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Research report

Age dependence of strain determinant on mice motor coordination

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Abstract

Evaluation of motor coordination and motor learning in mice remains a challenge as many factors may interact with the different tests used. Among these factors, genetic background has been reported to be a major determinant of mice performances in motor coordination tests. However, it is not known if the strain dependence of motor coordination and motor learning remains constant through life. In order to assess this point, we tested during 5 days male and female mice of three different strains (NMRI, C57BL/6J, and C57BL/6J × 129OlaHsd) in runway, rotarod, and thin rod tests at juvenile (first day of testing = postnatal day 19) and adult (3 months) age. We found a strong strain effect on motor performances and motor learning at juvenile age (C57BL/6J performing more poorly than the two other strains), whatever the tests used. Interestingly, the C57BL/6J mice were the best performing mice at the adult age. These strain rankings were observed either in male and female groups. These results demonstrate that the strain determinant on mice performances and motor learning is highly age dependent.

Theme: Motor systems and sensorimotor integration
Topic: Cerebellum

Keywords: Motor coordination; Strain; Age; Mice

1. Introduction

Ataxia is a common characteristic in many neurological disorders. As cerebellum plays a central role in motor control and especially in the fine tuning of movements [17,18,22], ataxia and other impairment of motor coordination are often associated with cerebellar dysfunction although other brain regions, such as vestibulum, motor cortex, striatum, or spinal cord, may also be involved. Quantifying motor coordination in mice models of ataxic disorders is crucial in the evaluation process of the model or of subsequent therapeutic approaches. Moreover, impairment of motor coordination in mice may be impossible to detect in standard rooming environments and appears when mice are challenged in tests designed to specifically evaluate motor coordination [1,2,19]. Accelerated rotarod, where the mice have to stay as long as possible on an accelerating rod, is the most commonly used test in this purpose [4,9]. Runway (where the mice have to run along a thin bar without slipping) and stationary horizontal thin rod test (where the mice have to stay as long as possible on a thin bar) have also been used recently [14]. The sometimes subtle differences between normal and impaired mice complicate the choice of an adequate test. Indeed, the discrimination between normal and slightly impaired mice requires a test which as to be not too easy for the impaired, but not too difficult for the normal mice. If these two conditions are not fulfilled, normal and impaired mice may wrongly appear similar, even with perfectly matched test groups. It is thus very important to know the factors that

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may potentially interfere with the different tests and the possible interactions between these factors. Among them, strain [7,8,10,15,20] has received much attention these last 10 years because of the employment of different mouse strains in transgenic technology. For instance, C57BL/6J, a frequently used strain, is considered to perform very well in motor coordination test in comparison with other strains, but it is not known if this superiority is constant for both genders and through ages. The importance of strains in genetic engineering goes far above motor coordination or behavioral performances, as different mutations may appear very impairing in certain genetic background and nearly asymptomatic in others [3]. Indeed, if gender and age [11,20] also appear to interact with motor coordination evaluation, their interactions with strain have not yet been reported to our knowledge. The choice and the interpretation of motor coordination test according to the strain, age, and gender of the evaluated mice remain thus widely empirical. To assess this point, we evaluated 3 currently used mice strains at juvenile and adult ages through 3 different motor coordination tests. The tests were performed during 5 consecutive days in order to evaluate the motor performances and the motor learning ability of the mice. We found that the strain determinant on motor performances is largely dependent on the age of the tested animals.

2. Materials and methods

Naive male and female juvenile (at the first day of the test the animals are P19) and naive adults (3 months old) of C57BL/6J (B6) (Iffa Credo, France), NMRI (Iffa Credo, France) strains, and 129/OlaHsd × C57BL/6J F2 crosses (129B6) (inbred strains were obtained from Harlan and Iffa Credo, France) were used in this study. The animals were housed in the same sex groups of three to four animals per cage in clear plastic cages maintained in a temperature- and humidity-controlled room on a 12-h light–dark schedule with food and water provided ad libitum. All experiments were conducted in the light phase of circadian cycle between 9:00 AM and 4:00 PM. Every day, animals were sequentially subjected to the following tests: runway, stationary horizontal thin rod, and accelerating rotarod test as described below. The study was approved by the Institutional Ethical Committee of the School of Medicine, Université Libre de Bruxelles, Belgium.

