Научно-практический журнал
ФАРМАЦИЯ И ФАРМАКОЛОГИЯ
(PHARMACY & PHARMACOLOGY)

ОРИГИНАЛЬНАЯ СТАТЬЯ

DOI: 10.19163/2307-9266-2024-12-1-63-73

Investigator's influence on the muscle strength assessment in animals in experiment: Comparison of automated "inverted grid" test and its classical variant

P.R. Lebedev[1], E.V. Kuzubova[1], V.M. Pokrovsky[1], A.I. Radchenko[1], S.I. Osipyan[1], Y.V. Stepenko[1], A.A. Apostol[1], L.M. Danilenko[1], A.A. Dolzhikov[1], T.G. Pokrovskaya[1], O.S. Gudyrev[1], Ya.S. Kochergina[2], O.V. Dudnikova[2]

[1] Belgorod State National Research University (BSU),
85, Pobeda Str., Belgorod, Russia, 308015
[2] Belgorod Office Chief Medical Examiners,
159, Volchanskaya Str., Belgorod, Russia, 308017

E-mail: Kuzubova@bsu.edu.ru

Received 10 Dec 2023 After peer review 27 March 2024 Accepted 11 April 2024

The aim of the work was to study the influence of the researcher on the muscle strength assessment in animals in the experiment by comparing the results of the automated "inverted grid" test and its classical variant.

Materials and methods. Male lines (Bla/J, *n*=20; FUS(1-359), *n*=20; Tau P301S$^{+/+}$, *n*=20) and their background controls (C57BL/6J, *n*=20; CD1, *n*=20) were selected for the study. The dynamics of changes in the muscle deficit of the animals was evaluated in the automated and classical variant of the "inverted grid" test.

Results. According to the results of the muscle strength assessment of mice with an edited genome of lines FUS(1-359)$^{+/-}$, Tau P301S$^{+/+}$, B6.ADysf^{prmd}/GeneJ, using the "inverted grid" test in the classical variant and the automated one, it was found that statistically significant differences were not obtained in comparison with the results obtained by the classical variant of the test. The standard error of the mean increases by 23–39% in the classical test compared to the automated one. It was shown that the standard error of the mean in the classical variant of the test in Tau P301S$^{+/+}$ mice was 6.24; 5.94; 5.88; 7.38 at 4 age points; in FUS(1-359)$^{+/-}$ mice, 4.49; 6.8; 6.98 and 4.1; B6.ADysf^{prmd}/GeneJ mice, 7.66; 7.58; 8.3 and 7.92, respectively.

Conclusion. Thus, the value of the standard error of the results study mean of the changes dynamics in the muscle strength when using the automated variant of the "inverted grid" test was reduced in comparison with the results of the classical variant of the test. The results of the study show that the automation of generally recognized behavioral tests is able to increase the accuracy of the obtained data reducing the influence of a human factor on the manipulation.

Keywords: "inverted grid"; automation; behavioral testing; neurodegeneration; transgenic animals

Abbreviations: ALS – amyotrophic lateral sclerosis; HD – Huntington's disease; PD – Parkinson's disease; AD – Alzheimer's disease; MD – motor deficit; PS – inverted grid; MS – muscle strength.

Для цитирования: П.Р. Лебедев, Е.В. Кузубова, В.М. Покровский, А.И. Радченко, С.И. Осипьян, Ю.В. Степенко, А.А. Апостол, Л.М. Даниленко, А.А. Должиков, Т.Г. Покровская, О.С. Гудырев, Я.С. Кочергина, О.В. Дудникова. Влияние исследователя на оценку мышечной силы у животных в эксперименте: сравнение автоматизированного теста «перевернутая сетка» и его классического варианта. *Фармация и фармакология.* 2024;12(1):63-73. **DOI:** 10.19163/2307-9266-2024-12-1-63-73

For citation: P.R. Lebedev, E.V. Kuzubova, V.M. Pokrovsky, A.I. Radchenko, S.I. Osipyan, Y.V. Stepenko, A.A. Apostol, L.M. Danilenko, A.A. Dolzhikov, T.G. Pokrovskaya, O.S. Gudyrev, Ya.S. Kochergina, O.V. Dudnikova. Investigator's influence on the muscle strength assessment in animals in experiment: Comparison of automated "inverted grid" test and its classical variant. *Pharmacy & Pharmacology.* 2024;12(1):63-73. **DOI:** 10.19163/2307-9266-2024-12-1-63-73

RESEARCH ARTICLE

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY
(ФАРМАЦИЯ И ФАРМАКОЛОГИЯ)

Влияние исследователя на оценку мышечной силы у животных в эксперименте: сравнение автоматизированного теста «перевернутая сетка» и его классического варианта

П.Р. Лебедев[1], Е.В. Кузубова[1], В.М. Покровский[1], А.И. Радченко[1], С.И. Осипьян[1], Ю.В. Степенко[1], А.А. Апостол[1], Л.М. Даниленко[1], А.А. Должиков[1], Т.Г. Покровская[1], О.С. Гудырев[1], Я.С. Кочергина[2], О.В. Дудникова[2]

[1] Федеральное государственное автономное образовательное учреждение высшего образования «Белгородский государственный национальный исследовательский университет», 308015, Россия, г. Белгород, ул. Победы, д. 85

[2] Областное государственное бюджетное учреждение здравоохранения «Белгородское бюро судебно-медицинской экспертизы», 308017, Россия, г. Белгород, ул. Волчанская, д. 159

E-mail: Kuzubova@bsu.edu.ru

Получена 10.12.2023 После рецензирования 27.03.2024 Принята к печати 11.04.2024

Цель. Изучить влияние исследователя на оценку мышечной силы у животных в эксперименте на примере сравнения результатов автоматизированного теста «перевернутая сетка» и его классического варианта.

Материалы и методы. Для исследования были выбраны самцы линий (Bla/J, *n*=20; FUS(1-359), *n*=20; Tau P301S$^{+/+}$, *n*=20) и их фоновый контроль (C57BL/6J, *n*=20; CD1, *n*=20). Динамику изменения мышечного дефицита животных оценивали в автоматизированном и классическом варианте теста «перевернутая сетка».

Результаты. По результатам оценки мышечной силы мышей с редактированным геномом линий: FUS(1-359)$^{+/-}$, Tau P301S$^{+/+}$, B6.ADysf^{prmd}/GeneJ при помощи теста «перевернутая сетка» в классическом варианте и автоматизированном было установлено, что статистически значимых различий в сравнении с результатами, полученными при проведении классического варианта теста, получено не было. Стандартная ошибка среднего возрастала в классическом тесте по сравнению с автоматизированным на 23–39%. Было показано, что стандартная ошибка среднего в классическом варианте теста на мышах линии Tau P301S$^{+/+}$ составила 6,24; 5,94; 5,88; 7,38 в 4-х возрастных точках; на мышах линии FUS(1-359)$^{+/-}$ – 4,49; 6,8; 6,98 и 4,1; B6.ADysf^{prmd}/GeneJ – 7,66; 7,58; 8,3 и 7,92 соответственно.

Заключение. Таким образом, значение стандартной ошибки среднего результатов исследования динамики изменения мышечной силы при использовании автоматизированного варианта теста «перевернутая сетка» было снижено в сравнении с результатами проведения классического варианта теста. Результаты исследования показывают, что автоматизация общепризнанных поведенческих тестов способна повысить точность получаемых данных снизив влияние человеческого фактора на проведение манипуляции.

