Comparison of the behaviour of three strains of laboratory mice in the climbing test

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Received/Érkezett: 17. 01. 2025.
Accepted/Elfogadva: 31. 03. 2025.

Abstract: In our study, we tested three commonly used strains of laboratory mice in a simple behavioural test. In the climbing test, we looked at the time of the first climb and the number of climbs of the different strains during the 5-minute test time, depending on sex and age. Based on our results, neither tribe nor sex affected climbing time and the number of climbs, but different ages did. The younger animals completed the test much sooner and climbed much more often than the adult animals. Based on our results, we can say that in a more straightforward behavioural test, different mouse strains do not have as much effect as age.

Keywords: inbred mice strain, BALB/c, C57BL/6, C3H, behavioural test

Introduction

The evolution of the laboratory mouse

Laboratory mouse strains were originally developed by selectively breeding distinct colour variants of the house mouse (*Mus musculus*) (Bonhomme and Guénet, 1996; Yonekawa et al., 1980). By the late 18th century, Japanese breeders had already established several inbred mouse strains — including albino, agouti, waltzer, and piebald varieties (Tokuda, 1935). These strains were later introduced to Europe and North America, where researchers began to document their phenotypes and uncover the genetic mechanisms behind specific traits (Darbishire, 1902; 1903; 1904; Koide et al., 1998; Little and Tyzzer, 1915; So and Imai, 1920; Tyzzer, 1915; Yerks, 1907). The precise Mus species used to develop these strains remains unclear, but genetic studies increasingly indicate that laboratory mice have a hybrid origin, derived from several *Mus musculus* subspecies: *M. m. domesticus, M. m. musculus, M. m. castaneus,* and *M. m. molossinus* (Bonhomme et al., 1987; Wade et al., 2002). As a result, behavioural differences observed between strains may reflect not only selective breeding but also differences in genetic background (Wade et al., 2002).

Behavior of different strains of mice

Among the many available strains, the C57BL/6, BALB/c, CBA/J and C3H/He lines are the most commonly used mouse strains in biomedical research (van de Lagemaat et al., 2017). Although there is predominantly a large literature evaluating the behaviour of these mouse lines (Bullock et al., 1997; Kim et al., 2005; Bortolato et al., 2013), comparing the behaviour of strains can be problematic because baseline comparisons of strains on common behavioural tests (e.g. sociability, novelty, despair, cognition, anxiety, learning and memory, etc.) show similarity (Bailey et al., 2006). Furthermore, it is difficult if the assays were tested in different laboratories under different conditions, as this may contribute to large differences or similarities in the behaviour of mouse strains (Korte et al., 2003; Mistry et al., 2014). In addition, genetic mutations from different backgrounds can also lead to different behavioural phenotypes in different strains (Kammenga, 2017), affecting the test results. Indeed, behavioural studies depend to some extent on the genetic background of the experimental animal (Grant et al., 1992) and knowledge of small behavioural differences between strains (Schauwecker, 2011; Brooke et al., 2010; Bothe et al., 2005) can significantly influence the result of our investigation.

Presentation of the mouse strains used in the study

In this study, we focused on three commonly used inbred laboratory mouse strains: C57BL/6, BALB/c, and C3H/HeJ. The C57BL/6 strain was developed by C. C. Little in 1921 and remains one of the most frequently used mouse strains in research. These mice are black with pigmented eyes and are employed in a wide variety of biomedical experiments. The BALB/c strain, characterised by its white (albino) appearance, was developed in the 1930s by G. D. Snell from H. J. Bagg's original "Bagg albino" line. This strain is also widely used in many types of research (Suckow et al., 2001). The C3H/HeJ strain, often referred to simply as C3H, was established by L. C. Strong in 1920 through a cross between a "Bagg albino" female and a DBA male (Strong, 1935). This strain is used broadly in research areas such as oncology, infectious diseases, and sensory or cardiovascular biology.

Despite representing distinct lineages, many laboratory mouse strains display a high degree of genetic similarity, with relatively low inter-strain genetic variability (Potter, 1978).

The aim of our study was to examine whether three commonly used laboratory mouse strains (C57BL/6, BALB/c and C3H/HeJ) show any behavioural differences in a simple climbing test, considering age and sex as additional factors. This question is relevant because even subtle behavioural differences among strains can influence the outcome and reproducibility of behavioural experiments. Understanding these potential differences helps improve the selection of animal models and contributes to the refinement of experimental design in behavioural studies.

Materials and Methods

The experiments were conducted at the rodent facility of the Kaposvár Campus, part of the Hungarian University of Agricultural and Life Sciences. A total of 60 mice were used, including 20 BALB/c, 20 C57BL/6, and 20 C3H strains. Both sexes were represented equally, with 30 adult and 30 juvenile mice selected for the study.

