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Methodological Characteristics of the Use of the Morris Water Maze for Assessment of Cognitive Functions in Animals

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We present here a review of the use of the Morris water maze to identify impairments to the cognitive functions of the brain as part of the evaluation of the toxic actions of nanoparticles. Model experiments showed that individual variability in animals' behavior has significant influences on the results obtained in the water test. The need for preliminary selection of individuals as a measure to reduce such influences is grounded and the type of behavior displayed by the animal in the test to be used as the criterion for selection is discussed.

Keywords: Morris water maze, cognitive functions, selection of individuals, nanoparticles, individual variability, animal behavior.

Current scientific studies in biology, physiology, and medicine involve many tasks associated with the need to evaluate impairments to the cognitive functions of the brain induced by various external and internal factors – diseases of the nervous system, the toxic actions of pharmaceuticals, brain traumas, etc. One toxic factor which has come under investigation relatively recently is the potential ability of metal nanoparticles to cross the blood-brain barrier [6]. It is possible, for example, that constant contact of industrial workers in nanoindustrial sectors with nanoparticles will in future lead to the appearance of new forms of work-related diseases associated with impairment to the functions of the human brain due to the toxic actions of nanoparticles entering neural tissues [1–3]. Currently this question can only be studied in animal experiments, so the search for and studies of changes in behavior in animals and their cognitive functions in conditions of chronic administration of nanoparticles is a very relevant task.

One of the key questions is that of the sensitivity of methods to even small impairments to cognitive functions, as, on the one hand, the discussion relates to animals whose mental capacities are lower than those of humans, and, on the other, the impairments themselves may be minor at the early stages of poisoning. The Vladimirskii Moscow Regional Research Clinical Institute previously lacked any experience of this type of study, so the question of selecting methods for assessing cognitive functions was acute. The aim of the present work was to present a critical analysis of the literature to identify grounds for selecting the most optimum test for identifying these impairments and assessing the potential sensitivity of the method chosen.

Analysis of Current Approaches and Selection of a Test Version

Experimental studies of animal behavior and the factors influencing it make wide use of dry or water mazes. A clear advantage of water mazes over dry mazes is that in use, the animal tries to get out of the water and this operates as a motivation driving the processes of learning and remembering. A number of versions of water mazes were developed from the beginning of the 20th century [12, 19, 24, 52, 57], and all were initially projected for investigation of spatial learning and spatial memory. The water maze as a type of test subsequently became a tool for studies of the properties of the operation of various parts of the brain and

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has also acquired wide use for assessing the effects of very diverse factors (medicines, ageing, diet, etc.) on brain function [16, 50].

The most widely used variant in physiology and pharmacology for addressing tasks of these types is the Morris water maze (MWM) [36, 37]. The type variant is the hidden platform test: the test apparatus consists of a round white or black basin of diameter 1.2–1.8 m, which is filled with water to below the brim at a temperature just above room temperature. The investigator places a vertical support with a small platform, the size of a rodent, on top of it at a selected point in the basin (in one of the four quadrants). The platform is located only slightly – about 1 cm – beneath the level of the water in the basin, so the animal can get out of the water by climbing onto it.

Animals are tested individually: the animal is launched to swim freely in the basin at some defined point in time. The animal is uncomfortable in the water and tries to escape, and the only possibility for doing so while swimming is to find the platform. However, it is not particularly easy to do this, as the platform is invisible in the water: either the water in the basin is colored the same color as the platform (dried milk or a nontoxic dye) or both the basin and the platform are black. If the individual fails to find the platform after a certain duration of swimming, the investigator places the animal on it and allows it to sit there for some time to allow the animal to see and try to remember the position of the platform relative to visible orientation markers outside the maze. These can be colored cards on the walls, any objects in the context of the laboratory, angled lights, the investigator himself, etc. After this rest, the individual is again launched to swim; in some experiments, the animals are given a break between trials – they are lifted from the platform, dried, and returned to their cages for a while [9, 11, 43].

Testing is performed over a number of sequential days. Each animals is used in a number of trials of swimming to the platform each day. If the animals remembers the position of the platform, it swims shorter distances from day to day, spending less time reaching the objective. The investigator measures the time taken by the animal to swim to the platform and identifies the parameters of the animal's movement trajectory (length, curvature, number of turns, etc.). This is now done by computerized analysis of video recordings of experiments obtained using a video camera suspended from the ceiling above the basin.

