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BEHAVIORAL REACTIONS AND COGNITIVE FUNCTIONS IN RATS WITH VASCULAR MODEL OF ALZHEIMER'S TYPE DEMENTIA AT THE DIFFERENT STAGES OF DISEASE BEFORE AND AFTER STEM CELL CORRECTION

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**Abstract.**

BEHAVIORAL REACTIONS AND COGNITIVE FUNCTIONS IN RATS WITH VASCULAR MODEL OF ALZHEIMER'S TYPE DEMENTIA AT THE DIFFERENT STAGES OF DISEASE BEFORE AND AFTER STEM CELL CORRECTION

Lukyanova Y., Nikolaeva O., Pavlova O., Vasyleva I., Naglov O., Shchegelskaya E., Omelchenko E.

**Background.** The last researches offer to conduct the study of Alzheimer disease (AD) mechanisms using diverse experimental models. However, it was not investigated the behavioral and cognitive impairment in rats at the different stages of vascular model of dementia of Alzheimer’s type developed by us.

**Subjects and methods.** The experiment was performed on 32 male WAG rats weighing 180-250 g which were divided into 4 groups. Rats from group 1 and 3 were injected by aqueous solution of sodium nitrite at a dose of 50 mg/kg of body mass intraperitoneally during 14 and 28 days respectively. Groups 2 and 4 were received 500,000 mesenchymal stem cells in suspension intravenously against the background of experimental nitrite-induced AD. To estimate the behavioral reactions and cognitive functions the Open Field Test (OFT) and Passive Avoidance test (PAT) were used.

**Results.** In all experimental groups in most cases it was found the significant decrease in vertical and horizontal activity (p <0,05) and an increase in the number of defecation in the OFT. Rats from group 3 had the drop in locomotor, research and orientation activity. In the OFT and PAT in groups 2,4 it was observed an improvement in research activity and significant cognitive functions recovery (p=0,012) after stem cell correction.

**Conclusions.** It was found the progression of the protective inhibition and cognitive impairment during experiment. The stem cells introduction had positive effects on brain function recovery.

**Key words:** Alzheimer disease, sodium nitrite, stem cells, cognition, behavior, brain, rats.

**Анотація.**

ПОВЕДІНКОВІ РЕАКЦІЇ ТА КОГНІТИВНІ ФУНКЦІЇ У ЩУРІВ З СУДИННОЮ ДЕМЕНЦІЄЮ АЛЬЦГЕЙМЕРІВСЬКОГО ТИПУ В РІЗНІ ТЕРМІНИ ЗАХВОРЮВАННЯ ДО ТА ПІСЛЯ КОРЕКЦІЇ СТОВБУРОВИМИ КЛІТИНАМИ

Лук’янова Є., Ніколаєва О., Павлова О., Васильєва І., Наглов О., Щегельська О., Омельченко О.

**Передумови.** Останнім часом вчені пропонують проводити вивчення механізмів хвороби Альцгеймера з використанням різноманітних експериментальних моделей. Однак, особливості поведінкових і когнітивних порушень у щурів з судинної деменцією альцгемерівського типу в різні терміни захворювання мало вивчені.

**Матеріали та методи.** Експеримент був проведений за участю 32 щурів-самців популяції WAG масою 180-250 г, які були розділені на 4 групи. Щурам групи 1 і 3 вводили водний розчин нітриту натрію в дозі 50 мг/кг маси тіла внутрішньоочеревинно протягом 14 і 28 днів відповідно. Групи 2 і 4 з модельованою деменцію альцгеймерівського типу судинного походження отримували 500 000 мезенхімальних стовбурових клітин у вигляді суспензії внутрішньовенно. Для оцінки поведінкових реакцій і когнітивних функцій були використані тести «Відкрите поле» (ВП) і «Умовний рефлекс пасивного уникання» (УРПУ).

**Результати.** У всіх експериментальних групах в більшості випадків було виявлено значуще зниження вертикальної і горизонтальної активності (р <0,05) та збільшення числа дефекацій в тесті ВП. У щурів групи 3 відзначалося зниження рухової, орієнтовно-дослідницької активності. В ОП і УРПУ в групах 2,4 спостерігалося поліпшення дослідницької активності і значуще відновлення когнітивних функцій (р = 0,012) після корекції стовбуровими клітинами.

