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In Search of Memory Tests Equivalent for Experiments on Animals and Humans

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Older people often exhibit memory impairments. Contemporary demographic trends cause aging of the society. In this situation, it is important to conduct clinical trials of drugs and use training methods to improve memory capacity. Development of new memory tests requires experiments on animals and then clinical trials in humans. Therefore, we decided to review the assessment methods and search for tests that evaluate analogous cognitive processes in animals and humans.

This review has enabled us to propose 2 pairs of tests of the efficiency of working memory capacity in animals and humans.

We propose a basic set of methods for complex clinical trials of drugs and training methods to improve memory, consisting of 2 pairs of tests: 1) the Novel Object Recognition Test – Sternberg Item Recognition Test and 2) the Object-Location Test – Visuospatial Memory Test. We postulate that further investigations of methods that are equivalent in animals experiments and observations performed on humans are necessary.

MeSH Keywords: Animal Testing Alternatives • Clinical Trials as Topic • Memory Disorders • Neuropsychological Tests

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Background

Many researchers have recently presented results of experiments on animals related to the mechanisms of learning and memory. Some of these investigations concern the essence of the processes of data remembering and retrieval. For instance, Akar et al. presented results that confirm the thesis that inhibition of nitric oxide synthetase disturbs emotional, visual, and olfactory memory in mice [1].

Other researchers reported experiments on animals as models of clinical situations encountered in humans [2,3]. Zeef et al. performed experiments on rats, reproducing the pathological mechanisms of Huntington's disease [2]. Darcet et al. performed experiments on mice, which establish a model of anxiety and depression [3].

There are increasing publications presenting the results of trails in animals of effectiveness of various substances and drugs on improvement of memory [4–16].

In parallel, many studies have been conducted on human memory capacity in various clinical situations [18,19]. There are also reports on attempts to improve the effectiveness of memory capacity in humans, both through the use of medication and by use of special training method [18–22].

Animal experiments on memory have been performed specifically to increase the possibility of evaluating and improving human memory [24].

Both in animal experiments and in observations and studies on humans, the researchers use tests that assess memory capacity and eventual improvements of storage and data retrieval.

To be able to transfer research findings from animal experiments to address problems of memory disorders in humans, it would be convenient to utilize, at least in part, memory tests, that are equivalent in both animal and human experiments since they deal with similar aspects of memory mechanisms. Therefore, we sought to determine which animal memory tests were applicable to humans.

Contemporary investigations on methods of assessment and improvement of memory are particularly important due to frequent memory impairments in old age, which is important for healthcare in an aging society. The quality of life in the elderly is especially affected by impairments in functioning of the working memory system (WMS) [25].

We performed a review of memory tests used by investigators engaged in experiments on animals as well as tests used by scientists engaged in research on humans, looking for methods that evaluate the basic equivalent mechanisms of data storing and processing. We also present a review focused on tests suitable for the assessment of working memory, which is essential for overall mental capacity.

In Search of Simple Methods Used in Studies of Memory in Humans

Ciecko-Michalska et al. evaluated impairments of memory among patients with liver cirrhosis and used the following tests: Auditory Verbal Learning Test (AVLT), Letter and Semantic Fluency Tests (LF and SF), Trail Making Test (TMT), Digit Symbol Test (DST), Block Design Test (BDT), and Mental Rotation Test (MRT) [18]. Olubunmi et al. utilized the automated neuro-psychological test battery (FePsy) to assess the memory and perceptual-motor skills of patients with chronic renal impairments [19].

Hirayama et al. investigated whether dietary supplementation with soy-derived phosphatidylserine, a naturally occurring phospholipid, improves memory and ADHD symptoms in children [20]. They investigated 36 children with symptoms of ADH aged 4–14 years, who had not previously received any drug treatment related to ADHD, and a group of 17 children who received placebo [20]. They examined short-term auditory memory and working memory using the Digit Span Test of the Wechsler Intelligence Scale for Children [20].

Alvares-Sabin et al. evaluated the effects on cognitive impairment of treatment using citicoline and tested 6 neurocognitive domains: attention and executive function, memory, language, spatial perception, motor speed, and temporal orientation [21].

On the basis of the review of many articles, we noted that the action of the WMS in humans is very often assessed by designation of neuropsychological tasks such as the Wisconsin Card Sort Task (WCST) [26], in which the subject sorts a stack of 128 cards into piles. Each card has a particular number of particular shapes of a particular color. The cards differ in the kind of presented shapes (forms), number of these forms, and colors. The psychologist decides at a particular moment of testing which criterion should be used for sorting and the patient must figure it out. The psychologist says "yes" or "no" to each card that is laid down. Once the patient is sorting along the correct criterion, the tester adopts a new criterion.