Different variants of the Morris water maze are used to address different research tasks. For example, many reports [22, 31, 32, 55, 58] cover studies in which the formation of long-term spatial memory in test animals is verified at the end of the type tests by running a transfer test, in which the conditions are the same other than that the platform has been removed. The "place recall" test allows the actions of manipulations of the animal (brain surgery, substance administration, etc.) on the use of information assimilated in previous Morris water maze trials to be identified [45].

For economy in relation to the number of experimental animals used, a single group of animals can be used for repeated cycles of training in the Morris test (relearning phases). In each cycle, the platform is moved to the basin quadrant opposite to that used in the previous test, as platform position memory can persist in the animals for two months after a test cycle [32]. Completion of each cycle (main test variant and, often, a subsequent transfer test) is followed by allowing the animals a rest period of 1–2 weeks [50]. However, when this is the case, it is important to take cognizance of the fact that each training cycle generally runs more quickly as the animals accumulate information relating to the space in and around the basin during previous cycles and can use this information in subsequent cycles. This behavior reflects a particular brain function – working memory [4], and this is also studied in the water maze (working memory procedures) [49–51]. It should be noted that this version of the test involves overlap between the processes forming spatial memory and the effects of another kind of stress factor (bright flashing light): if the rat is placed beneath the flashing light for the whole of the 15-min break between swimming trials, they do not remember the position of the platform. However, when the individual is allowed to rest without flashing lights for 3–5 min after swimming and the flashing light is then switched on, spatial memory does form [11]. This observation points to a possible advantage of test protocols including planned breaks for rests between trials.

There are also modifications of the basic test and the test for working memory in which the researcher does not help the animal find the platform for a prolonged period – of up to 5–6 min, during which the animal is swimming freely – and most individuals do find the platform independently. The duration of the experiment in these conditions is two days, with the animal taking part in six trials per day. These protocols can be used for studies of the role of biochemical processes taking place in the brain (different substances are given directly into the brain) on the formation and retention of spatial memory [7].

Factors affecting Morris water maze results. In planning Morris water maze experiments and analyzing the results, there is a need to monitor the characteristics of the test animals and include them in the evaluation. The animals' weight, level of development, and age can influence swimming speed. Test performance is also affected by gender, species, or genetic strain. Males generally yield better results than females, and this is almost unrelated to differences in muscle strength [16]. Animals kept in a rich environment perform the test better than those kept in standard conditions [48]. Finally, stressed or sick animals have been observed to cope less well with the test; diet has also been shown to have an effect [16].

Intergender differences. In most experiments, male rodents display better spatial learning in the Morris test than females. However, there are indications that by age six

months males and females are equally successful in performing the test, and this has led to the suggestion that young animals differ as a result of differences in the rate of maturation [10]. A recent study on mice of the outbred strain ICR demonstrated faster performance of the test by females, though control testing one day after the end of the main test showed that both genders remembered the platform position correctly [22].

Sex hormones evidently play a role in gender differences in cognitive abilities. Injection of testosterone to rats during the first week of life leads to better results in adult females than adult males, which is opposite to the situation seen in the control group: testosterone has been proposed to influence the establishment of memory during early postnatal ontogeny [43]. Data on the actions of estrogens on spatial memory are contradictory. On the one hand, ovariectomized females coped with the test better than intact animals [15], while females in proestrus (low concentration of estrogens) demonstrated superior performance to females in estrus (high concentration of estrogens) [53]. However, on the other hand, administration of estradiol into the hippocampus decreased test productivity both in ovariectomized females and in males [38, 39]. Stimulation or inhibition of spatial memory by estrogens may depend on the hormone dose used, as different doses of estrogens are detected by different receptors [16]. Thus, administration of small estradiol doses improves results in the Morris test in ovariectomized mice, while administration of high doses, comparable with blood estrogen levels in proestrus, was not reflected in the test results [41]. It is of note that estrogen therapy decreased learning success in the Morris test in ovariectomized mice with unmanipulated (wild type) genomes, while no such effect was seen in mice with knockout of the estrogen α receptor [42].