**Висновки.** У всіх групах було виявлено прогресування захисного гальмування і когнітивних порушень протягом експерименту. Введення стовбурових клітин позитивно вплинуло на відновлення функції мозку.

**Ключові слова:** хвороба Альцгеймера, нітрит натрію, стовбурові клітини, когнітивні функції, поведінка, мозок, щури.

**Аннотация.**

ПОВЕДЕНЧЕСКИЕ РЕАКЦИИ И КОГНИТИВНЫЕ ФУНКЦИИ У КРЫС С СОСУДИСТОЙ МОДЕЛЬЮ ДЕМЕНЦИИ АЛЬЦГЕЙМЕРОВСКОГО ТИПА В РАЗНЫЕ СРОКИ ЗАБОЛЕВАНИЙ ДО И ПОСЛЕ КОРРЕКЦИИ СТВОЛОВЫМИ КЛЕТКАМИ

Лукьянова Е., Николаева О., Павлова Е., Васильева И., Наглов А., Щегельская Е., Омельченко Е.

**Предпосылки.** В последнее время ученые предлагают проводить изучение механизмов болезни Альцгеймера с использованием разнообразных экспериментальных моделей. Однако, особенности поведенческих и когнитивных нарушений у крыс с сосудистой моделью болезни Альцгеймера, нами разработанной, в разные сроки заболевания не изучены.

**Материалы и методы.** Эксперимент был проведен при участии 32 крыс-самцов популяции WAG массой 180-250 г, которые были разделены на 4 группы. Крысам группы 1 и 3 вводили водный раствор нитрита натрия в дозе 50 мг/кг массы тела внутрибрюшинно в течение 14 и 28 дней соответственно. Группы 2 и 4 с моделированной деменцией альцгеймеровского типа сосудистого происхождения получали 500 000 мезенхимальных стволовых клеток в виде суспензии внутривенно. Для оценки поведенческих реакций и когнитивных функций использовались тесты «Открытое поле» (ОП) и «Условный рефлекс пассивного избегания» (УРПИ).

**Результаты.** Во всех экспериментальных группах в большинстве случаев было обнаружено значимое снижение вертикальной и горизонтальной активности (р <0,05) и увеличение числа дефекаций в тесте ОП. У крыс группы 3 отмечалось снижение двигательной, ориентировочно-исследовательской активности. В ОП и УРПИ в группах 2,4 наблюдалось улучшение исследовательской активности и значимое восстановление когнитивных функций (р = 0,012) после коррекции стволовыми клетками.

Выводы. Во всех группах было обнаружено прогрессирование защитного торможения и когнитивных нарушений в течение эксперимента. Введение стволовых клеток оказало положительное влияние на восстановление функции мозга.

**Ключевые слова:** болезнь Альцгеймера, нитрит натрия, стволовые клетки, когнитивные функции, поведение, мозг, крысы.

**Introduction**

According to the World Health Organization data (2019) around 50 million people have dementia worldwide and there are nearly 10 million new cases every year. Alzheimer disease (AD) is the most common form of dementia and may contribute to 60–70% of cases [1]. Incremental increase of dementia of Alzheimer’s type leads to researching the mechanisms of disease’s start and progression. The main amyloid cascade hypothesis involves the excessive production of amyloid plaques with their subsequent accumulation and future nerve cells apoptosis [2]. It is obvious that this theory cannot alone exactly elaborate the all steps of neurodegeneration. So that scientists propose other supplemental pathways of progressive neurons injury and loss. It is well known that oxidative stress, neuroinflammation cause endothelial dysfunction that could play the crucial role in development neurodegeneration [3].

The last researches offer to conduct the study of AD mechanisms using diverse experimental models. There are plenty of transgenic and non-transgenic animal AD models in vivo and in vitro tissue, cell, molecular simulation models [4]. One of the most common psychopharmacological model of Alzheimer’s type dementia is induced by scopolamine. It is published that the activity of choline acetyltransferase was dropped in the cortex of AD patients [5]. It was associated with brain lesions and clinical performance [6; 7]. The cholinergic hypothesis of AD was accepted and chronic administration of scopolamine during 28 days was allowed for further AD researching [8; 9].