Outside the testing periods, the animals were housed in standard T4 laboratory plastic rodent boxes under controlled laboratory conditions (temperature: 20–22 °C). The lighting followed a 12-hour light/12-hour dark cycle, using reversed daylight. Illumination was provided automatically: during the light phase, OSRAM L36W/640 fluorescent tubes were used, while in the dark phase, Philips TL-D 36W/10 red fluorescent lights were applied. Bedding consisted of cleaned wood shavings (LIGNOCELL, J. Rettenmaier & Söhne GmbH), and animals received complete rodent feed (Ssniff S8106-SO11 Spezialdiäten GmbH) along with ad libitum access to water.

The behavioral testing apparatus was designed by the authors. It included a 27 cm tall rod with a diameter of 0.9 cm, mounted at a 90-degree angle onto a round base with a diameter of 10 cm. Both the climbing rod and base were made of untreated, unsanded wood. For the experiments, five identical climbing rods were positioned within a circular plastic container (65 cm in diameter and 55 cm in height). The arrangement of the rods was consistent across all tests (Figure 1).

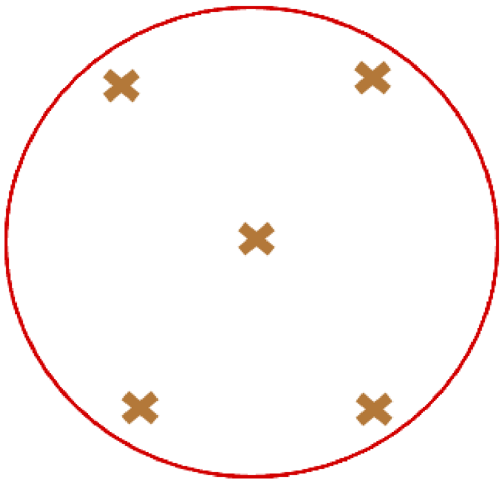

Figure 1 Arrangement of climbing rods in the plastic container

Each selected mouse was individually placed into the testing container. After every trial, the climbing rods and the container were wiped down with an alcohol swab to eliminate scent traces, ensuring that the behavior of subsequent mice was not influenced by residual odors. The entire duration of each test session was 15 minutes. The first 10 minutes served as an acclimatization or "rest" period, allowing the mouse to explore and become familiar with its new surroundings. After this habituation phase, a 5-minute observation period followed, during which behavioral data were collected.

During the 5-minute test phase, the observers recorded whether the mouse climbed the rod, how many times it climbed, and the exact time point (in seconds) of the first climb. Behavior was assessed through direct observation and manually documented by two trained observers. No video recordings were made. Mice were categorized by strain and age group: young mice were between 28 and 35 days old, while adults were approximately 500 days old. Both male and female mice were included in the study.

To analyze the time of the first climb, a survival analysis was performed using the Kaplan-Meier estimator (Kaplan and Meier, 1958), where the survival function S(t) represents the probability that a mouse had not climbed the rod by time "t": $S(t) = P(T > t)$. Differences in survival curves across strains, sexes, and age groups were evaluated using the Log-rank test. The influence of strain, sex, and age on climbing latency was further assessed using the Cox Proportional Hazards model. Since the number of climbs followed a Poisson distribution, Poisson regression was applied to evaluate the effect of the independent variables. All statistical analyses were conducted using IBM SPSS Statistics version 29.0.

Results and discussion

The survival function for the three laboratory mouse strains is shown in Figure 2. The curves show the proportion of the mouse strain that climbed the rod. Based on the log-rank test, there is no significant difference between the three strains of mice ($p = 0.118$).