Oscillations in hormone levels during the estrous cycle have been identified by researchers as a factor complicating interpretation of results in behavioral tests [25, 27, 28]. However, testing of cognitive functions did not reveal any differences between the phases of the menstrual (estrous in rodents) cycle in either humans [26] or rats [49]. Furthermore, most members of groups of females kept together have been noted to be in the same phases of the cycle; the behavioral characteristics of female mice can be evaluated without interference from the estrous cycle [35].

Interspecies differences. The Morris test was initially developed to study spatial memory in rats (*Rattus norvegicus*) [36, 37]. However, it is currently also used widely in mice (*Mus musculus*), especially in relation to extending their use as objects for genetic modifications (transgenic mice) [42, 55].

Mice in the Morris water maze demonstrate behavior notably different from that seen in rats: they are much more inclined to noncognitive swimming strategies – passive drifting and thigmotaxis – which hinders performance of the test by mice. Some investigators [55] have suggested that rats perform the test better than mice because of their better swimming ability and more stable performance in sequences of trials but not because of any differences in their cognitive processes. However, we note here that interspecies differences may be due to some difference in the cellular and molecular mechanisms of memory. Comparison of the behavior of rats and mice in a selection of place orientation tests showed that these species coped with the "dry" version of the test (various radial mazes) equally well, but mice were inferior to rats in "water" tasks (Versions of the Morris maze) [54]. Thus, differences between species in the test do not arise from interspecies differences in spatial learning ability [16]. Better performance of the Morris test by laboratory rats is due to their origination from gray rats – an initially semi-aquatic species for which swimming is not a non-standard activity, as it is for mice [5].

Mice and rats behave differently in situations provoking laboratory animals to display restlessness or inducing stress. For example, comparison of animal models producing restlessness led to the conclusion that mice produce better results in tests based on an exploration paradigm, while rats perform better in tests based on a punishment paradigm [13]. Mice may be defeated by tests for active avoidance (and in the Morris test) simply by behaving passively, even electing not to make any active attempt to find a route to escape from the adverse factor (these are the behaviors noted above – passive drift and thigmotaxis) [16].

Differences between genetic strains. Many studies have demonstrated that features of the laboratory animal strains selected for the Morris text are reflected in the test results. Some studies have suggested a link between differences in Morris test performance by different rat strains and lack of maturity in albinos (especially inbred): bicolor Long–Evans rats cope better with the task than inbred albino Fischer 344 rats [33]. However, comparison of several albino strains showed that calm Sprague–Dawley rats learned better in the water maze than hyperstressed Wistar–Kyoto, such that the features of brain functioning typical of a strain also affect Morris test results [17, 29]. It is all the more noteworthy that Tokai High Avoider rats identified on the basis of good performance of avoidance tests cope with the task better than rats of the ancestral Wistar strain [47].

A similar comparison was performed in several mouse strains – 129/Ola, BALB/c, C57BL/6, and FVB/N [44]. It is interesting to note that no difference was seen between pigmented C57BL/6 and albino BALB/c. All mouse strains apart from FVB/N performed well in the basic variant of the test, the transfer test, and the visible platform test. The poor results of FVB/N mice were linked by the authors with decreased visual acuity in this strain, due to the fact that the genotype contains alleles inducing retinal degeneration. Some mouse strains had different levels of success performing the test in basins of different diameters [14].

Differences in age. Starting from early studies, it has repeatedly been noted that success in performing the Morris

test decreases with age. This may be due partly to age-related decreases in swimming ability, mobility, and investigative behavior [16]. Aging laboratory animals (rats and mice), like elderly humans, show problems with cognitive abilities in many situations, and many studies have demonstrated a link between poor performance of the Morris test and structural changes in various parts of the brain, including the hippocampus [20, 23, 40, 58]. Estradiol treatment of adult and old female rats improved results in the Morris test, while this effect was not seen in young rats, which is evidence for an effect of age-related characteristics of brain biochemistry on test results [31]. Fischer 344 rats aged from 1.5 months to 26 months performing the basic test and the visible platform test showed smooth and near-linear decreases in the level of learning success with age [34]. However, there was a significant spread of results among individuals, and age as a factor explained only part of this spread. It was suggested that the nature of these results could also be explained by an age-related decrease in brain functions not associated with spatial learning ability, i.e., functions determining noncognitive behavioral strategies. Thus, this provides further support for the non-synonymous nature of the Morris test results and the level of spatial learning ability.