Ключевые слова: «перевернутая сетка»; автоматизация; поведенческое тестирование; нейродегенерация; трансгенные животные

Список сокращений: БАС – боковой амиотрофический склероз; БГ – болезнь Хантингтона; БП – болезнь Паркинсона; БА – болезнь Альцгеймера; ДД – двигательный дефицит; ПС – перевернутая сетка; МС –мышечная сила.

INTRODUCTION

The reproducibility and repeatability of experimental results in the biomedical research is a hot topic of discussion among researchers and has been intensely debated over the latest 10 years [1]. In the works by other authors it was shown that from 50 to 90% of the experimental data are non-reproducible and controversial in their conclusions [2]. Several reasons for the poor repeatability and reproducibility of the experimental results are pointed out at once. They are: a bias in the interpretation of the results [3], an incorrect approach to the results statistical processing [4], lack of randomization [5], non-compliance with the rules of the animal housing (different crowding of animals in the cage) [6], neglect of validation of the equipment and auxiliary materials [7]. Working with animals requires a special approach to the study design, randomization of the group and minimization of the experimenter's influence on the results of phenotypic tests [8].

Conducting a behavioral study is an integral part for characterizing neurodegenerative and neuropsychiatric diseases in animal models. Over the past 30 years, behavioral testing has become ubiquitous in neurological and genetic animal studies [9].

From 1940 to 1989, a PubMed library search on "mice" and "behavior" found about 1800 articles, mostly related to behavior genetics, drug testing, and neurobiology, but only one article reported a behavior

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2024-12-1-63-73

analysis of transgenic mice [10]. From 1990 to 2023, the number of articles on behavior testing of genetically modified mice alone grew to 28 000 [11].

In studying the dynamics of neurological diseases symptoms in transgenic animals, various behavioral tests are used, one of which are the tests aimed at detecting motor disorders that provide a good phenotypic characterization of the disease. In order to interpret the therapy efficacy of a model disease of an experimental animal on humans, it is necessary to obtain, in addition to molecular, also a phenotypic confirmation of the disease identity [12]. This tool is an integral component of modern protocols for studying disease mechanisms using experimental models and genetically modified laboratory animals [13]. Behavioral research provides a quantitative and qualitative marker of human disease symptoms and is a preclinical tool for evaluating the efficacy of new therapies [14].

Creating an animal with an edited genome that mimics the human disease phenotype is important for the study of basic pathophysiological cascades and the development of new methods for the pathology treatment.

There are various genetically modified mouse models for studying pathophysiological aspects of neurodegeneration and myodystrophies. The most commonly used models of these diseases are transgenic mouse models of amyotrophic lateral sclerosis (ALS), Huntington's disease (HD) [15], Parkinson's disease (PD) [16, 17], and Alzheimer's disease (AD) [18]. The motor dysfunction present in these transgenic mice can be categorized as motor deficits (MD), impaired balance, coordination or muscle strength (MS) [19].

The tests that can show phenotypic manifestations of MD are "CatWalk", "Narrowing track", "Staircase", "Staircase with rungs", "Open field". One of the most used tests to assess a motor impairment is the "inverted grid" (IG), which is used to assess the limb strength of mice, their coordination and endurance. The test involves engaging all four of the animal's limbs to fixate to a wire grid, allowing a non-invasive measurement of the animal's MS, showing a sustained limb tension, counteracting the effects of gravity. The stimulus for keeping the animal on the grid is a fear of heights. The parameters of interest are assessed by placing the animal on an ID and determining the duration of its fixation on the grid. The test evaluates the MS, a movement coordination and balance of the animal by measuring how long it can hold on the inverted screen before falling down [20].

In the experiments related to the study of therapy for muscular dystrophies, a number of symptoms of the disease are investigated, such as MD associated with the progression of muscle atrophy in the proximal limb muscles [21]. To study the dynamics of the disease, scientists use behavioral tests that can interpret the clinical improvement of the animal's health from therapy. One of such tests is the «inverted grid».

Creating automated forms of settings for conducting behavioral studies of animals, is able to objectify the study, eliminate the adverse effect of a researcher-animal interaction on the test, optimize the duration of the test and reduce labor costs for its implementation. The protocol automation allows to obtain more data for a statistical comparison between the groups without additional manipulations with the animal [22, 23].

This article describes a comparative study of a classical variant of the "inverted grid" test and its automated form to use it to determine MD and MS dynamics in mice with a confirmed phenotype of human neurodegenarative and myodystrophic diseases.

THE AIM of the work was to study the influence of the researcher on the muscle strength assessment in animals in the experiment by comparing the results of the automated inverted grid test and its classical variant.

MATERIALS AND METHODS

Animals

Males of the lines Bla/J (*n*=20), FUS(1-359)$^{+/-}$ (*n*=20), Tau P301S$^{+/+}$ (*n*=20) and their background controls (C57BL/6J, *n*=20; CD1, *n*=20) were selected for the study and kept in groups according to their age, genetic background and group in the experiment. The animals were kept in the SPF vivarium of Belgorod State National Research University (BSU) under the conditions of artificially regulated daylight hours (12 h of dark and 12 h of light) at a temperature regime from +22 to +26°C, a relative humidity in the housing system from 50 to 65%, had a free access to food and water. The work was guided by ethical principles for the treatment of laboratory animals in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (ETS No. 170). All painful animal manipulations were performed in accordance with the regulatory standards: Directive 2010/63/EU of the European Parliament and of the Council of the European Union dated September 22, 2010 on the protection of the animals used for scientific purposes. Experimental studies were approved by the Bioethical Commission of the BSU (Minutes No. 15/10 of 29.10.2021).

Study design

For a comparative study of the MD registration and MS change dynamics using the classical "inverted grid" test and its automated form in male transgenic animals and their background controls of the B6.ADysf^{prmd}/GeneJ, FUS(1-359)$^{+/-}$, and Tau P301S$^{+/+}$ lines at 4 age points (the manifestation period of clinical disease signs) for each cohort of mice (Fig. 1).

Experimental groups of the B6.ADysf^{prmd}/GeneJ mouse line were established at weeks 50, 51, 52 and 53, respectively.

1) The group for studying the dynamics of MD and MS in the B6.ADysf^{prmd}/GeneJ$^{-/-}$ line (*n*=10) using the classical variant of the IG (Bla/J) test.

2) The group for studying MD and MS dynamics in the C57BL/6J line (*n*=10) using the automated variant of the IG (b/C57BL/6J) test. The group is control to the Bla/J$^{+/+}$ line.

3) The group for studying the dynamics of MD and MS in the line B6.ADys^{fprmd}/GeneJ$^{-/-}$ (*n*=10) using the automated variant of the IG (Bla/J) test.

4) The group for studying MD and MS dynamics in the C57BL/6J line (*n*=10) using the classical variant of the IG (b/C57BL/6J) test. The group is control to the Bla/J line.

Experimental groups of the FUS(1-359) mouse line were established at weeks 11, 12, 13 and 14, respectively.

1) The group for studying the dynamics of MD and MS in the FUS(1-359)$^{+/-}$ line (n=10) using the classical variant of the IG (FUS(1-359)) test.