We have developed another model of dementia of Alzheimer’s type where the endothelial dysfunction triggered amyloid formation and cognitive impairment. This model caused by chronic administration of aqueous solution of sodium nitrite intraperitoneal at dose at 50 mg/kg of body mass during 2 weeks [10]. It was not investigated the behavioral and cognitive impairment at the different stages of disease and after stem cells injections.

**The aim of our study** was to assess the changes of behavioral reactions and cognitive functions in rats with vascular model of Alzheimer’s type dementia at the different stages of disease before and after stem cells administration.

**Subjects and methods**

The experiment was performed on 32 male WAG rats weighing 180-250 g which were divided into 4 groups. Rats from group 1 (sodium nitrite 2 weeks, n=8) and group 3 (sodium nitrite 4 weeks, n=8) were injected by aqueous solution of sodium nitrite at a dose of 50 mg/kg of body mass intraperitoneally during 14 and 28 days (2 and 4 weeks), respectively, resulted in the development of dementia of Alzheimer’s type of vascular genesis. Group 2 (sodium nitrite 2 weeks + stem cells, n=8) and group 4 (sodium nitrite 4 weeks + stem cells, n=8) were received 500,000 mesenchymal stem cells (MSCs) in suspension intravenously against the background of experimental nitrite-induced AD.

All institutional and national guidelines for the care and use of laboratory animals were strictly followed. The Ethics and Bioethics Commission of the Kharkiv National Medical University (October 10, 2018, minutes of the meeting №8) confirmed that the design and manipulations during this experiment were compliant to bioethical requirements of EU Directive 2010/63/EU on the protection of animals used for scientific purposes and the Council of Europe Convection for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (ETS123).

Open Field test (OFT) was used to characterize behavioral reactions such as the locomotion, anxiety, neophilia (ability to adapt rapidly to extreme change) and exploration. Our OFT apparatus was circle area with holes into the walls and the field marked with a grid and square crossings. The main variables recorded during the next 300 sec were: crossings (number of times the line of a square is crossed with all 4 legs), head dipping (number of putting rat’s head into the hole), rearings (number of times the animal stands on its hind legs), grooming (frequency of grooming activity), number of defecation [11-13]. The animals were tested in the beginning of experiment before sodium nitrite injections, just after the sodium nitrite injections and before stem cells injections and in 2 weeks after sodium nitrite and stem cells injections

Cognitive functions were evaluated using Passive Avoidance Test (PAT). In PAT the formation of the conditioned reflex was fixed during 180 sec. If animal crosses from the light to the dark compartment with mild foot shock next day after training the passive avoidance response or conditioned reflex is not formed (0). If rat avoids the entry to the dark compartment and stays at the light compartment the passive avoidance response is formed (1) [14; 15].

Primary culture of MSCs was obtained from bone marrow cell suspension flushed out of rat femurs. The cells were washed in Hanks’ balanced salt solution, centrifuged at 450g for 10 min and plated in 75-cm2 culture flasks at a density of 4x105 cells/cm2 in DMEM/F12 (1/1) containing 2mM L-glutamine, 10% FBS (SIGMA-ALDRICH, cat.n. F7524) and 2 μl/ml, Antibiotic Antimycotic Solution (SIGMA-ALDRICH, cat.n. A5955). The medium with nonadherent cells was discarded after 24 hours of the culture and fresh medium was added to the adherent fibroblast-like MS cells. They were cultured at 37ºC and 5% CO2 in air in an CO2 - incubator for 14 days in the medium changed every 3 days. All reagents for culture were purchased from SIGMA-ALDRICH [16].

To evaluate the behavioral reaction changes inside each group during different period (before sodium nitrite injections, just after s sodium nitrite injections and before stem cells injections, in 2 weeks after sodium nitrite injections) the one-way test ANOVA was used. To estimate the stem cells efficiency on cognitive functions the Pearson's chi-squared test was used.If p values were below 0.05, the difference was statistically significant. All numerical data were analyzed using IBM SSPS Statistics.

**Conflict of interests**

The authors of the article declare no conflict of interest.