2.1. The runway test

In this test, mice ran along an elevated runway with low obstacles intended to impede the progress of mice. The runway was 100 cm long, either 1.2-cm or 0.7-cm width for adults or juvenile mice, respectively. We used two different sizes for the width of the runway test in order to adapt the test to the size of the mice. Using a 1.2-cm width test for the juvenile mice, this one was too easy and not discriminating enough. Obstacles being of 1-cm diameter wood rod took place every 10 cm along the runway, the width of the obstacles being adjusted to the width of the runway. The number of slips of the right hind legs was counted. Mice were placed on one brightly illuminated extremity of the runway and had to run to the other side where they retrieve their cage. Animals were given four trials per day during 5 consecutive days.

2.2. The stationary horizontal thin rod test

This test consists of a horizontal fixed thin rod of wood (diameter 0.6 cm) placed 30 cm above the cage of the animals. Mice were transversely placed on the rod and their latency to fall was measured. Animals staying during 60 s were taken from the rod and recorded as 60 s. Mice were given four trials per day during 5 consecutive days.

2.3. The accelerating rotarod

The rotarod apparatus (accelerating model Ugo Basile) consisted of a plastic roller (3 cm in diameter) with small grooves running along its turning axis. On the first day, mice were given a training session. During this training session, every mouse was placed on the rotarod at a constant speed (4 rpm) for a maximum of 60 s. Afterwards, mice received four trials per day during 5 consecutive days. During each test session, animals were placed on the rod rotating at a constant speed (4 rpm) and, as soon as all the animals were placed on the rod, the rod started to accelerate continuously from 4 to 40 rpm over 300 s. The latency to fall off the rotarod was recorded. Animals staying during 300 s were taken from the rotarod and recorded as 300 s.

2.4. Statistical procedure

As all strains presented strong motor learning through days, strains were compared every day by a one-way ANOVA test and for all days by a two-way repeated measures ANOVA test. The same procedure was used for single-gender groups. In addition, two-way non-repeated measures ANOVA tests (Strain × Gender) were also used to compare mice every day.

Results are expressed as mean ± SEM and were considered significant if $P < 0.05$. All analyses were performed on Statistica 6.0.

3. Results

3.1. Accelerating rotarod

At juvenile age, a strong effect of genetic background was observed on rotarod performances from day 1 to 5 ($F(2,38) = 19.99$, $P < 0.000001$ compared with one-way ANOVA, day 1) (Fig. 1A). The highest performance at

