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Additional quantitative criterion of the accurate selection of animals in Morris water maze

Elena N. Petritskaya, Alexandra L. Ivlieva, Maria V. Lopatina, Alexey A. Glazkov and *Dmitry A. Rogatkin[1)]

Laboratory of Medical and Physics Research, MONIKI named after M.F. Vladimirskiy, Shepkina str. 61/2, Moscow, Russia
[1)]rogatkin@medphyslab.com

ABSTRACT

During the study on influence of silver nanoparticles on cognitive functions in mice, which were evaluated in the Morris water maze (behavioral test), additional result was obtained: the percentage of the brain mass to the body mass of each animal significantly correlated with results of the behavioral test. The general sample of mice was divided to three groups - capable, middle and incapable, according to types of their behavior during the test. The nature of behavioral strategies noticeably determined an extent of individual ability to learn in the water maze. After delivery of nanoparticles, all animals were euthanized, and their brain, blood, liver and kidneys were extracted for estimation of amounts of silver in it. As a result, it was found that more capable mice had significantly larger percentage of the brain mass to the mass of the animal's body. Thus, correctness and effectiveness of the applied method of testing and applied technique of differentiation of these three groups by ability to learn were proved, as well as the additional quantitative criterion for estimation of correctness of this selection in the behavioral test was obtained.

1. INTRODUCTION

Recently, many investigations on possible toxicity of nanoparticles began, and one of its potential dangerous traits is potential ability to overpass the blood-brain barrier (Lockman et al., 2002; Trickler et al., 2010). In perspective sectors of nanoindustry, the workers constantly contact with nanoparticles, and it can cause emergence of new professional diseases, which can be connected with brain dysfunctions induced by toxic influence of nanoparticles on brain cells (Ycas, 1994; Abaeva et al., 2010; Gmoshinski et al., 2013). In our study we investigated the influence of prolonged admission of nanoparticles on cognitive abilities in mice, which were associated with types of behavior during a test in the Morris water maze. Mice were tested in the Morris water maze, then the total sample was divided into three groups according to amounts of successful performance of the test: capable, middle and incapable. After a few months of exposure to nanoparticles the experimental and the control animals of each group were compared. The method was described in detail in (Ivlieva et al., 2017).

After the final testing, all animals were euthanized in order to measure amount of nanoparticles in their inner tissues by the neutron activation analysis (Antsiferova et al., 2015). However, when we related morphometric data to the types of behavior which were found in the first selective test, and then compared it between all three groups, very interesting result was obtained: the ratio of the brain mass to the body mass significantly correlated with the selective test results. Although it is known that encephalization quotient (EQ) is higher in more “intellectual” animals (Ycas, 1994; Perepelkina et al., 2013), and, also, that laboratory animals which live in the enriched environment usually have higher body mass and thicker cortex than animals which live in relatively isolated conditions (Bloom et al., 2005), we have considered our result is very interesting, and, therefore, decided to share it extracting from other results obtained.

2. METHODS

Morris water maze was used for investigation of influence of nanoparticles on cognitive functions in mice (Morris, 1984). SHK mice of both genders, of average mass in males 34,71±5,13 g and in females 27,51±5,64 g and of 8 month age (at the time of euthanasia) were used. During the first selective test before the administration of nanoparticles, we met with the effect of different individual capabilities of learning (Ivlieva et al., 2017). Thereby we divided the general sample of mice to three groups - capable, middle and incapable, according to the types of behavior that was shown by mice during the first selective test. Then, experimental mice had been drinking silver nanoparticles’ solution (in pure water) of 50 μg/ml concentration during 2 month. Control mice drank pure water. After delivery of nanoparticles all animals were tested again in the same Morris water maze, and then were euthanized, and their brain, blood, liver and kidneys were extracted for estimation of amounts of silver in them (Antsiferova et al., 2015). Additionally, individual brain mass (BRM) and total body mass (TBM) of each animal were measured, and individual percentages of the brain mass to the body mass (100%*BRM/TBM) were calculated and compared statistically in Statistica 12 (Statsoft inc.) between the aforementioned groups without considering of the gender. For each group the Mean, standard deviation (SD), median (Me), and quartiles (Qi) were computed. Kruskal-Wallis ANOVA criterion was used to compare the groups. Differences were considered to be statistically significant when $p<0.05$.