**Results and discussion**

Analyzing the behavioral reactions during one-way analysis of variance the Levene's test showed a large dispersion of data with identical variation of samples in group 1 (significant point> 0,05), which indicates a single adherence to the conditions of the experiment. Throughout the experiment, there is a significant decrease in the number of head dipping into the holes by almost 3 times, which indicates a decrease in research activity. A significant increase in the number of defecations by almost 3 times confirms an increase in anxiety in rats (p<0,05). An insignificant decline in the number of rearings and grooming is a non-specific manifestation of a decrease in orientational-research activity, which correlates with a decrease in number of head dipping into the holes. [Poshivalov, V. P. (1978). Jetiologicheskij atlas dlja farmakologicheskih issledovanij na laboratornyh gryzunah. Moscow.]. At the same time, there is a decrease in locomotive activity, which is accompanied by an insignificant decrease in the number of crossings (Table 1).

***Table 1***

*Open Field Test (OFT) behavioral reaction results in group 1*

*(sodium nitrite 2 weeks)*

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Behavior reactions | Period of time | Mean±CI (confidence interval) | SD (standart deviation) | Levene's test | Significant point | ANOVA, p-value |
| crossing | before sodium nitrite injection | 36,69±9,85 | 16,31 | 0,069 | 0,934 | 0,622 |
|  | after sodium nitrite injection | 38,46±9.69 | 16,03 |
|  | in 2 weeks after sodium nitrite injection | 29,5±22,52 | 14,15 |
| head dipping into the holes | before sodium nitrite injection | 3,85±1,15 | 1,91 | 1,931 | 0,165 | 0,044 |
|  | after sodium nitrite injection | 1,85±1,63 | 2,70 |
|  | in 2 weeks after sodium nitrite injection | 1,25±1,52 | 0,96 |
| rearing | before sodium nitrite injection | 2,5±1,95 | 2,33 | 2,993 | 0, 077 | 0,059 |
|  | after sodium nitrite injection | 5,63±3,09 | 3,7 |
|  | in 2 weeks after sodium nitrite injection | 1,75±2,72 | 1,71 |
| defecations | before sodium nitrite injection | 1±0,74 | 1,22 | 0,212 | 0, 81 | 0,019 |
|  | after sodium nitrite injection | 0,92±0,94 | 1,55 |
|  | in 2 weeks after sodium nitrite injection | 3,25±2,39 | 1,5 |
| grooming | before sodium nitrite injection | 0,69±0,57 | 0,95 | 3,345 | 0,05 | 0,332 |
|  | after sodium nitrite injection | 0,69±0,57 | 0,95 |
|  | in 2 weeks after sodium nitrite injection | 0 | 0 |

Values are mean±confidence interval (CI) for the mean.

Variances for each group do not statistically significantly differ. ANOVA results may be considered correct (Levene's test, significant point > 0,05).

The influence of time on behavior results is statistically significant (ANOVA, p-value<0,05).

Rats from group 2 show a significant decrease in locomotion. Comparing the number of head dipping into the holes at different periods of time the explorative activity in 2 weeks after the stem cells administration is less expressed than before the experiment, but higher than before the stem cells correction. It can be assumed that the recovery of explorative activity in animals is associated with the effect of stem cells. Reducing the number of defecations and the number of crossings hypothesizes the animal stress becomes less. However, according to Kaluev A.V. (2002), Markel "A.L. (1981), this type of reaction signifies the development of defensive inhibition in response to stress factors (pain factor resulting from injections and open field testing). [Kaluev, A. V. (2002). Gruming i stress. Moscow: Aviks; Markel', A. L. (1981). K ocenke osnovnyh harakteristik povedenija krys v teste otkrytogo polja. Zhurn. Vyssh. Nervn. Dejatel'nosti, 31(2), 301-307.]. (Table 2).