2) The group for studying the dynamics of MD and MS in FUS(1-359)$^{+/-}$ (*n*=10) using the automated variant of the IG (FUS(1-359)) test.

3) The group for studying the dynamics of MD and MS in the CD-1 line (*n*=10) using the automated variant of the IG (CD-1) test. The group is control to the FUS(1-359)$^{+/-}$ line.

4) The group for studying dynamics of MD and MS in CD-1 line (*n*=10) using the classical variant of the IG (CD-1) test. The group is control to the FUS(1-359)$^{+/-}$ line.

Experimental groups of the Tau P301S$^{+/+}$ mouse line were established at weeks 16, 17, 18 and 19, respectively.

1) The group for studying the dynamics of MD and MS in the Tau P301S$^{+/+}$ line (*n*=10) using the classical variant of the IG (Tau P301S$^{+/+}$) test.

2) Group of studying the dynamics of MD and MS in the Tau P301S$^{+/+}$ line (*n*=10) using the automated variant of the IG (Tau P301S$^{+/+}$) test.

3) The group for studying the dynamics of MD and MS in the C57BL/6J line (*n*=10) using an automated variant of the IG (C57BL/6J) test. The group is control to the Tau P301S$^{+/+}$ line.

4) The group for studying the dynamics of MD and MS in the C57BL/6J line (*n*=10) using the classical variant of the IG (C57BL/6J) test. The group is control to the Tau P301S$^{+/+}$ line.

Three lines of mice exhibiting symptoms of the disease affecting the motor function were used. The FUS line (1-359)$^{+/-}$ has a nervous system with an expressed transgenic sequence encoding an aberrant form of a human FUS protein with a deleted nuclear localization signal under the neuron-specific Thy-1 promoter [24]. The most common disease-associated mutations of the FUS gene affect the nuclear localization signal of the C-terminus of the encoded protein, causing its accumulation in the cytoplasm and at least a partial depletion of its nuclear pool [25]. This mouse line models amyotrophic lateral sclerosis. This neurodegenerative disease is characterized by a loss of neurons in the motor areas of the brain and spinal cord. The FUS(1-359)$^{+/-}$ line is characterized by the manifestation presence of the motor impairment symptoms and MS in a mono-allelic mutant animal at the 15th week of life [26].

The study made use of the Tau P301S$^{+/+}$ transgenic mice that express human Tau (1N4R) with the P301S mutation [27]. The identification of disease-causing mutations in *MAPT*, the *Tau* gene, in cases of frontal temporal dementia, has shown that a dysfunction of the Tau protein is sufficient to cause neurodegeneration and dementia [28]. These mice show a progressive neurofibrillary tangle pathology and neurodegeneration in the brain and spinal cord. In the spinal cord, the Tau pathology leads to the dramatic loss of motor neurons (approximately 50%) and an early, progressive and severe motor impairment [29]. The manifestation of lower limb MD begins at about 3 months of age [30].

A subline of B6.ADysf^{prmd}/GeneJ (Bla/J) mice in which a spontaneous insertion in intron 4 had been detected by chance, was also used. This type of pathology is a phenotypically heterogeneous progressive muscular dystrophy caused by mutations in the *DYSF* gene, which encodes a transmembrane protein dysferlin involved in a sarcolemma repair. It is also involved in the membrane repair, in the intracellular vesicle system and in the development of T-channels in the skeletal muscle [31]. The diseases are characterized by muscle weakness and atrophy that progress slowly and symmetrically in the proximal limb girdle muscles at about the 50th week of the animal's life [32].

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2024-12-1-63-73

Assessment of muscle strength and motor deficit

The dynamics of changes in the muscle deficit of the animals were assessed in the automated and classical variants of the IG test [33]. In case of using the automated variant, the animal was placed in the center of a 25×25 cm grid with the holes of 5×5 mm width and the thickness of 0.5 mm. Turning the net with the animal was performed at a preset speed using an automated platform. The net was mounted on supports with an adjustable height (from 30 to 100 cm in 10 cm increments), above a cage with a thick layer of bedding. The animal was placed on the net, after the animal had steadily grasped the net with all four paws, the protocol was started on the management controller. After that, the net was turned with the animal by 180° by means of a servo drive. In this case, the weight of the net with the laboratory animal was registered by the corresponding strain gauge sensor, and a jump-like change in the weight of the net was interpreted as a fall of the animal from the net with the registration of the time of the actual presence on it and showing off this result on the display. After the completion of the protocol, the animal was removed from the test by sliding the cage out of the setup.

The automated variant of the test was made on the equipment according to the patent for invention No. 2815584 “Automated device for conducting the behavioral “inverted grid” test “.

When the classical variant of the setup was used, the inverted grid was a 25×25 cm wire mesh of 5×5 mm mesh size with a wire diameter of 0.5 mm, surrounded by a 4 cm partition to prevent the mouse from attempting to climb over to the other side. The test was used to assess a movement coordination and MS of the both pairs of limbs. The mice were placed in the center of a wire mesh that had been inverted and placed 50 cm above a soft surface. The time of the animal’s fall was recorded or the animal was removed from the net if the time reached 180 sec [31].

Statistical processing

Statistical processing was performed using GraphPad Prism Software 8.0 program (GraphPad Software Inc., USA). Depending on the type of the traits distribution and equality of variance, the significance of the obtained results was evaluated using parametric (ANOVA) or nonparametric (Mann-Whitney *U*-test). The unpaired Student’s *t*-test was used to identify differences in the