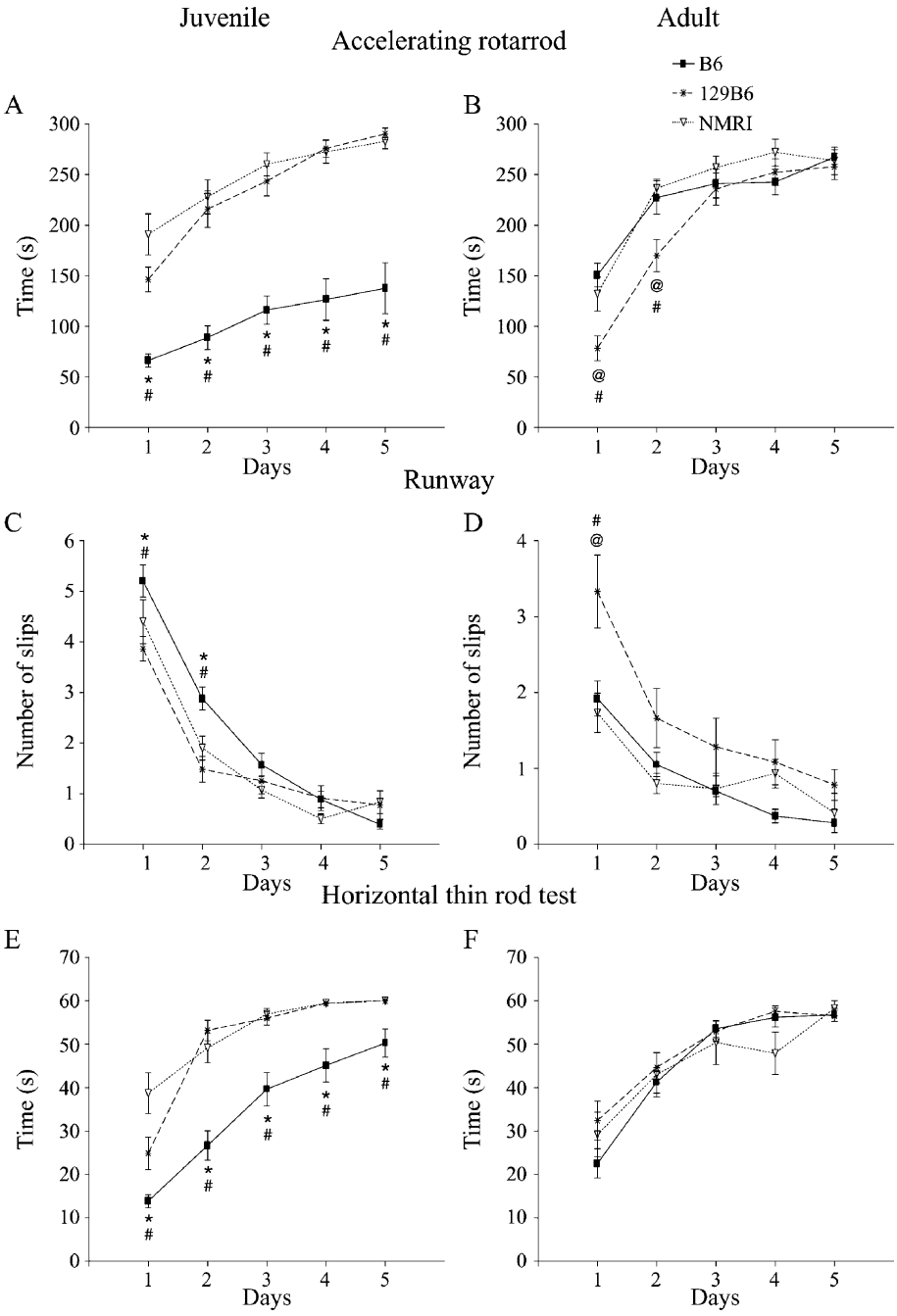

Fig. 1. Performances of juvenile and adult B6 (-■-), 129B6 (-*-), and NMRI (-▽-) mice (genders pooled). At juvenile age, the highest performing mice on the accelerating rotarod are the 129B6 and the NMRI, whereas B6 behaves poorly (A). At adult age, the best performing mice are the B6 and the NMRI, outperforming the 129B6 mice on day 1 and day 2 (B). The runway test confirms the ranking of rotarod at juvenile age, the better performing strains being the 129B6 and the NMRI (C). At adult age, the best performing strains were the B6 and the NMRI (D). On the horizontal thin rod test, the highest performing strains at juvenile age are the 129B6 and the NMRI, B6 behaving poorly (E). At adult age, the superiority of 129B6 on B6 mice disappeared (F). Results expressed as mean ± SEM; *$P < 0.05$ compared to the 129B6, #$P < 0.05$ compared to the NMRI, and @$P < 0.05$ compared to the B6.

juvenile age was achieved by the 129B6 and the NMRI groups, with no significant differences between these strains whereas B6 behaves poorly. This strong effect of genetic background on rotarod performances was equally observed in female ($F(2,19) = 8.10$, $P = 0.003$ one-way ANOVA, day 1) and in male ($F(2,16) = 11.46$, $P = 0.0008$ one-way ANOVA, day 1).