***Table 2***

*OFT behavioral reaction results in group 2*

*(sodium nitrite 2 weeks + stem cells)*

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Behavior reactions | Period of time | Mean±CI (confidence interval) | SD (standart deviation) | Levene's test | Significant point | ANOVA, p-value |
| crossing | before sodium nitrite injection | 41,5±15,9 | 19,02 | 1,107 | 0,349 | 0,031 |
|  | after sodium nitrite injection, before stem cells injections | 38,75±12,92 | 15,45 |
|  | in 2 weeks after sodium nitrite and stem cells injection | 19,5±12,76 | 15,26 |
| head dipping into the holes | before sodium nitrite injection | 5,5±2,72 | 3,25 | 2,215 | 0,134 | 0,010 |
|  | after sodium nitrite injection, before stem cells injections | 1,75±1,53 | 1,83 |
|  | in 2 weeks after sodium nitrite and stem cells injection | 2,5±1,26 | 1,51 |
| rearing | before sodium nitrite injection | 2,88±1,44 | 1,73 | 1,661 | 0,214 | 0,892 |
|  | after sodium nitrite injection, before stem cells injections | 3,13±2,16 | 2,59 |
|  | in 2 weeks after sodium nitrite and stem cells injection | 2,63±1,54 | 1,85 |
| defecation | before sodium nitrite injection | 1,38±1,26 | 1,51 | 0 ,092 | 0 ,913 | 0,68 |
|  | after sodium nitrite injection, before stem cells injections | 1,75±1,244 | 1,49 |
|  | in 2 weeks after sodium nitrite and stem cells injection | 1,13±1,04 | 1,25 |
| grooming | before sodium nitrite injection | 1,13±1,04 | 1,25 | 2,595 | 0,098 | 0,667 |
|  | after sodium nitrite injection, before stem cells injections | 1,38±1,54 | 1,85 |
|  | in 2 weeks after sodium nitrite and stem cells injection | 0,75±0,74 | 0,89 |

Values are mean±confidence interval (CI) for the mean.

Variances for each group do not statistically significantly differ. ANOVA results may be considered correct (Levene's test, significant point > 0,05).

The influence of time on behavior results is statistically significant (ANOVA, p-value<0,05).

In rats from group 3 there is a drop in all indicators against the background of an increase in number of defecation (an increase in anxiety levels). Conspicuous is the fact that the number of crossings and head dipping into the holes with heterogeneous data variance are reduced by 4 and 11 times [respectively](https://www.multitran.com/m.exe?s=respectively&l1=1&l2=2)  (p < 0,001). It is probably associated with large damage of brain tissue (Table 3).

***Table 3***

*OFT behavioral reaction results in group 3 (sodium nitrite 4 weeks)*

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Behavioral reactions | Period of time | Mean±CI (confidence interval) | SD (standart deviation) | Levene's test | Significant point | ANOVA, p-value |
| crossing | before sodium nitrite injection | 40,71±10,23 | 11,06 | 6,783 | 0,006 | 0,000 |
|  | after sodium nitrite injection | 19,71±15,22 | 16,46 |
|  | in 2 weeks after sodium nitrite injection | 10,43±4,1 | 4,43 |
| head dipping into the holes | before sodium nitrite injection | 4,86±2,23 | 3,06 | 5,166 | 0,017 | 0,000 |
|  | after sodium nitrite injection | 0,71±0,88 | 2,41 |
|  | in 2 weeks after sodium nitrite injection | 0,43±0,49 | 0,95 |
| rearing | before sodium nitrite injection | 4,29±3,45 | 3,73 | 4,825 | 0,021 | 0,006 |
|  | after sodium nitrite injection | 0,43±0,49 | 0,53 |
|  | in 2 weeks after sodium nitrite injection | 0,57±0,73 | 0,79 |
| defecation | before sodium nitrite injection | 0,57±0,73 | 0,79 | 2,482 | 0,112 | 0,362 |
|  | after sodium nitrite injection | 0,86±1,35 | 1,46 |
|  | in 2 weeks after sodium nitrite injection | 1,43±0,9 | 0,98 |
| grooming | before sodium nitrite injection | 2,83±3,75 | 2,48 | 4,507 | 0,026 | 0,335 |
|  | after sodium nitrite injection | 2±0,73 | 2 |
|  | in 2 weeks after sodium nitrite injection | 0 | 0 |

Values are mean±confidence interval (CI) for the mean.

Variances for each group do not statistically significantly differ. ANOVA results may be considered correct (Levene's test, significant point > 0,05).

The influence of time on behavior results is statistically significant (ANOVA, p-value<0,05).