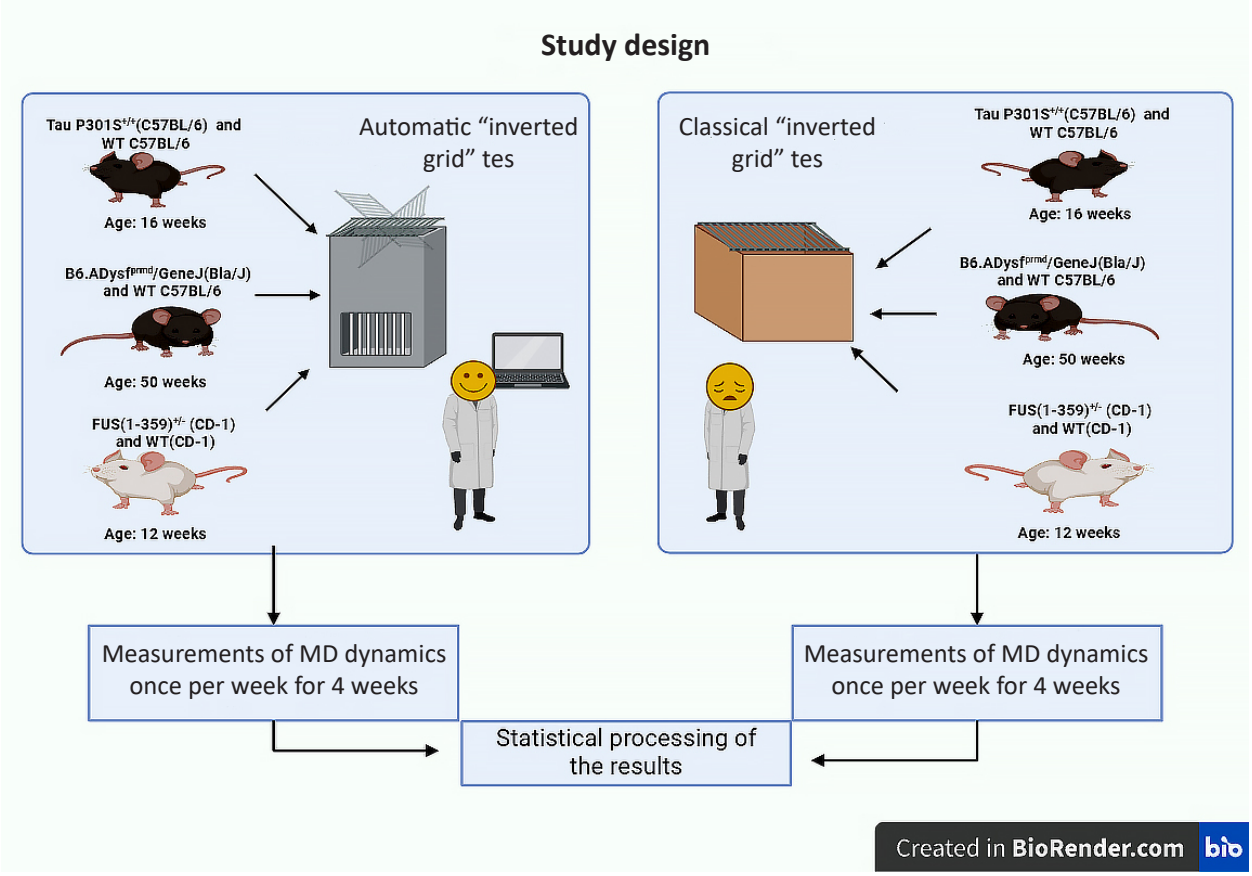

Figure 1 – Study design
Note: Experimental (Bla/J, *n*=20; FUS(1-359)$^{+/-}$, *n*=20; Tau P301S$^{+/+}$, *n*=20) and control (C57BL/6J, *n*=20; CD1, *n*=20) mice.
MD – motor deficits.

intergroup comparisons. The results were considered reliable at $p \leq 0.05$.

RESULTS

In a comparative study of the classical variant of the IG test and its automated form to determine MD and MS dynamics in mice with a confirmed human neurodegenarative and myodystrophy phenotype, the following results were previously reported [21, 34, 35].

As a result of the IG test, the degree of an increase in the MD symptoms and a decrease in the MS in Tau P301S$^{+/+}$ line mice (Fig. 2) compared to the control group (C57BL/6J), in case of the automatic variant of the IG test (Tau P301S$^{+/+}$) was 32.17, 48.67, 67.67 and 82.17% (p <0.001) at 16, 17, 18, 19 weeks of age, respectively. In case of a classical variant test, the group (Tau P301S$^{+/+}$) had 22.17, 39.34, 61.83 and 72.17% (p <0.0001) at 16, 17, 18, 19 weeks of age, respectively, compared to the control group (C57BL/6J). Thus, the difference in the results obtained from the two variants of the IG test were 10, 9.33, 5.84, and 10% at 16, 17, 18, and 19 weeks of age, respectively.

As a result of the IG test, the degree of an increase in MD symptoms and a decrease in MS in the FUS(1-359)$^{+/-}$ mice (Fig. 3) compared to the control group (CD-1), in case of the automatic variant of the test (FUS(1-359)$^{+/-}$ was 21.83, 53.17, 72.67 and 90.5% (p <0.0001) at 11, 12, 13, 14 weeks of age, respectively. In case of the classical variant test, the FUS(1-359)$^{+/-}$ group had 16.17, 40.67, 61.67 and 73.33% (p <0.0001) at 11, 12, 13 and 14 weeks of age, respectively, compared to the control group (CD-1). Thus, the difference in the results obtained from the two variants of the IG test was 5.67, 12.5, 11 and 17.17% at weeks 1–4 of the study.

As a result of the IG test, the degree of an increase in MD symptoms and a decrease in MS in the Bla/J line mice (Fig. 4) compared to the control group (C57BL/6J) in case of the automatic variant of the IG (Bla/J) was 25.83, 33.51, 33 and 58.67% (p <0.0001) at 12, 13, 14 and 15 weeks of age, respectively. In case of the classical variant test, the group (Bla/J) had 41.17, 44.67, 51.83, and 59.33% (p <0.0001) at 50, 51, 52, 53 weeks of age, respectively, compared to the control group (C57BL/6J). Thus, the difference in the results obtained from the two variants of the IG test were 15.34, 11.67, 0.5, and 0.67% at weeks 1–4 of the study.

Using the automation example of the well-known "inverted grid" test, the authors wanted to show the differences in the results obtained when using the automated complex and the classical test on three sublines of transgenic animals (Fig. 5). It was shown that the standard error of the mean in the classical variant of the test on Tau P301S$^{+/+}$ line mice was 6.24, 5.94, 5.88, and 7.38 at 4 age points, and on the FUS(1-359)$^{+/-}$ line mice it was 4.49, 6.8, 6.98, and 4.1; Bla/J was 7.66, 7.58, 8.3, and 7.92, respectively. The analysis of the data from the automated variant of the IG test showed the standard error of the mean as 5.1, 4.93, 3.42, and 2.26 in the Tau P301S$^{+/+}$ line, respectively, and 4.24, 4.52, 5.19, and 2.9 in the FUS(1-359)$^{+/-}$ mice; Bla/J, 5.24, 4.52, 4.7, and 4.85, respectively. Thus, automating the test and reducing the experimenter's influence decreased the standard error of the mean in mice of the Tau P301S+/+ line by 36.6%, FUS(1-359)$^{+/-}$ by 23.1%, B6. ADysf^{prmd}/GeneJ by 38.5%.

It is generally accepted that the experimenter influences the outcome of the experiment and the interpretation of its results, so it is important to automate this variable and minimize the experimenter's influence on both the experiment itself and its interpretation [36, 37].

DISCUSSION

Behavioral testing of animals in experimental studies to determine MD and MS has been used to estimate the efficacy of pharmacological therapies in many disease models. The first experimental article that mentioned the IG test date by 1986[1].

The tests known as "Open field", "Inverted grid", and "Rotating rod", are widely used in the experimental work related to the effectiveness estimation of the diseases therapeutic correction, the main symptoms of which are coordination disorders and motor deficits. Nowadays, there is a possibility for researchers to automate classical tests, which minimizes a human contact with a animal thus making the study more objective and accurate; it also contributes to increasing the throughput capacity of the installation. Strictly speaking, this work shows that it is possible to automate a large number of behavioral tests widely known to the research community, which will increase the intensity of experiments.

The examples include the Cat walk test, the essence of which is to automate the registration of the animal's paws position during its gait by means of the infrared radiation, which had been done before, by applying ink to the subject's paws and placing it on sheets of paper.

[1] Kordower JH, Felten SY, Felten DL, Gash DM. Behavioral sequelae following MPTP administration in mice. A neurotoxin producing a parkinsonian syndrome. Orlando: Academic Press; 1986. P. 413–417.