In strong contrast with the juvenile ranking of strains, the B6 and NMRI mice were the best performing strains among the adult mice, as they statistically outperformed the 129B6 mice on day 1 and day 2 ($F(2,38) = 9.36$ $P = 0.0005$, one-way ANOVA day 1) (Fig. 1B). Because of rapid motor learning in all strains (Fig. 1B), two-way repeated measures ANOVA test failed to demonstrate a

significant effect of background on all days of performances ($F(2,38) = 2.55$ $P = 0.09$).

3.2. Runway assay

At juvenile age, the runway test confirmed the ranking of rotarod, demonstrating a strong effect of genetic background on mice performances on day 1 and day 2 ($F(2,37) = 3.27$, $P < 0.05$ one-way ANOVA day 1), the best performing strains being the 129B6 and NMRI as compared to the B6, with no significant differences between the two former ones (Fig. 1C). This effect was equally observed in male and female but did not reach statistical significance in single genders groups. However, two-way ANOVA (Strain × Gender) revealed a significant effect of strain on motor performance on day 1 ($F(2,34) = 3.12$, $P = 0.04$, day 1) with no significant effect of gender ($F(1,34) = 0.01$, $P = 0.91$ day 1). Because of motor learning in all strains, there were no statistical differences observed from day 3 to 5. However, the two-way repeated measures ANOVA test revealed a significant effect of genetic background on runway performances through all days ($F(2,37) = 3.26$, $P = 0.049$).

As observed in the accelerated rotarod, runway assay at the adult age confirmed the inversion of the juvenile strain ranking. Indeed, the best performing strains were the B6 and NMRI. One-way ANOVA demonstrated a significant effect of genetic background on runway performances on day 1 ($F(2,39) = 5.88$, $P = 0.006$) (Fig. 1D). Because of motor learning in all strains, the differences observed from day 2 to 5 did not reach statistical significance. However, the two-way repeated measures ANOVA revealed a significant effect of genetic background on runway performances through all days ($F(2,39) = 4.22$, $P = 0.02$).

3.3. Horizontal thin rod test

The horizontal thin rod test confirmed the ranking of runway assay and accelerated rotarod in the juvenile mice. Once again, 129B6 and NMRI outperformed B6 mice from day 1 to 5 ($F(2,38) = 12.55$, $P < 0.0001$, one-way ANOVA day 1) (Fig. 1E). This strong effect of genetic background on thin rod performances in juvenile mice was equally observed in females ($F(2,19) = 9.88$, $P = 0.001$, one-way ANOVA day 1) and in males ($F(2,16) = 5.75$, $P = 0.01$, one-way ANOVA day 1). If all strains were able to increase their performance during the test, 129B6 and NMRI reached their maximum after 4 days while the B6 mice never reached a plateau and continued to increase their performances all over the 5 days of the test, as previously observed in the rotarod assay. However, they remained statistically worse than the two other strains every single day.

As for the runway and the rotarod assays, the superiority of the 129B6 on the B6 mice disappeared at adult age, but the tendency did not inverse in the thin rod test. No effect of the genetic background could be pointed out on the mice performances in this test ($F(2,43) = 1.40$, $P = 0.26$, day 1 one-way ANOVA, $F(2,43) = 0.59$, $P = 0.56$ two-way repeated measures ANOVA) (Fig. 1F).

3.4. Weight effect

The constantly observed inversion of performance ranking between the B6 and the 129B6 led us to hypothesize that a difference in the body development level reached by these strains at day 19 could be involved in the differences observed between the juvenile groups. Indeed, the comparison of mice weight at day 19 demonstrated as strong effect of genetic background on the juveniles' weight ($F(2,197) = 197$, $P < 0.0001$), the B6 (5.8 ± 0.5 g) being smaller than the 129B6 (7.6 ± 0.7 g) and the NMRI (10.7 ± 0.8 g) mice. However, we could not point out any significant linear correlation between mice weight and any of the tests performances in any background. Moreover, the differences between the 129B6 and the B6 backgrounds remained highly significant when comparing the "small" 129B6 (with a body weight below the average weight of the 129B6) with the "big" B6 (body weight above the average weight of the B6) mice. Comparisons between "small" and "big" animals of the same background did not reveal any significant difference in any of the three tests.