Researchers use also often the Sternberg Item Recognition Paradigm (SIRP) in humans [27,28] in which a small group of items, called the "positive set", is presented for the subject to memorize. After a delay, a single item is presented that may or may not have been shown before. The subject is asked to respond 'yes' or 'no', indicating their recognition of the item. This procedure is repeated over several trials in which the number

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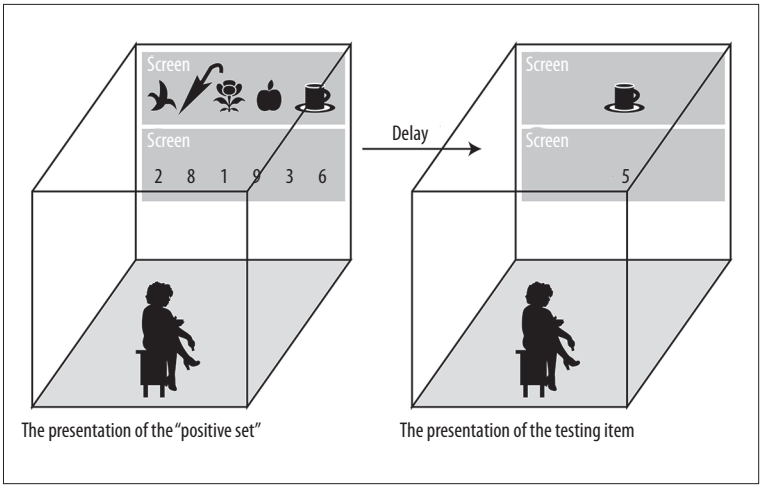

Figure 1. The Sternberg Item Recognition Paradigm or the Stenberg Short-Memory Test. A subject should accomplish a rather simple task. A small set of items, called the positive set, is presented, for instance on the screen, and should be memorized. The positive set can be composed for numbers, words, or images. After a delay, a single test item is presented. The subject should decide if the object was present among the "positive set". This procedure is repeated several times and the number of items in the "positive set" is varied. The subject should respond as fast as possible without making errors.

of items in the positive set is varied. Subjects are asked to respond as fast as they can without making errors (Figure 1).

The essence of the recently proposed methods of memory training is using the basic mental activity enforced by SIRP, which consists of a visual-to-code (symbol) switching [30]. An overview of methods used in humans can also be found in well-known textbooks.

The search for equivalent animal/human memory tests should be facilitated by a schema of contemporary distinguished domains of mental abilities. Strauss et al., in a well-known textbook [33], enumerated the following types of tests: General Cognitive Functioning, Neuropsychological Batteries and Assessment of Premorbid Intelligence, Achievement Tests, Executive Function, Attention, Memory, Language Tests, Test of Visual Perception, Tests of Somatosensory Function, Olfactory Function and Body Orientation, Test of Motor Function, Assessment of Mood, Personality and Adaptive Function, and Test of Response Bias and Suboptimal Performance

In their textbook, Strauss et al. list memory tests using the following methods: Autobiographical Memory Interview (AMI), Benton Visual Retention Test (BVRT), Brief Visuospatial Memory Test (BVMT), Brown-Peterson Task, Buschke Selective Reminding Test (SRT), California Verbal Learning Test (CVLT), Children's Memory Scale (CMS), Doors and People Test (DPT), Hopkins Verbal Learning Test (RMT), Recognition Memory Test (RMT), Rey-Osterrieth Auditory Verbal Learning Test (RAVLT), Rey Complex Figure Test (POCF), Rivermead Behavioral Memory Test (RBMT), Ruff-Light Trail Learning Test (RULIT), Sentence Repetition Test (SRT), Wechsler Memory Scale (WMS-III), and Wide Range Assessment of Memory and Learning (WRAML.2).

Most of these tests involve the determination of the tasks by oral commands. Only the Recognition Memory Test (RMT) and Rivermead Behavioral Memory Test (RBMT) use simple mental processes that are used by both humans and animals. Among the described methods, there are probably only 4 tests related to the assessment of the working memory system: Brief Visuospatial Memory Test (BVMT), Raven's Progressive Matrices, Category Test, and Wisconsin Card Sorting Test.

The examples of methods used by investigators of memory in humans reveal that tests used in such studies are inappropriate for use in experiments in animals because usually they involve more complex mental functions. It would probably be easier to find guidance related to this kind of test from researchers implementing animal experiments.

Data on Memory Tests Used by Researcher Performing Experiments on Animals

Akar et al. used 5 tests of memory in their experiments: Novel Object Recognition Test (NORT), Passive Avoidance Test (PA), Social Transmission of Food Preference Test (STFP), Open Field Test, and Free Exploratory Paradigm [1]. The administration of NORT and PA test are illustrated in Figures 2 and 3.

Hasanein and Shahidi used the Passive Avoidance Test (PA) in studying the influence of a selected substance on memory deficit in diabetic rats [4].

Zeef et al. investigated the impairments of spatial and object recognition memory in experiments on transgenic rats, an established model of Huntington's disease [2]. They tested the memory function using the Object Location Test (OLT) and the Object Recognition Task (ORT) [2]. These tests are similar to the Object-Location Test (Figure 4).