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2024-12-1-63-73

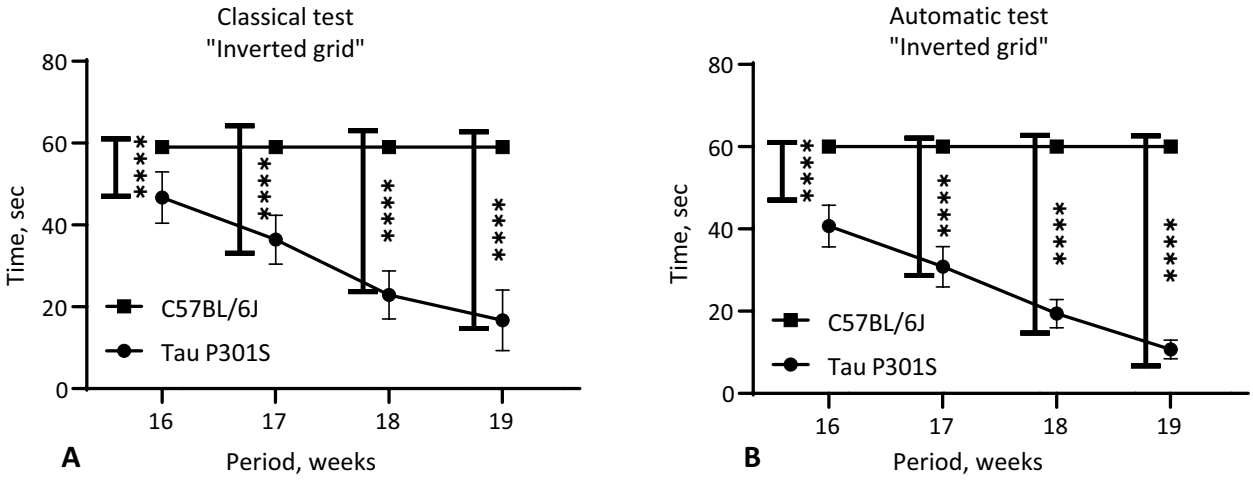

Figure 2 – Examination of motor deficits in Tau P301S$^{+/+}$ line using automated and classical variants of "inverted grid" test

Note: medians and standard error of the mean are presented. Samples were tested for normality, and statistical significance was assessed using the Mann-Whitney *U*-test (****p <0.0001). A – classical IG test, B– automated IG test.

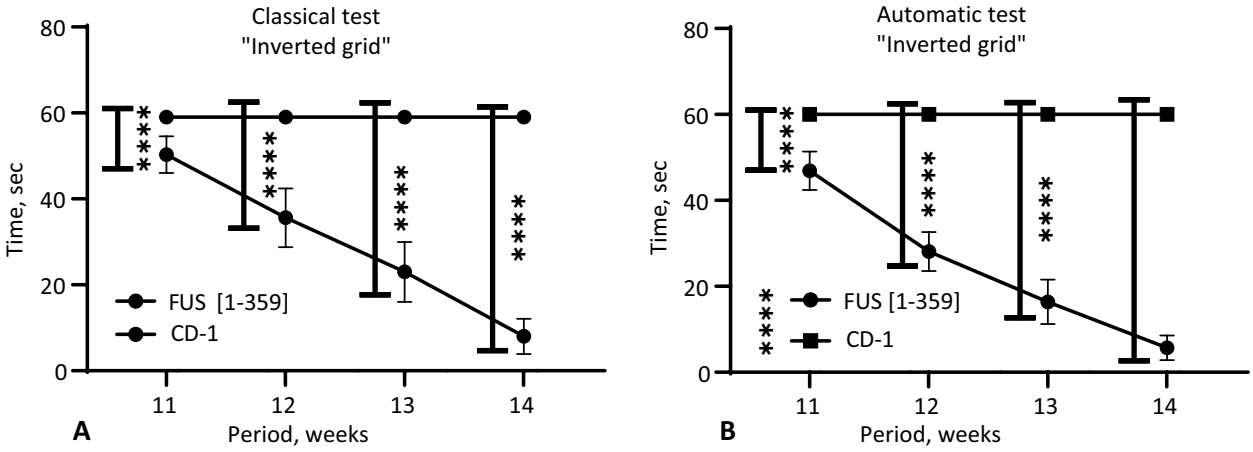

Figure 3 – Examination of motor deficits in the FUS(1-359)$^{+/-}$ line using automated and classical variants of the "inverted grid" test

Note: medians and a standard error of the mean are presented. Samples were tested for normality and the statistical significance was assessed using the Mann-Whitney *U*-test (****p <0.0001). A – classical IG test, B – automated IG test.

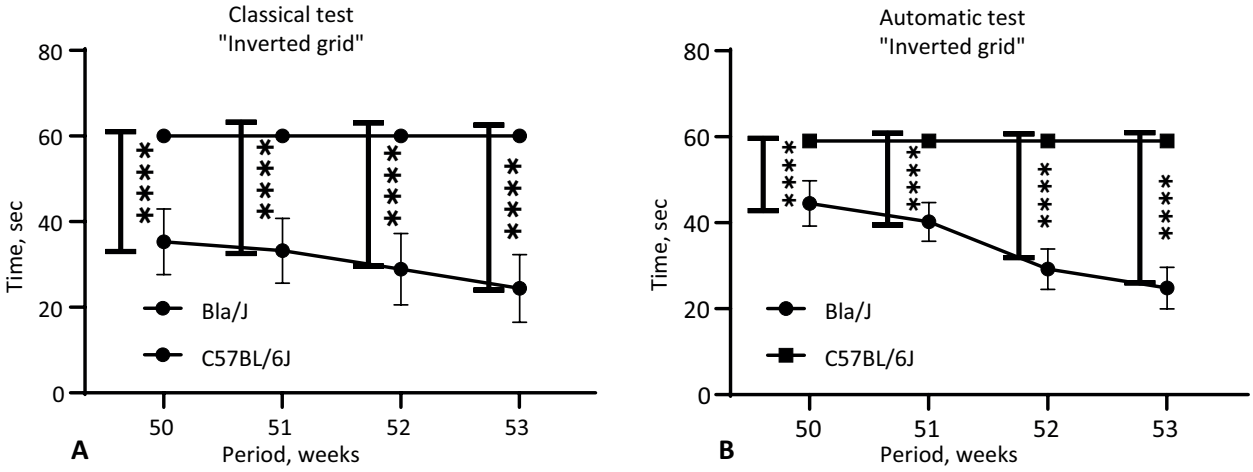

Figure 4 – Examination of motor deficits in the Bla/J line using automated and classical variants of the "inverted grid" test

Note: medians and a standard error of the mean are presented. Samples were checked for normality, and statistical significance was assessed using the Mann-Whitney *U*-test (****p <0.0001). A – classical IG test, B – automated IG test.