4. Discussion

The major finding of this paper is that the strain dependence of motor coordination performances in mice is highly age-related, whatever the test used. Mice were tested at adult and juvenile (at the first day of the test animals are P19) ages. It is noteworthy that this later age corresponds to the age at which most electrophysiological studies are performed. Among the strains tested, the B6 and the 129B6 are frequently tested in genetic research, as most gene-targeted mice are produced on a mixed genetic background of C57BL/6 and various substrains of strain 129 (129B6). In order to obtain a more homogeneous genetic background, mutant mice are usually backcrossed into B6 mice to produce a congenic strain. The third strain studied in this article is the NMRI, a strain frequently used in pharmacological studies [16]. This third strain is remarkable for its good performances in motor coordination tests at juvenile and adult ages. Indeed, the NMRIs were always associated with the best performing strain whatever the age studied or the test used.

It is noteworthy that the three tests performed in this present study constantly gave the same strain ranking, which suggests that despite their rather different design, they roughly evaluate the same motor performances. However, when comparing impaired and control mice, it is frequent to observe significant differences in one test and no differences in another one [19]. In the present study, thin rod test failed to point out any significant differences

between strains at the adult age. We believe that this is due to the fact that this test was very easy for adult mice, and thus being better performant than the worse strain was nearly impossible. We previously demonstrated such a mechanism in the B6 calretinin-deficient mice ($Cr^{-/-}$), as $Cr^{-/-}$ mice could only be discriminated from control by a modified runway test presenting increased difficulties, while no significant differences could be pointed out by the standard runway assay (B. Bearzatto, unpublished results).

At juvenile age, whatever the test used, the best performing strains were the 129B6 and the NMRI without any difference between both of them. On the contrary, the juvenile B6 strain was always the worse performing strain while at adulthood this so poorly performing B6 strain became more performing than the 129B6 strain and reached the level of the NMRI mice. Moreover, juvenile B6 mice never reached the performances of the two other strains in the horizontal stationary thin rod and the rotarod tests even after 5 days of training.

In the cerebellum of adult animals, every Purkinje cell is innervated by a single climbing fiber. This one-to-one relationship between a Purkinje cell and a climbing fiber is preceded by a developmental stage in which each Purkinje cell is innervated by multiple climbing fibers. The elimination of synapses formed by supernumerary climbing fibers occurs postnatally and depends on the presence of intact parallel fibers—Purkinje cell synapses. The state of monoinnervation is established at the end of the third postnatal week [12,13] and a defective elimination of supernumerary climbing fibers underlies motor coordination impairment [5]. It could thus be possible that the low performances of the B6 animals at juvenile age are due to slight differences in cerebellar development time course with some or more persistent multiple climbing fiber innervation in this strain at P19–P23.

All mice were able to increase their performances in the three tests through days, which demonstrate their learning capacity. As the maximal performance is limited in all tests (i.e., 0 in the runway, 60 s in the thin rod, and 300 s in the rotarod), the first 2 days of learning constitute the best period to discriminate between strains. This feature was also reported in the comparison between ataxic and control mice, as motor learning frequently fades away the differences between impaired and control mice [2,19].

The accurate discrimination between an impaired and a control group does not only depend on the precise age and background matching of the studied groups, but also on the difficulty level of the test used in this purpose: a too easy task would place the impaired at the level of control mice, while a too difficult one would not allow the controls to express their superiority. Thus, the choice and the interpretation of a test must be driven by the performances expected for the age and the background of the studied mice. For instance, if one would test the early effect of a mutation expressed on a C57B6 background, a rather easy task such as the thin rod test or a modified runway assay would be adequate. Contrastingly, if the same background is studied at an older age, a much more difficult test design will be required to accurately discriminate the groups.

Other factors may also affect the strain dependence of motor coordination in mice, such as the circadian circle, the temperature of the rooming, the level of stress during the testing, or the former experience in other tests [6,8,21]. In order to help the investigators in the choice of the most appropriate test design in a precise situation, further studies are required to better define the most adapted testing conditions to each strain.

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