Advantages and disadvantages of the Morris water maze. The wide area of application of the Morris test results from a number of advantages that this method has over other behavioral tests for small laboratory animals. Firstly, it does not require any preliminary training or preparation of the animals (such as stimulation by starvation), though in some studies animals have been given one "training" day with the platform visible [44, 58]. The test can be used over a relatively short period (a few days) in small numbers of animals (from five individuals).

Secondly, various modifications of the test allow different problems to be addressed. For example, the basic test reflects the process of learning, while the extinction test relates to the opposite process. In addition, in contrast to the "dry" versions of the same tests – the radial maze, the open field, and others – the water maze nullifies the effects of olfactory stimuli and markers on the animal's behavior.

The use of video recording and analysis of videos to determine trajectory and swimming speed, different types and strategies of behavior not associated with spatial memory (thigmotaxis, passive drift) can be identified, and animals' difficulties with motor function and vision can be detected. One particular variant of the test with the platform visible allows the problem of impaired visual orientation, which prevents the basic method from being used, to be avoided [50]. The platform has to be moved to a different part of the basin and only the first training (not repeat training) can be studied; the same approach can create conditions for comparing the effects of different doses of substances in a single experimental group (see above). Finally, immersion of the animal into water is a stress factor, though relatively mild compared with the starvation and electric shock used in other studies [50]. Test results are not influenced by common aspects of experiments such as subcutaneous injections (the fact of performing them) [45].

It is very important to note that when using the Morris test, there can be marked reductions in the distracting influence of the experiment on the test animal: the investigator can hide behind a curtain or screen and the animals can be monitored by video camera. Finally, the water maze can be used in small laboratories and is relatively simple technically for investigators to learn [50].

At the same time, the Morris water maze has drawbacks associated with difficulties in interpreting the contribution of spatial learning and various types of behavior of the test animals. When the transfer test is run (i.e., without the platform), the animals starts to spend less time in that part of the basin in which the platform had previously been located, which may indicate that this is direct evidence of extinction of spatial memory. However, in a considerable proportion of cases, the cause of increases in time spent on seeking the platform or extinction of searches at the previous location of the platform is not linked in any way with the animals' spatial memory. Along with remembering the structure of the space around itself, and often before this, the animal uses "noncognitive" behavioral strategies: for example, chaotic movement around the whole of the basin or exploring a fragment of the basin back and forth ("scanning") [18, 21, 56]. An extensive study in mice showed that about half of the variance in the results could be attributed to differences in thigmotaxis – a type of behavior in which the animal stays close to the basin wall; the mice would then make a long-lasting swim, spending little time close to the target. Some 19% of the results could be explained by the "passive drift" strategy – the mice swam slowly and showed a tendency to stay passively in the water until "rescued" by the experimenter. Differences in spatial memory came only in third place among the factors considered, accounting for only 13% of the spread in results. Finally, this order of factors reflects only their statistically observed influence on the data obtained in an actual experiment and not their functional priority. However, two thirds of the results could nonetheless be explained by factors not directedly related to spatial learning and memory, and thus termed "noncognitive" [55].

In this situation, it can be suggested that individual variability in the animals' use of noncognitive strategies is greater than that in the processes of acquiring spatial memory (on the basis of the greater influence of noncognitive strategies as a factor on the spread of the data). Significant amounts of data were collected for the study described above (data from 115 individuals), though the vast majority of studies use much smaller cohorts, making significant separation of the effects of spatial memory from noncognitive behavioral strategies difficult.

Thus, analysis of overall published data demonstrated the absence of evidence-based general recommendations supporting the greater suitability of any particular variant of

the Morris water test for assessing the effects of substances on brain function, so we selected the simplest of the available methods and assessed its suitability for our purposes.

A Model Experiment

Method. Experiments were performed in a standard apparatus – a round white basin 1.5 m in diameter, filled with water made opaque by addition of dried milk [37]; the basic version of the test was performed. Clear orientation markers were positioned around the basin. The time taken by the animal to reach the platform was measured in seconds.