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY
(ФАРМАЦИЯ И ФАРМАКОЛОГИЯ)

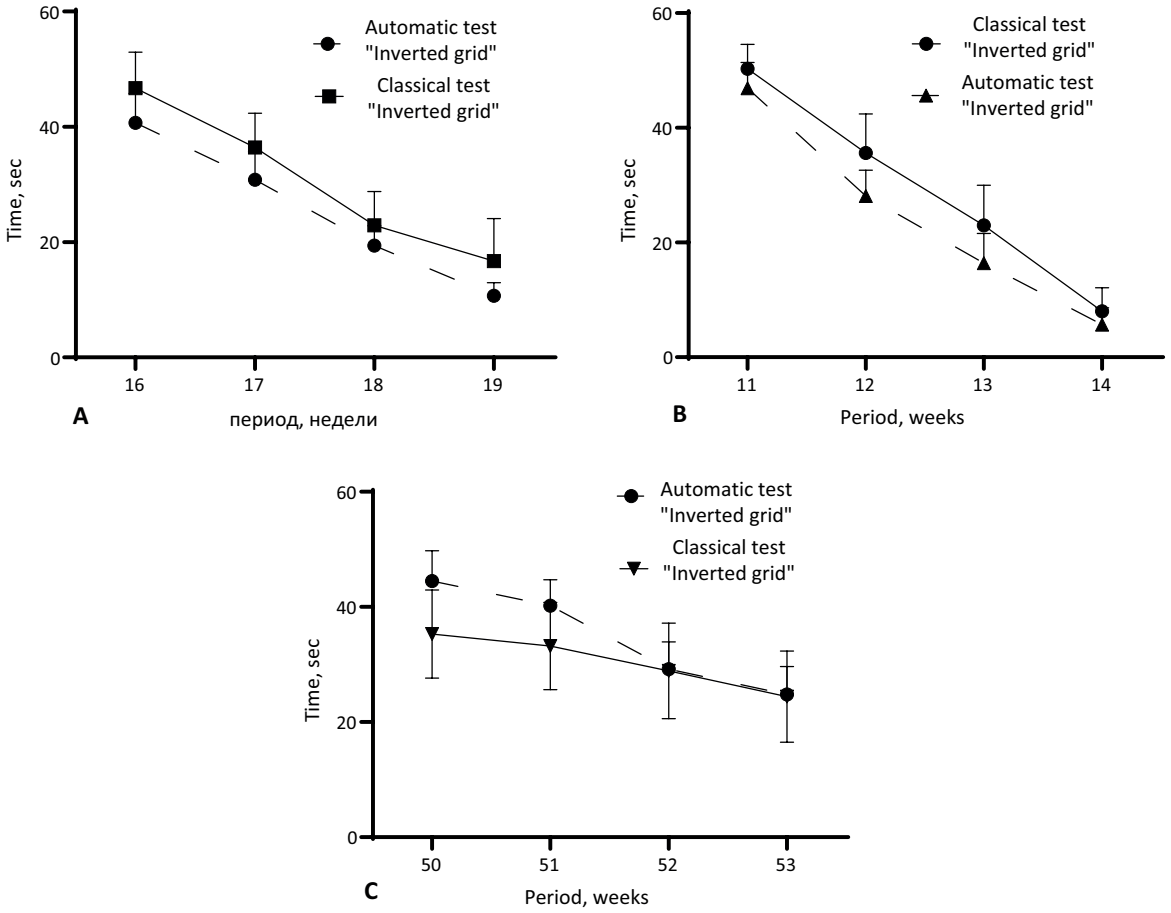

Figure 5 – Comparison of motor deficit study results in line A – Tau P301S$^{+/+}$, B –FUS(1-359)$^{+/-}$, C – Bla/J, using automated and classical variants of "inverted grid" test

Many research groups are working to develop a fully automated system for behavioral testing. For example, the system "IntelliCage" has been developed [11]. Apart from automating the work, it avoids potentially disturbing conditions for animals and has a wide range of different study protocols, but except the considerable advantages, this system cannot completely replace all the known classical behavioral tests. Another prominent example is the complete automation of a home cage for continuous testing of mice [38]. Undoubtedly, this development is a valuable tool for comprehensive phenotyping, but a large amount of data to process and the speed of tests are not suitable for all experimental work.

In some cases, the tests that currently already use automatic tracing of the animal movement (EthoVision and VideoMot) are also being automated and improved. For example, a new tool "Minopontikos" was proposed for the Morris Water Maze test [39]; it allows combining different methods of calculation and interpretation of results.

The carried out automation of the "inverted grid" has shown that in addition to reducing the standard error of the mean compared to the classical test by 23-39%, it also increases the efficiency factor by reducing the time spent on the test. Consequently, thereby the throughput of the test is increasing and the number of new indirect numerical values obtained are increasing, too (e.g., recording the angle at which the animal loses its fixation with the net). On average, a standard manual phenotypic testing of 30–40 mice (including recording of the spontaneous activity and data analysis) takes 20 h of working time. Another important advantage of the setup is the standardization of the mesh rotation speed and height of its installation relative to the cage with a shock-absorbing cover, which allows reducing the difference in the experienced stress among the mice.

Study limitations

The results of the study may be affected by a small sample size, conducting the research on experimental and control animals at different times of the circadian rhythm, unsatisfactory animal housing, including a high stress load on the experimental subject, a close proximity of the experimenter near the device during testing.

CONCLUSION

Nowadays, an integral part of the experimental work with animals is the use of phenotypic tests. However, at the same time, inaccuracies in estimating

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2024-12-1-63-73

the parameters of the animal behavior sharply reduce the value of the results of the whole experiment and lead to significant financial costs. That is why all over the world, the development of systems of automatic registration and behavior estimation is actively carried out. The work has been done on the "inverted grid" test automation.

During the comparative characterization of the classical test with the automated one, the value of the standard error of the study mean result of the MS dynamics change, was reduced in comparison with the results of the classical variant of the test when using the automated variant of the "inverted grid" test. The results of the study show that the automation of generally recognized behavioral tests is able to increase the accuracy of the obtained data, reducing the influence of the human factor on the manipulation.

FUNDING

The work was supported by the Ministry of Science and Higher Education of the Russian Federation, Agreement No. 075-15-2021-1346. The development of the automated system was financially supported by the Ministry of Education and Science of the Russian Federation, Agreement No. FZWG-2021-0016.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS CONTRIBUTION

Petr R. Lebedev – automation of the "inverted grid" test; Elena V. Kuzubova – article writing, research design development; Vladimir M. Pokrovsky – literature analysis, article writing; Alexandra I. Radchenko – evaluation and performance of behavioral tests; Sergey I. Osipyan – preparation of animal cohorts, genotyping; Yulia V. Stepenko – interpretation of results; Alina A. Apostol – observation and care of animals, animal handling; Lyudmila M. Danilenko – study design development; Aleksander A. Dolzhikov – design of graphical material; Tatiana G. Pokrovskaya – consultation on the issues of conducting individual stages of experimental work; Oleg S. Gudyrev – statistical processing of data; Yana S. Kochergina – consultation on the issues of conducting individual stages of experimental work; Olga V. Dudnikova – data analysis. All authors confirm that their authorship complies with the ICMJE international criteria (all authors have made a substantial contribution to the conceptualization, research and preparation of the article, read and approved the final variant before publication).