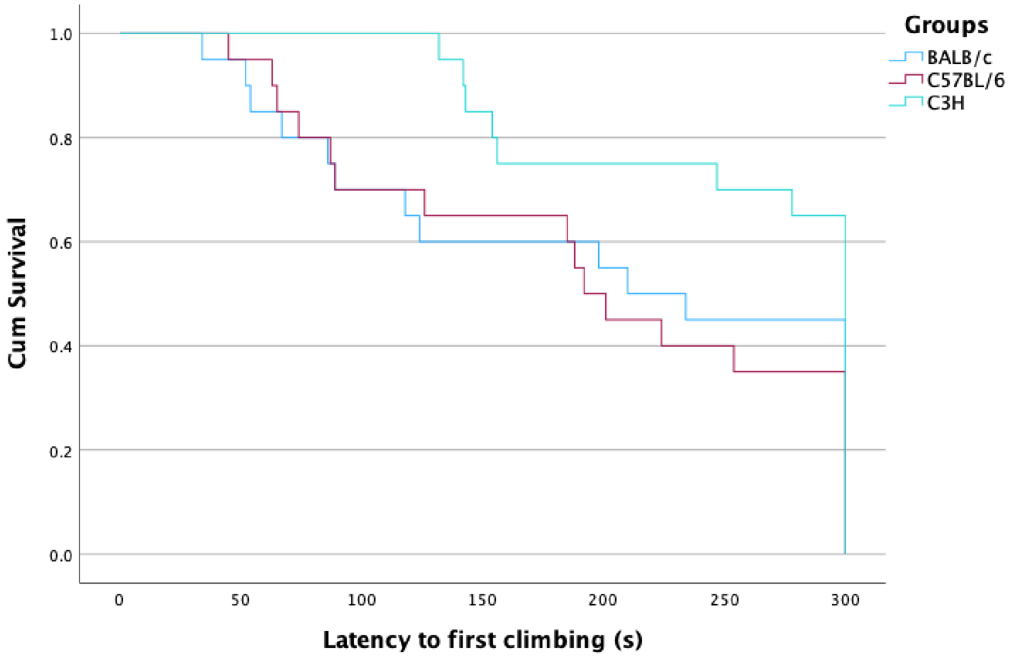

Figure 2 The survival curve showing the climbing of the three strains of laboratory mice.

The figure shows that the first climb of the BALB/c and C57BL/6 mice occurred close to the 50th second, while the first climb of the C3H mice only occurred around the 130th second. 55% of the BALB/c mice, 65% of the C57BL/6 mice, and only 35% of the C3H mice climbed the rod during the 5-minute test.

Breaking down the climbing by sex, based on the Log-rank test, there was no significant difference in climbing between male and female mice ($p = 0.869$) (Figure 3).

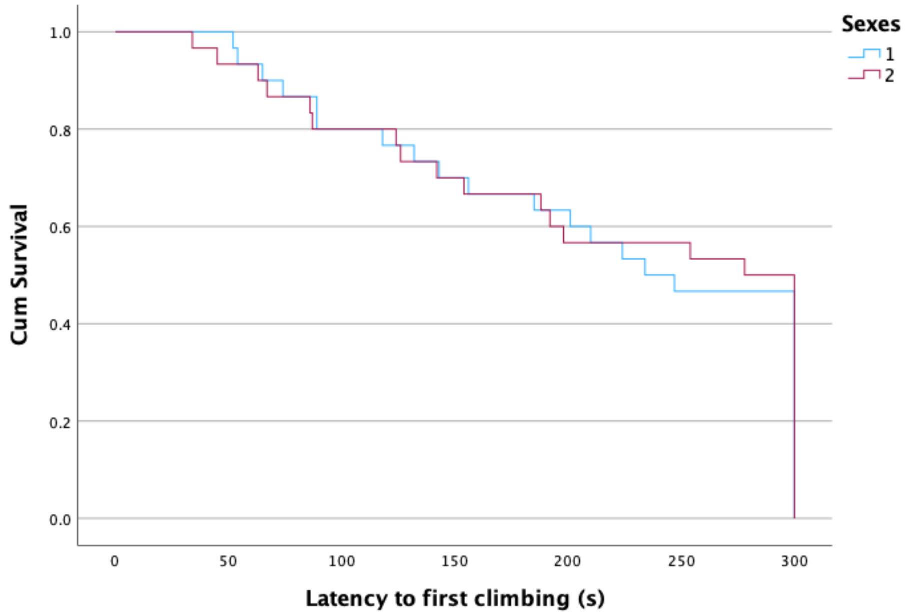

Figure 3 Climbing survival curve of male and female mice.

The figure clearly shows a big difference in the time of the first climb between the groups of male and female mice; the first climb of both sexes took place around the 50th second; 53% of the male mice climbed the rod, 50% of the females.

Based on the log-rank test, there was a significant difference in climbing between adult and young mice ($p < 0.001$) (Figure 4).