Testing was performed for three days using three swimming trials per day with groups of 10–12 individuals. Mice were launched from one of four defined points around the basin, with random alternation of launch points. The duration of each trial was 180 sec; the individual sat on the platform for 20 sec and was then removed from the platform, dried, and returned to its cage until the next launch; the interval between launches was 20–30 min.

A total of 56 mongrel SHK mice aged 3.5–4 (mean wight 28.2 ± 3.4 g) months were used; these animals had no previous experience of behavioral tests. Animals were kept in the animal house at the Moscow Regional Research Clinical Institute and received normal daily feed and unlimited water. Data from four individuals were excluded because of failure to complete the experimental protocol: two mice kept jumping off the platform without sitting on it, and the other two used passive drift for more than two days in a row, i.e., they did not explore the surroundings. The overall cohort for statistical analysis consisted of 52 individuals (26 males and 26 females). The mean time spent on the platform per day was determined for each individual; daily means for the cohorts were subsequently compared. Data were processed statistically in Statistica 8.0 and differences were regarded as significant at $p < 0.05$.

Study results. We did not see any statistically significant differences between the first and subsequent experimental days (Wilcoxon test for linked pairs, $p = 0.077$; signs test, $p = 0.055$), either for the group as a whole ($n = 52$) (see Fig. 1, *A*) or for cohorts by gender (Wilcoxon test for linked pairs: ($p = 0.13$ for males and $p = 0.36$ for females; $n = 26$ for each group). There were also no differences between the daily cohorts of males and females (Mann–Whitney test, $p > 0.3$; two-cohort Kolmogorov–Smirnov test, $p > 0.1$). As the cohorts being compared belonged to different experimental days, we tested the role of day as a factor able to influence differences between the daily cohorts (Friedman test). The effects of day as a factor were found to be insignificant for both the whole group ($p = 0.17$) and for the separate genders ($p = 0.1$ for males, $p = 0.29$ for females).

Visual observation of swimming mice showed that different animals behaved differently during testing; the characteristics of individual behavior were stable in all three swimming trials on each experimental day, though they could change from one day to another. On the basis of individual behavioral characteristics in the test, the overall set of individuals was divided into three groups (see Table 1). Animals were classified as *capable* if on at least one day they displayed behavioral strategies whose use gave a high probability of finding the platform ("scanning," targeted search – classification as per [21]). These individuals accounted for one third of the cohort (31%, $n = 16$) and their mean swimming time to the platform was 81.8 ± 27.3 sec. This group showed a marked decrease in time by days (see Fig. 1, *B*) and differences between the first and last days were significant (Wilcoxon test for linked pairs, $p = 0.006$; signs test, $p = 0.006$).

Individuals consistently using random search – a type of behavior with an intermediate probability of finding the platform – constituted the *intermediate* group ($n = 25$). These animals found the platform in a mean of 124.5 ± ± 4.3 sec. There were almost no differences between days (Wilcoxon test for linked pairs, $p = 0.27$; signs test $p = 0.1$; see Fig. 1, *C*). We note that within this group, a more successful set of individuals could be identified, which found the platform within 2 min on two of the three days (mean 98 ± 28 sec for all days; $n = 12$), while the other mice coped with the task mostly during the third minute of swimming (mean for all days 149 ± 31 sec; $n = 13$). It should also be noted that the random search strategy was displayed by most mice on the first day of the experiment – on primary exploration of the basin. It was also noted that the mice swam more actively on the first day of the experiment than on the subsequent days.

Finally, the rest of the individuals in the cohort ($n = 11$), *incapable* individuals, showed types of behavior in which finding the platform was very unlikely on at least one day: these are the so-called "noncognitive" strategies (passive drift or thigmotaxis – [16]). Before reaching the platform, these mice swam for a mean of 160 ± 15 sec by the end of the trial, but more often failed to find it before the end of the allotted time. Platform reaching time in this group increased by day (see Fig. 1, *D*): after the initial (day 1) familiarization with the apparatus, the individuals transferred to the behavioral strategies noted above, which rarely led to finding the platform. Differences between the first and last days were significant (Wilcoxon test for linked pairs, $p = 0.018$; signs test, $p = 0.023$).