REFERENCES

1. Peirson SN, Brown LA, Pothecary CA, Benson LA, Fisk AS. Light and the laboratory mouse. J Neurosci Methods. 2018;300:26–36. DOI: 10.1016/j.jneumeth.2017.04.007
2. Fitzpatrick BG, Koustova E, Wang Y. Getting personal with the "reproducibility crisis": interviews in the animal research community. Lab Anim (NY). 2018;47(7):175–177. DOI: 10.1038/s41684-018-0088-6
3. Munafò MR, Nosek BA, Bishop DVM, Button KS, Chambers CD, du Sert NP, Simonsohn U, Wagenmakers EJ, Ware JJ, Ioannidis JPA. A manifesto for reproducible science. Nat Hum Behav. 2017;1(1):0021. DOI: 10.1038/s41562-016-0021
4. Voelkl B, Würbel H. A reaction norm perspective on reproducibility. Theory Biosci. 2021;140(2):169–176. DOI: 10.1007/s12064-021-00340-y
5. García-Berthou E, Alcaraz C. Incongruence between test statistics and P values in medical papers. BMC Med Res Methodol. 2004;4:13. DOI: 10.1186/1471-2288-4-13
6. Manouze H, Ghestem A, Poillerat V, Bennis M, Ba-M'hamed S, Benoliel JJ, Becker C, Bernard C. Effects of Single Cage Housing on Stress, Cognitive, and Seizure Parameters in the Rat and Mouse Pilocarpine Models of Epilepsy. eNeuro. 2019;6(4):ENEURO.0179-18.2019. DOI: 10.1523/ENEURO.0179-18.2019
7. Replicating scientific results is tough – but essential. Nature. 2021;600(7889):359–360. DOI: 10.1038/d41586-021-03736-4
8. Smith AJ. Guidelines for planning and conducting high-quality research and testing on animals. Lab Anim Res. 2020;36:21. DOI: 10.1186/s42826-020-00054-0
9. Nigri M, Åhlgren J, Wolfer DP, Voikar V. Role of Environment and Experimenter in Reproducibility of Behavioral Studies With Laboratory Mice. Front Behav Neurosci. 2022;16:835444. DOI: 10.3389/fnbeh.2022.835444
10. Finger S, Heavens RP, Sirinathsinghji DJ, Kuehn MR, Dunnett SB. Behavioral and neurochemical evaluation of a transgenic mouse model of Lesch-Nyhan syndrome. J Neurol Sci. 1988;86(2-3):203–13. DOI: 10.1016/0022-510x(88)90099-8
11. Lipp HP, Krackow S, Turkes E, Benner S, Endo T, Russig H. IntelliCage: the development and perspectives of a mouse- and user-friendly automated behavioral test system. Front Behav Neurosci. 2024;17:1270538. DOI: 10.3389/fnbeh.2023.1270538
12. Rusmini P, Cortese K, Crippa V, Cristofani R, Cicardi ME, Ferrari V, Vezzoli G, Tedesco B, Meroni M, Messi E, Piccolella M, Galbiati M, Garrè M, Morelli E, Vaccari T, Poletti A. Trehalose induces autophagy via lysosomal-mediated TFEB activation in models of motoneuron degeneration. Autophagy. 2019;15(4):631–651. DOI: 10.1080/15548627.2018.1535292
13. Wang C, Chen S, Guo H, Jiang H, Liu H, Fu H, Wang D. Forsythoside A Mitigates Alzheimer's-like Pathology by Inhibiting Ferroptosis-mediated Neuroinflammation via Nrf2/GPX4 Axis Activation. Int J Biol Sci. 2022;18(5):2075–2090. DOI: 10.7150/ijbs.69714
14. Salmina AB, Gorina YaV, Bolshakova AV, Vlasova OL. Analysis of Main World Trends in Objectivization of Protocols for Behavioral Testing of Laboratory Animals with Brain Pathology. Journal Biomed. 2023;19(1):34–46. DOI: 10.33647/2074-5982-19-1-34-46. Russian

15. Yamin HG, Menkes-Caspi N, Stern EA, Cohen D. Age-Dependent Degradation of Locomotion Encoding in Huntington's Disease R6/2 Model Mice. J Huntingtons Dis. 2021;10(3):391–404. DOI: 10.3233/JHD-210492
16. Rai SN, Singh P. Advancement in the modelling and therapeutics of Parkinson's disease. J Chem Neuroanat. 2020;104:101752. DOI: 10.1016/j.jchemneu.2020.101752
17. Mustapha M, Mat Taib CN. MPTP-induced mouse model of Parkinson's disease: A promising direction of therapeutic strategies. Bosn J Basic Med Sci. 2021;21(4):422–433. DOI: 10.17305/bjbms.2020.5181
18. Zang C, Liu H, Shang J, Yang H, Wang L, Sheng C, Zhang Z, Bao X, Yu Y, Yao X, Zhang D. Gardenia jasminoides J.Ellis extract GJ-4 alleviated cognitive deficits of APP/PS1 transgenic mice. Phytomedicine. 2021;93:153780. DOI: 10.1016/j.phymed.2021.153780
19. Osmon KJ, Vyas M, Woodley E, Thompson P, Walia JS. Battery of Behavioral Tests Assessing General Locomotion, Muscular Strength, and Coordination in Mice. J Vis Exp. 2018;(131):55491. DOI: 10.3791/55491
20. Zolotarev YA, Shram SI, Dadayan AK, Dolotov OV, Markov DD, Nagaev IY, Kudrin VS, Narkevich VB, Sokolov OY, Kost NV. HLDF-6 peptides exhibit neuroprotective effects in the experimental model of preclinical Parkinson's disease. Neuropeptides. 2022;96:102287. DOI: 10.1016/j.npep.2022.102287
21. Bohlen M, Hayes ER, Bohlen B, Bailoo JD, Crabbe JC, Wahlsten D. Experimenter effects on behavioral test scores of eight inbred mouse strains under the influence of ethanol. Behav Brain Res. 2014;272:46–54. DOI: 10.1016/j.bbr.2014.06.017
22. Hånell A, Marklund N. Structured evaluation of rodent behavioral tests used in drug discovery research. Front Behav Neurosci. 2014;8:252. DOI: 10.3389/fnbeh.2014.00252
23. de Munter J, Babaevskaya D, Wolters EC, Pavlov D, Lysikova E, V Kalueff A, Gorlova A, Oplatchikova M, Pomytkin IA, Proshin A, Umriukhin A, Lesch KP, Strekalova T. Molecular and behavioural abnormalities in the FUS-tg mice mimic frontotemporal lobar degeneration: Effects of old and new anti-inflammatory therapies. J Cell Mol Med. 2020;24(17):10251–10257. DOI: 10.1111/jcmm.15628
24. Korobeynikov VA, Lyashchenko AK, Blanco-Redondo B, Jafar-Nejad P, Shneider NA. Antisense oligonucleotide silencing of FUS expression as a therapeutic approach in amyotrophic lateral sclerosis. Nat Med. 2022;28(1):104–116. DOI: 10.1038/s41591-021-01615-z
25. Lysikova EA, Kukharsky MS, Chaprov KD, Vasilieva NA, Roman AY, Ovchinnikov RK, Deykin AV, Ninkina N, Buchman VL. Behavioural impairments in mice of a novel FUS transgenic line recapitulate features of frontotemporal lobar degeneration. Genes Brain Behav. 2019;18(8):e12607. DOI: 10.1111/gbb.12607
26. Korde DS, Humpel C. Spreading of P301S Aggregated Tau Investigated in Organotypic Mouse Brain Slice Cultures. Biomolecules. 2022;12(9):1164. DOI: 10.3390/biom12091164
27. Larson T, Khandelwal V, Weber MA, Leidinger MR, Meyerholz DK, Narayanan NS, Zhang Q. Mice expressing P301S mutant human tau have deficits in interval timing. Behav Brain Res. 2022;432:113967. DOI: 10.1016/j.bbr.2022.113967
28. Macdonald JA, Bronner IF, Drynan L, Fan J, Curry A, Fraser G, Lavenir I, Goedert M. Assembly of transgenic human P301S Tau is necessary for neurodegeneration in murine spinal cord. Acta Neuropathol Commun. 2019;7(1):44. DOI: 10.1186/s40478-019-0695-5
29. Zarate N, Gundry K, Yu D, Casby J, Eberly LE, Öz G, Gomez-Pastor R. Neurochemical correlates of synapse density in a Huntington's disease mouse model. J Neurochem. 2023;164(2):226–241. DOI: 10.1111/jnc.15714
30. Korokin MV, Kuzubova EV, Radchenko AI, Deev RV, Yakovlev IA, Deikin AV, Zhunusov NS, Krayushkina AM, Pokrovsky VM, Puchenkova OA, Chaprov KD, Ekimova NV, Bardakov SN, Chernova ON, Emelin AM, Limaev IS. B6.A-DYSF^{PRMD}/GeneJ mice as a genetic model of dysferlinopathy. Pharmacy & Pharmacology. 2022;10(5):483–496. DOI: 10.19163/2307-9266-2022-10-5-483-496
31. Seo K, Kim EK, Choi J, Kim DS, Shin JH. Functional recovery of a novel knockin mouse model of dysferlinopathy by readthrough of nonsense mutation. Mol Ther Methods Clin Dev. 2021;21:702–709. DOI: 10.1016/j.omtm.2021.04.015
32. Lysikova EA, Funikov S, Rezvykh AP, Chaprov KD, Kukharsky MS, Ustyugov A, Deykin AV, Flyamer IM, Boyle S, Bachurin SO, Ninkina N, Buchman VL. Low Level of Expression of C-Terminally Truncated Human FUS Causes Extensive Changes in the Spinal Cord Transcriptome of Asymptomatic Transgenic Mice. Neurochem Res. 2020;45(5):1168–1179. DOI: 10.1007/s11064-020-02999-z
33. Vikharevaa EA, Chichevaa MM, Evgen'evb MB, Funikovb SYu, Deikinc AV, Bachurina SO, Ustyugov AA The role of heat shock proteins on life duration and behavioral functions of mice with FUS-proteinopathy. Neuroscience. 2021;38(1):37–42. DOI: 10.31857/S1027813321010143. Russian
34. Begam M, Collier AF, Mueller AL, Roche R, Galen SS, Roche JA. Diltiazem improves contractile properties of skeletal muscle in dysferlin-deficient BLAJ mice, but does not reduce contraction-induced muscle damage. Physiol Rep. 2018;6(11):e13727. DOI: 10.14814/phy2.13727
35. Gessesse GW, Tamrat L, Damji KF. Amsler grid test for detection of advanced glaucoma in Ethiopia. PLoS One. 2020;15(3):e0230017. DOI: 10.1371/journal.pone.0230017
36. Sturman O, Germain PL, Bohacek J. Exploratory rearing: a context- and stress-sensitive behavior recorded in the open-field test. Stress. 2018;21(5):443–452. DOI: 10.1080/10253890.2018.1438405
37. Mathiasen JR, Moser VC. The Irwin Test and Functional Observational Battery (FOB) for Assessing the Effects of Compounds on Behavior, Physiology, and Safety Pharmacology in Rodents. Curr Protoc. 2023;3(5):e780. DOI: 10.1002/cpz1.780
38. Ho H, Kejzar N, Sasaguri H, Saito T, Saido TC, De Strooper B, Bauza M, Krupic J. A fully automated home cage for long-term continuous phenotyping of mouse cognition and behavior. Cell Rep Methods. 2023;3(7):100532. DOI: 10.1016/j.crmeth.2023.100532
39. Chernyuka DP, Zorinb AG, Derevtsovaa KZ, Efimovac EV, Prikhodkod VA, Sysoev YI, Vlasovaa OL, Bolsunovskaiab MV, Bezprozvanny IB. Automatic analysis of THE «Morris water maze» behavioral test data. Pavlov Journal of Higher Nervous Activity. 2021;71(1):126–35. DOI: 10.31857/S0044467721010044. Russian