In rats from group 4 in 2 weeks after stem cells injection it was found the insignificant raise of number of head dipping into the holes groups compared to period of time after finishing of sodium nitrite administration and before stem cells injection. Thus, the decrease in horizontal and vertical locomotion in contrast to increased anxiety level is not corrected by stem cells. It is possible that protective inhibition in this case is due not only to the administration of sodium nitrite, but also to chronic pain stress [Povedencheskaja aktivnost' krys v «otkrytom pole» posle svetovoj ili temnovoj deprivacij i fizicheskogo pereutomlenija. (2016). Bjulleten' Sibirskoj Mediciny, 15(3), 16-23. doi:10.20538/1682-0363-2016-3-16–23].

***Table 4***

*OFT behavioral reaction results in group 4*

*(sodium nitrite 4 weeks + stem cells)*

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Behavioral reactions | Period of time | Mean±CI (confidence interval) | SD (standart deviation) | Levene's test | Significant point | ANOVA, p-value |
| crossing | before sodium nitrite injection | 34±11,33 | 13,55 | 1,475 | 0,252 | 0,004 |
|  | after sodium nitrite injection, before stem cells injections | 18,63±8,2 | 9,8 |
|  | in 2 weeks after sodium nitrite and stem cells injection | 13,63±7,98 | 9,55 |
| head dipping into the holes | before sodium nitrite injection | 4,13±3,14 | 3,76 | 1,982 | 0,163 | 0 ,201 |
|  | after sodium nitrite injection, before stem cells injections | 1,63±1,09 | 1,3 |
|  | in 2 weeks after sodium nitrite and stem cells injection | 2,88±2,02 | 2,42 |
| rearing | before sodium nitrite injection | 3,5±2,36 | 2,83 | 2,409 | 0,114 | 0 ,137 |
|  | after sodium nitrite injection, before stem cells injections | 1,25±1,07 | 1,28 |
|  | in 2 weeks after sodium nitrite and stem cells injection | 1,75±1,99 | 2,38 |
| defecation | before sodium nitrite injection | 0,375±0,62 | 0,74 | 1,213 | 0,317 | 0 ,073 |
|  | after sodium nitrite injection, before stem cells injections | 0,75±0,87 | 1,04 |
|  | in 2 weeks after sodium nitrite and stem cells injection | 1,63±1,09 | 1,3 |
| grooming | before sodium nitrite injection | 0,75±0,97 | 1,16 | 0,398 | 0,677 | 1 |
|  | after sodium nitrite injection, before stem cells injections | 0,75±0,74 | 0,89 |
|  | in 2 weeks after sodium nitrite and stem cells injection | 0,75±1,46 | 1,75 |

Values are mean±confidence interval (CI) for the mean.

Variances for each group do not statistically significantly differ. ANOVA results may be considered correct (Levene's test, significant point > 0,05).

The influence of time on behavior results is statistically significant (ANOVA, p-value<0,05).

The variances of all results of behavioral reactions in control group were not significantly different.

During PAT a conditional reflex was formed in 81.25% of cases in rats from group 2, 4 who received stem cells. While in group 1, 3 the conditional reflex was not formed in 37.5% of cases. These results demonstrate positive effects of stem cells on cognitive recovery in rats (Table 5).

***Table 5***

*Passive Avoidance Test (PAT): cognitive function comparison between group with stem cell injections (group 2+group 4) and group without stem cells injections (group 1+group 3)*

|  |  |  |  |
| --- | --- | --- | --- |
| Test | Sodium nitrite 1, 3 weeks groups without stem cells | Sodium nitrite 2, 4 weeks groups with stem cells | Altogether |
| Failed | 10 | 3 | 13 |
| Passed | 6 | 13 | 19 |
| Altogether | 16 | 16 | 32 |

Actual and expected results are statistically different. (Pearson's chi-squared test, p=0,012(<0,05)

**Conclusion**

1. In all experimental groups with chronic stress caused by prolonged intraperitoneal administration of aqueous solution of sodium nitrite and single intravenous administration of stem cells suspension, the protective inhibition develops. It is accompanied by a decrease in vertical and horizontal activity and an increase in the number of defecation, as determined by testing in the open field.

2. In all rats with a 4-week disease model, the drop in locomotor, research and orientation activity was found. Probably, it means that the brain tissue is damaged significantly.

3. In the OFT in all animal from groups 2,4 it was observed an improvement in research activity in comparison with that before stem cells introduction.

4. In the PAT higher percentage of rats from group 2,4 passed this test which indicates the significant cognitive functions recovery after stem cell correction.

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