The role of experimental day as a factor was assessed for each of these groups of individuals (Friedman's test). A significant influence of day on differences between the daily cohorts was seen for capable and incapable individuals ($p = 0.003$ and $p = 0.02$, respectively) but not for intermediate individuals ($p = 0.24$). Significant differences in daily cohorts for values by experimental day were seen between intermediate and capable individuals (Mann–Whitney test, $p < 0.048$ for all three days; two-cohort Kolmogorov–Smirnov test on days 1 and 3, $p = 0.01$). Differences between intermediate individuals and the incapable group were significant on days 2 and 3 (Mann–Whitney test, $p < 0.027$; two-cohort Kolmogorov–Smirnov test, $p < 0.025$).

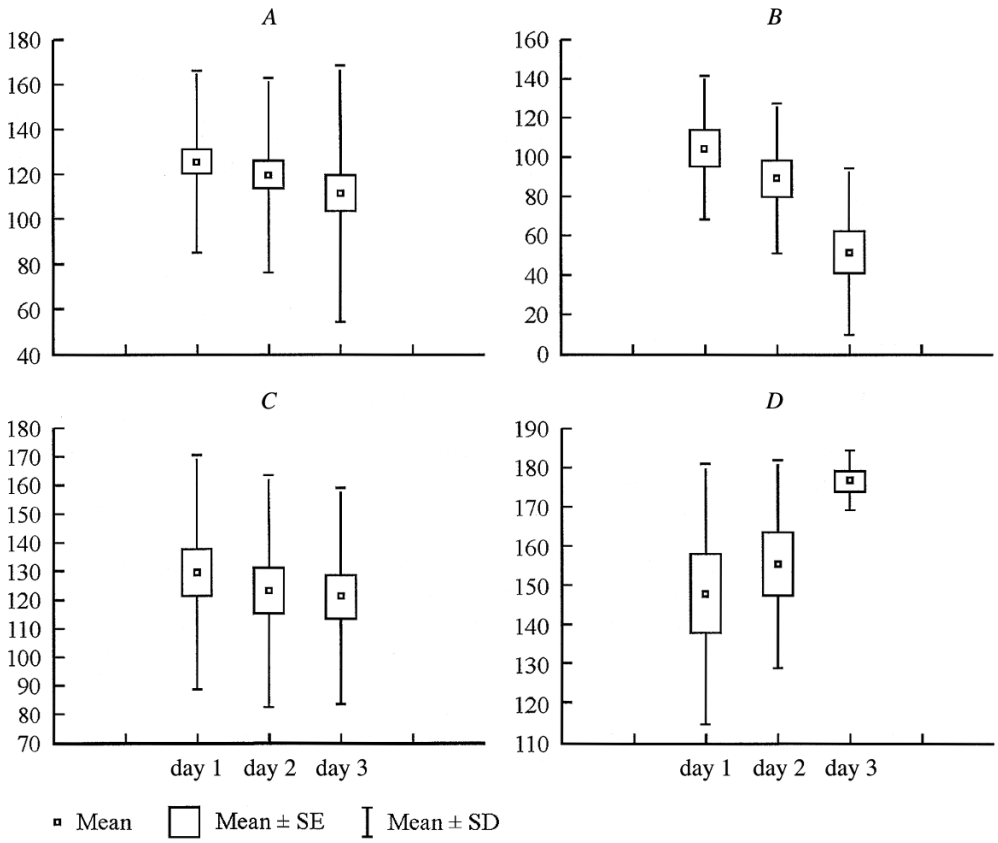

Fig. 1. Spread of Morris test results by experimental days. *A*) Whole group; *B*) capable individuals; *C*) intermediate individuals; *D*) incapable individuals. The ordinate shows mean platform finding time, sec. The small square shows the mean group value by day, the large rectangle shows the standard error of the mean, and the interval shows the standard deviation.

Discussion of results. The data presented here lead to the suggestion that individual behavioral features in the experimental animals had significant influences on Morris test results. The method used here is insensitive to changes in differences between experimental days, including those due to the random composition of the cohort of experimental individuals. As individuals with preferences for different types of behavior and with initially nonuniform changes in platform finding were taken into the analysis as a single cohort, statistical calculations smoothed the differences between experimental days. These results were regarded as insignificant and led to the conclusion that there were no visible changes in the group by day.