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2024-12-1-63-73

AUTHORS

Petr R. Lebedev – junior researcher of the Laboratory of Genetic Technologies and Gene Editing for Biomedicine and Veterinary Medicine, Belgorod State National Research University. ORCID ID: 0000-0001-9102-3360. E-mail: Artkelt98@yandex.ru

Elena V. Kuzubova – junior researcher of the Laboratory of Genetic Technologies and Gene Editing for Biomedicine and Veterinary Medicine, Belgorod State National Research University. ORCID ID: 0000-0003-2425-5027. E-mail: 1015artek1015@mail.ru

Vladimir M. Pokrovsky – assistant of the Department of Pharmacology and Clinical Pharmacology, Belgorod State National Research University. ORCID ID: 0000-0002-1493-3376. E-mail: pokrovskiy@bsu.edu.ru

Alexandra I. Radchenko – junior researcher of the Laboratory of Genetic Technologies and Gene Editing for Biomedicine and Veterinary Medicine, Belgorod State National Research University. ORCID ID: 0000-0002-4554-2116. E-mail: sandrinkaradchenko@gmail.com

Sergey I. Osipyan – researcher of the Laboratory of Genetic Technologies and Gene Editing for Biomedicine and Veterinary Medicine, Belgorod State National Research University. ORCID ID: 0009-0008-4837-3507. Email: osipyansergey23@gmail.com

Yulia V. Stepenko – junior researcher of the Laboratory of the Laboratory of Genetic Technologies and Gene Editing for Biomedicine and Veterinary Medicine, Belgorod State National Research University. ORCID ID: 0000-0002-7414-7326. E-mail: julia.v.stepenko@gmail.com

Alina A. Apostol – researcher of the Laboratory of Genetic Technologies and Gene Editing for Biomedicine And Veterinary Medicine, Belgorod State National Research University. ORCID ID: 0000-0003-3290-2917. E-mail: alinakum835@gmail.com

Lyudmila M. Danilenko – Doctor of Sciences (Pharmacy), Professor of the Department of Pharmacology and Clinical Pharmacology, Belgorod State National Research University. ORCID ID: 0000-0001-6101-8712. E-mail: danilenko_l@bsu.edu.ru

Aleksander A. Dolzhikov – Doctor of Sciences (Medicine), Professor of the Department of Human Anatomy and Histology, Belgorod State National Research University. ORCID ID: 0000-0003-2798-513X. E-mail: dolzhikov@bsu.edu.ru.

Tatiana G. Pokrovskaya – Doctor of Sciences (Medicine), Professor of the Department of Pharmacology and Clinical Pharmacology, Belgorod State National Research University. ORCID ID: 0000-0001-6802-5368. E-mail: pokrovskaia@bsu.edu.ru.

Oleg S. Gudyrev – Candidate of Sciences (Medicine), Associate Professor of the Department of Pharmacology and Clinical Pharmacology, Belgorod State National Research University. ORCID ID: 0000-0003-0097-000X. E-mail: gudyrev@mail.ru.

Yana S. Kochergina – medical expert at Belgorod Office Chief Medical Examiners. ORCID ID: 0009-0003-8167-5666. E-mail: yana5568@mail.ru

Olga V. Dudnikova – medical expert at Belgorod Office Chief Medical Examiners. ORCID ID: 0009-0005-8949-5058. E-mail: Olenka_Normanskikh@mail.ru