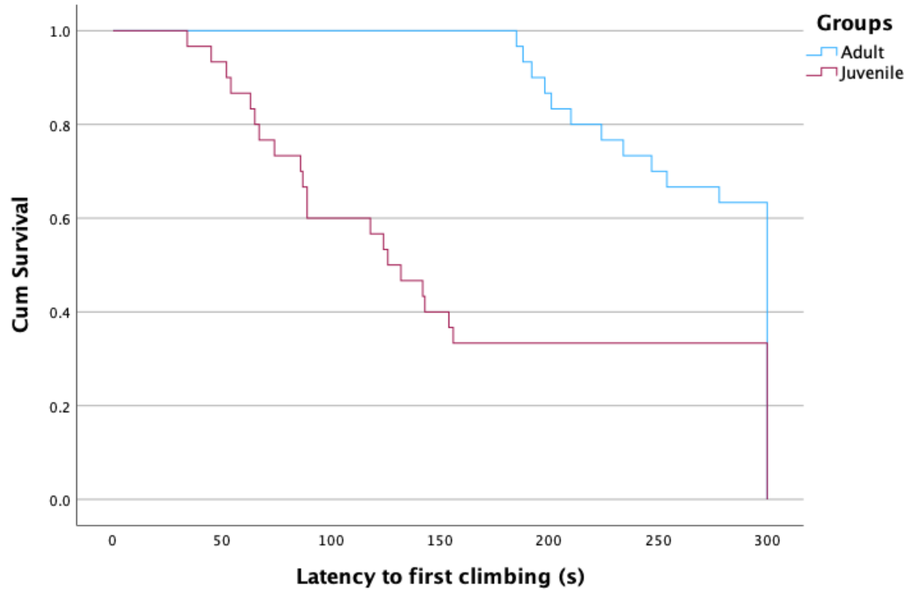

Figure 4 Survival curve showing climbing of young and adult mice.

The figure shows that the first climb of the young mice took place before the 50th second, while the first climb of the adult mice took place somewhere around the 190th second. Furthermore, young mice climb the rod at a higher rate than adult mice; 36% of the adults climbed up, while 63% of the young climbed up the rod during the 5-minute test.

Table 1 Maximum Likelihood estimates of Cox regression coefficients (B) and Hazard ratios (Exp(B)) examining the effects of mouse strain, sex, and age of mice on the duration of their first climbing.

Items	B	SE	Sig.	Exp(B)
Mouse strain	-0.225	0.155	0.145	0.798
Sexes	-0.010	0.258	0.969	0.990
Age	0.677	0.264	0.010	1.968

B = Positive coefficients, SE = Standard error, p = Significant level, Exp (B) = Hazard ratio

Based on the Cox regression, mouse strain and gender did not significantly affect the time of the first climb, but age had a significant effect. Based on the Poisson regression, in the activity of how many times the mice climbed up during the test time, no significant difference was found between the mouse strains ($p = 0.417$), just as there is no significant difference between the sexes ($p = 0.363$). However, between the ages, we found a significant difference in activity ($p = 0.004$); the young mice climbed the rod several times during the test period.

From our results, we can see that the difference in behaviour between the strains of laboratory mice is minimal; this can be explained by the fact that there is no big genetic difference between the strains (Kirby et al., 2010). The expression of the behavioural phenotype of a laboratory mouse depends not only on its genotype (Morice et al. 2004; Young et al. 2002), but also on several environmental variables, including laboratory variables (Crabbe et al. 1999) such as the experimenter (Chesler et al. al. 2002), or the position of the mouse cage before testing (Izidio et al. 2005). Furthermore, our results confirm Bailey et al. (2006), where it was stated that the inbred strains of laboratory mice performed similarly on general behavioural tests.

In the climbing test we used, all three mouse strains performed well, which can also be explained by the fact that the laboratory mice come from house mice (*Mus musculus*), which we know from our previous research that they can be considered excellent climbers even within the Mus genus (Bárdos et. al. 2023), which is due to the widespread distribution of house mice, that they occur all over the world and live mainly in human settlements (Brown, 1953), where it is also necessary to use the vertical space of the buildings, thus having a good climbing ability is necessary.

In our study, we did not find differences between the strains of mice, but also between the sexes in the climbing test, which can be explained by the fact that there is no significant sexual dimorphism in mice (Haisová-Slábová et al. 2010), the lifestyle of male and female house mice is very similar, which it is also manifested in the fact that, for example, both sexes build nests (Lisk et al., 1969), as both it does not collect food in the same way, so the ability to climb is not limited to one or the other sex.

On the contrary, based on our studies, we can state that the age of the mice is an important influencing factor when designing the behavioural tests since the difference is clearly visible that the young laboratory mice climbed much faster and in a much higher proportion in the climbing test than the adults. These results are consistent with a study in C57BL/6 mice, where adult and aged mice were found to be less active in open field tests than young mice (Sprott and Eleftheriou, 1974). The aforementioned statement is the same as the results of the study by DeFries and Weir (1964), where they found that 40-day-old mice were much more active than the other group, where the activity of 120-day-old mice was also observed in the open-filed test.

In summary, we can say that in simple behavioural tests, the type of laboratory mice and which strain they belong to are less important; of course, the phenotypic characteristics of each strain, such as the albino eyes of BALB/c, can influence the results of each test in strong lighting, but under appropriate housing conditions in general, it can be said that the behaviour of laboratory mouse strains is very similar, which is due to a small degree of genetic variation between strains (Bailey et al., 2006). The age of the animals, on the other hand, significantly affects the results of the tests. Thus, when designing a behavioural test, we must take into account the age of the mice to be tested.

Conclusions

When designing behavioural tests with mice, the behavioural differences between different strains of mice are insignificant, especially under appropriate housing conditions; on the other hand, the age of the mice to be tested must be taken into account since young animals can be much more active than adult individuals. It is important to conduct behavioural tests with mice of a similar age to ensure the results' authenticity.

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