The recent study reported by Solov'eva et al. [8] divided a cohort of individuals into two groups on the basis of platform finding time: those whose times were below the median were assigned to the "well trained" group and the others to the "poorly trained" group. Mixed experimental and control groups were then compared: half the individuals were taken from one "ability group " and the other half from the other. Comparison of the results of repeat (after substance administration) testing revealed a significant overall difference between the control and experimental groups. However, there were no significant differences between the "well trained" and "poorly trained" animals from the experimental and control groups. Almost the reverse situation was seen when a different substance concentration was used.

The authors of [8] explained the differences in terms of the characteristics of the influences of different substance concentrations on individuals of the two groups. However, with this experimental protocol, the result could be significantly affected by the conditionality of the principle of identifying "groups by ability" – the exact boundaries are relative, i.e., subject to the influences of random factors and applicable only to a particular cohort of individuals. The very small number of individuals in the groups (six individuals, three "well trained" and three "poorly trained") also suggests that the influences of the test substance might be affected by the random distribution of individuals by group. When individuals are selected, a measure decreasing the influences of individual variability in test performance on the nature of the results obtained requires not only a concrete time boundary, but also a more universal boundary less subject to random influences, such as the preferred type of behavior in the test.

TABLE 1. Morris Test Results by Day

Groups defined by behavior during testing	Individual No.	Gender	Mean platform finding time by day, sec			Behavior during swimming
			day 1	day 2	day 3	
Capable	1	Males	92.0	44.7	26.0	Day 1 – random search; day 2 – targeted search; day 3 – “scanning”
	2	»	59.0	73.3	23.3	Day 1 – random search; days 2 and 3 – “scanning”
	3	»	170.3	94.3	61.0	Days 1 and 2 - random search; day 3 – targeted search
	4	»	84.0	27.0	14.7	Day 1 – random search; day 2 – “scanning;” day 3 – targeted search
	5	»	75.3	97.3	44.0	Days 1 and 2 – random search; day 3 – targeted search
	6	»	51.7	45.3	21.3	Ditto
	7	Females	89.7	95.7	27.7	»
	8	»	111.3	71.0	15.7	Day 1 – random search; days 2 and 3 – targeted search
	9	»	113.7	47.0	71.3	Days 1 and 2 – random search; day 3 – targeted search
	10	Males	110.3	153.3	168.0	Day 1 – random search; day 2 – “scanning;” day 3 – random search
	11	»	156.3	142.7	40.0	Days 1 and 2 – random search; day 3 – tendency to “scanning”
	12	»	92.7	108.3	36.0	Days 1 and 2 – random search; day 3 – targeted search
	13	»	82.7	85.3	130.7	Day 1 – random search; day 2 – “scanning;” day 3 – random search
	14	Females	180.0	115.0	37.7	Days 1 and 2 – random search; day 3 – “scanning”
	15	»	90.7	83	43	Day 1 – random search; days 2 and 3 – “scanning”
	16	»	115.7	145.3	64.7	Days 1 and 2 – random search; day 3 – “scanning”
Intermediate	17	»	83.0	66.7	79.3	Random search
	18	Males	87.3	120.3	139.7	»
	19	»	88.3	76.0	72.7	»
	20	»	71.3	132.3	60.3	»
	21	»	180.0	180.0	108.3	»
	22	Females	180.0	121.7	119.7	»
	23	»	134.0	66.3	121.7	»
	24	»	145.7	84.3	58.0	»
	25	»	101.3	99.0	121.7	»
	26	»	158.7	107.0	82.7	»
	27	»	34.0	91.7	128.7	»
	28	»	119.7	106.0	110.0	»
	29	Males	147.3	72.3	83.3	»
	30	»	180.0	180.0	180.0	»
	31	»	175.7	142.7	108.0	»
	32	»	165.3	180.0	113.0	»
	33	»	135.0	166.0	131.3	»