3. RESULTS AND DISCUSSION

During the first selective test before the administration of nanoparticles, we met with the effect of different individual capabilities of learning in the Morris water maze and different behavioral strategies (Ivlieva et al., 2017). The nature of these strategies noticeably determined an extent of individual ability to learn in the water maze, which was evidenced by quantitative parameters of movement of animals in different groups. Mice that were more capable used behavioral strategies, which were effective for completion of the task. They explored the pool actively and soon, after a few trials, began to search for the hidden platform. Therefore, afterward, they often found it. Middle mice moved in the pool in random directions. Thus, they found the platform by

accident. Incapable mice did not explore the pool at all; therefore, they rarely found the platform. Thereby we divided the general sample to three groups according to the types of behavior - capable, middle and incapable. After delivery of nanoparticles, during the second control testing, behavioral strategies of mice did not change much neither in the experimental nor in the control groups. Therefore, at the evaluation of brain mass the presence or absence of the fact of nanoparticles administration was not taken into account.

In the whole sample of mice the percentage of the brain mass to the body mass in groups increased from the incapable (1,27±0,24%) via the middle (1,42±0,29%) to the capable (1,73±0,46%) animals (Fig.1). Statistically significant differences are revealed between all three groups (p=0,047). The differences between capable and incapable mice turned up significant in paired comparison at p=0,040 (Table 1).

Table 1. Percentages of brain mass to body mass in groups.

Sample	Groups by ability to learn	Number of mice in the group	Mean±SD, %	Me, %	Q1; Q3, %
All mice	Capable	9	1,73±0,46	1,65	1,50; 2,07
	Middle	11	1,42±0,29	1,31	1,16; 1,65
	Incapable	14	1,27±0,24	1,25	1,11; 1,44

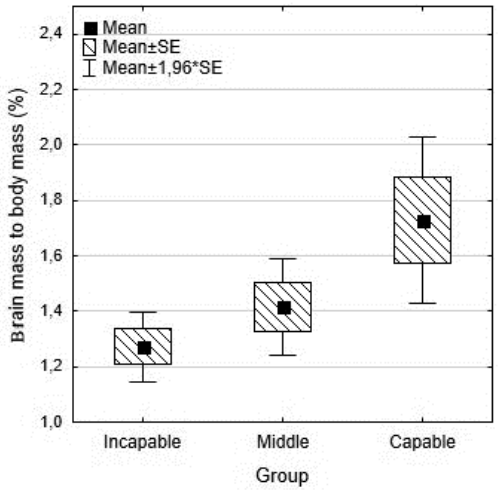

Fig.1. Percentages of the brain mass to the body mass in three groups divided according to results of the behavioral test in the Morris water maze. Legend: point – mean for the group; rectangle – standard deviation; interval - 95% confidence interval.

As we see, the percentages of the brain mass to the body mass increases in groups in accordance with increasing of the cognitive abilities of animals, i.e. with amounts of

successful performances of the test, which, to a noticeable extent, is connected with the behavioral strategies of animals in the Morris water maze.

The majority of other researchers, known to us, which examined relations between characteristics of the brain and other parameters of the individual, have estimated a correlation between the brain and body mass and/or body mass index (i.e. with the height also). On the intraspecific level, mostly in humans, the correlations between the high/excess body mass and brain volume (Gunstad et al., 2008), and between the body mass and functioning of the white matter (Xu et al., 2013) were found. In groups of rats, which were distinguished by the body mass, individuals with small, middle and high brain masses were detected: accordingly, fluctuating percentages of brain mass to body mass were found, and the ratios of the volume of cortex to the volume of "remaining part" of the brain had been changing during the brain mass increasing (Ryzhavskii, Litvintseva, 2015).

There is less data about correlations between the brain mass and behavioral characteristics within species. Nevertheless, in one study (Markina et al., 2003) the result similar to ours was demonstrated: mice with higher brain mass surpass mice with lower brain mass in the level of success in learning. In that experiment in T-shaped maze, mice of the line, which was selected by bigger brain mass, performed the task (choice of the right direction; food motivation) more often than mice of the line with smaller brain mass and showed a decrease in the time spent to the test by days. Indeed, the big brain mass by itself does not say anything neither about increased number of neurons (for example, the mass can be bigger because of glial cells) nor about number of synaptic connections. However, when it is combined with the data about ability to learn in the T-shaped maze (Markina et al., 2003) or in the Morris water maze (this study); these data acquire the definite meaning. The main point of our study is unwittingly obtained additional quantitative confirmation: our technique of selection of animals into three groups is effective and indicates the real cognitive abilities of animals correlating with their brain mass.

4. CONCLUSIONS

During the research on influence of silver nanoparticles on cognitive functions of animals which were estimated in the Morris water maze, additional result was obtained: the percentage of the brain mass to the body mass significantly correlated with the results of the behavioral test. Mice that were more capable had significantly larger percentage of the mass of their brain to the mass of their body. Aside from the point of view that we consider this fact alone to be very interesting and deserving a publication as an addition to other known data on this thematic; these results additionally indicate correctness and effectiveness of the applied technique of testing and differentiation of these three groups of animals by their ability to learn. Unwittingly, it is possible to obtain an additional quantitative criterion for estimation of correctness of animals' selection in the test.

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