segments of the limbs) [3, 13]. As is known and mentioned above, the cause of peripheral nerve myelinopathy in the case of CMT1A is a mutation of the *PMP22* gene that encodes the corresponding protein. Such mutation can lead to an increase in the number of Schwann cells and excessive mitotic activity, peripheral demyelination, and progressing muscle atrophy [7].

The results of the behavioral test used in our study show that adult mice of strain C57Bl/6 meet significant difficulties in locomotion via the inclined beam to a goal (shelter). These animals spend much more time for reaching this shelter, and their movements are accompanied with numerous motor errors (slippings of the hindlimbs from the beam). At the same time, after training, control C57Bl mice performed this test with no considerable difficulties.

It should be noted that older (20 week old) males and females with neuropathy overcame the test distance about 26% slower than younger animals (15 week old), and the number of hindlimb slippings was greater by 42% and 17% in older males and females, respectively, as compared with the respective index in 15-week-old animals. These facts may indicate that the intensity of pathological shifts in motor behavior increases over time. Walsh et al. [14] reported that the amplitude of compound muscle action potentials and action potentials generated by sensory neurons decrease in aging C57Bl/6 mice of both sexes. In our study, we also observed that females with HSMN passed the beam test more slowly (by about 24%) than males of the same age category. These data may indicate that the examined pathology is noticeably more pronounced in female animals than in males. In a study on humans, Padua et al. [15] noted that women with CMT usually demonstrate a higher degree of motor disability than men of the same age. This difference can be related to the presence in women of a steroid hormone, progesterone, which promotes PMP22 overexpression and, therefore, contributes to a stronger increase in the level of nerve fiber demyelination [16]. Caruso et al. [1] examined the effects of $GABA_A$ receptor modulators in the male and female CMT1A experimental models. These authors demonstrated that $GABA_A$ receptors are expressed in both sciatic nerve fibers and Schwann cells, and activation of these receptors by neuroactive steroids, such as 3α5α-tetrahydroprogesterone and 3α-diol, increases the level of PMP22 expression. Thus, the latter effect is sex-dependent. In a primary culture of rat Schwann cells, the expression of PMP22 is stimulated by 3α5α-tetrahydroprogesterone only in cultures obtained from sciatic nerves of female rats [17].

The above-described behavioral study on transgenic mice with peripheral neuropathy showed that such rather simple testing technique helps to obtain demonstrative quantitative results with respect to changes in motor activity during the development of the pathology and to objectively estimate the level of pathological changes caused by HSMN, including gender and age specificities. Such a technique (and/or similar ones) may help experimenters to assess the effectiveness of therapeutic approaches in the treatment of this pathology at different stages of development of the latter. Thus, along with electrophysiological techniques for diagnosing this pathology, gait analysis is one of the main behavioral approaches to determine the extent of peripheral nerve damage in experimental animals.

All experimental procedures on animals were carried out in accordance with the Council Directive 86/609/EEC of November 24, 1986, and with permission of the Bioethics Committee of the Bogomolets Institute of Physiology (Kyiv, Ukraine).

The authors, I. O. Govbakh, D. O. Zavodovskiy, N. V. Bulgakova, O. M. Tsupykov, D. A. Vasylenko, and A. V. Maznychenko, confirm the absence of conflicts in relations with persons and organizations having any relation to the study and in interrelations of the authors.

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