TABLE 1. Continued

Groups defined by behavior during testing	Individual No.	Gender	Mean platform finding time by day, sec			Behavior during swimming
			day 1	day 2	day 3	
Intermediate	34	»	123.3	180.0	180.0	»
	35	»	124.7	96.3	94.7	»
	36	Females	57.0	103.3	123.7	»
	37	»	154.3	180.0	180.0	»
	38	»	139.3	86.7	129.7	»
	39	»	123.0	123.7	180.0	»
	40	»	180.0	180.0	180.0	»
	41	»	145.0	131.3	140.3	»
Incapable	42	Males	160.7	123.0	163.3	Day 1 – mainly thigmotaxis; days 2 and 3 – random search
	43	»	131.3	126.0	180.0	Days 1 and 2 – random search; day 3 – tendency to passive drift
	44	»	180.0	180.0	180.0	Days 1 and 2 – thigmotaxis; day 3 – random search
	45	»	87.3	167.3	159.3	Days 1 and 2 – random search; day 3 – tendency to thigmotaxis
	46	»	126.3	126.7	180.0	Days 1 and 2 – random search; day 3 – thigmotaxis
	47	Females	180.0	180.0	180.0	Days 1 and 2 – thigmotaxis; day 3 – tendency to passive drift
	48	»	161.0	180.0	180.0	Day 1 – random search; days 2 and 3 – thigmotaxis
	49	»	104.0	144.3	180.0	Days 1 and 2 – tendency to passive drift; day 3 – passive drift
	50	»	180.0	122.3	180.0	Thigmotaxis
	51	»	180.0	180.0	180.0	»
	52	»	133.0	178.0	180.0	Day 1 – random search; days 2 and 3 – tendency to thigmotaxis

This raises the questions: what causes the demonstrable feature of the type of behavior? Can this change in any set of test conditions? Some studies have addressed performance of the Morris test by animals with differences in their behavior: a significant difference was seen in test performance by animals with different levels of anxiety – a quality which depends on the individual level of an innate characteristic of the nervous system (arousability) [9]. The influences of the characteristics of brain operation on Morris test performance by different laboratory animal strains have been noted [17, 29, 44], as have the ifluences of innate qualities. At the same time, the Morris water maze is suitable for assessing the "level of operation" of spatial learning and spatial memory functions (it is understood that this level will be determined separately for each individual). These functions are performed by limbic system structures (the hippocampus, etc.) and the limbic system, among others, is responsible for the emotions – a significant proportion of the temperament, from the physiological point of view, corresponds to the Pavlovian type of higher nervous activity.

On the basis of these points, it can be suggested that the type of behavior displayed by an individual in the Morris water test is determined (to at least some extent) by the innate properties of nervous system functioning of a particular individual, or the type of higher nervous activity that an individual has. And since the type of nervous activity remains unaltered throughout an individual's life, it would appear that the preferred type of behavior in the Morris water maze in each individual will also remain unaltered in any test protocol, so it can be used as a criterion for separating individuals into those performing the test well and those performing poorly. Our results indicate that this selection principle is correct: marked and significant differences were seen in the ranges of values and dynamics of the parameter of interest between groups of individual identified on the basis of their type of behavior in the test. Capable mice found the target relatively quickly and displayed marked changes in swimming time by experimental day, such that this group can be used in studies of effects on the brain requiring evaluation and alteration of the dynamics of the parameter and its range. The more successful proportion of the intermediate group could also be included in such studies as a separate "borderline" group for identifying changes in the range of the parameter.

It also follows that incapable individuals using "ineffective" behavioral strategies (such as passive drift) will not in principle find the platform in any systematic way, so changes by day will either not be seen in this group, as was the case in [30, 46] or will consist of increases in the time taken to swim to the platform. These individuals are consequently unsuitable for experimental investigations of any kind of action on the brain in cases in which the experimental protocol aims to detect changes in the dynamics of swimming time or where an increase in the range of a parameter would be expected. However, if the result of the study treatments is expected to be a decrease in the swimming time compared with controls, these individuals can be included in the test as a separate group.

Thus, we can suggest that decreasing the influences of individual behavioral features on test results needs prior selection of individuals using each type of behavior as the selection criterion. We will test this hypothesis in future studies.

Conclusions

The literature on the use of the Morris test for different experimental task was reviewed. The literature contains no general recommendations supporting the greater suitability of any particular version of the Morris test for the task of assessing the influences of substances on brain functions. The present studies selected the simplest of the methods, and this was used in a model experiment to assess the applicability of this method in upcoming studies of the physiological effects of nanoparticles on cognitive functions. The experimental results indicated that the initial method was recognized as insensitive. Prior selection of individuals on the basis of individual characteristics of behavior in the test is proposed as a measure to increase the sensitivity of the method.

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