Darcet et al. assessed cognitive performances by "behavioral tests measuring episodic (novel object recognition test, NORT), associative (One-Trial Contextual Fear Conditioning, CFC), and

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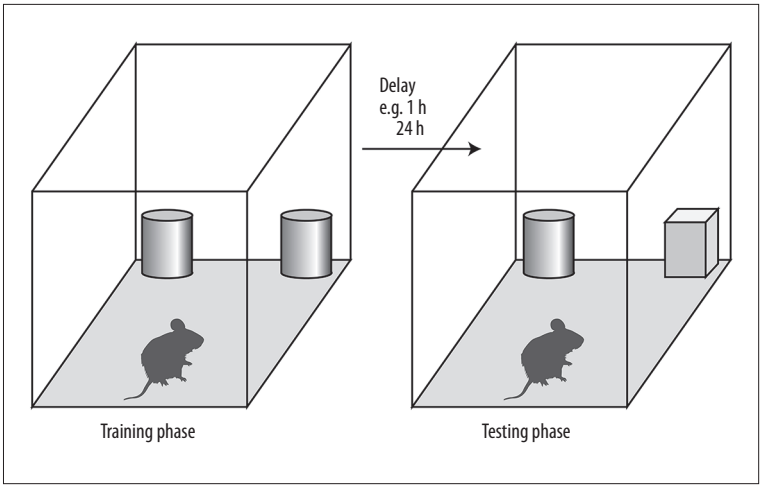

Figure 2. The Novel Object Recognition Test. The protocol of NORT in the training phase allows the experimental animals (usually mice or rats) to explore 2 identical objects. After of a delay (1 h or even 24 h) the animal is exposed to 2 different object: 1 familiar from the training phase and 1 novel object. The time that the animal spends to explore the novel object is recorded.

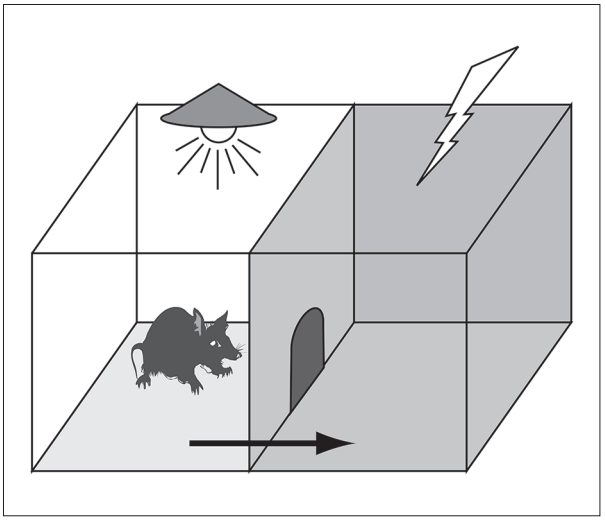

Figure 3. The passive Avoidance Test. It is performed in a cage composed of 2 parts: lighted and dark chambers. An animal may initially freely explore the light and dark compartments. Mice have a natural tendency to enter dark environments. Afterwards, a mild electric foot shock is delivered in 1 of these chambers. The animal learns to avoid the compartment in which an aversive stimulus was previously delivered. The latency to pass the gate in order to avoid the shock is used as an indicator of learning and memory.

visuospatial (Morris Water Maze and Barnes Maze) learning and memory" [3]. The method of administration is similar, but simpler – the Y maze test is illustrated in Figure 5.

Budzynska et al. describe their methodology, writing that "Male Swiss mice were tested for anxiety in the elevated plus maze test (EPM), and for cognition using passive avoidance (PA) procedures" [5].

The same team, in their former work, evaluated the impact of substances that affect the cholinergic system in mice, using the Modified Elevated Plus Maze (mEPM) Test, emphasizing that this test allows examination of various processes of memory (acquisition, consolidation, and retrieval). As an index of memory, the authors used the time necessary for mice to move from the opened arm to the enclosed arm [6].

Nassiri-Asl et al. tried to verify the hypothesis that flavonoids, compounds present in fruits and vegetables, prevent neurodegenerative diseases[7]. These investigators assessed the effect of quercetin (3,3',4',5,7-pentahydroxyflavone) on oxidative stress and memory retrieval using a step-through passive avoidance task in kindled rats [7].

Chauveau et al. tested whether Ciproxifan (histamine H3 receptor type antagonists) improves working memory in sleep-restricted mice. They tested the function of working memory by appropriate tasks using spatial spontaneous alternation behavior [8].

Bardgett et al. examined the impact of antagonists of H(3)-type histamine receptors on the various properties of memory. They tested whether ciproxifan could reverse the behavioral effects of MK-801, a drug used in animals to mimic the hypoglutamatergic state suspected to exist in schizophrenia. Four behaviors were chosen for study: locomotor activity, ataxia, prepulse inhibition (PPI), and delayed spatial alternation [9].

Akkerman et al. examined the temporal profile of pharmacologically enhanced episodic memory, using the object recognition task [10].

Prickaerts et al. investigated the effects of 2 cyclic GMP-specific phosphodiesterase enzyme type 5 inhibitors, sildenafil and vardenafil, using the object recognition task to assess memory performance of rats [11]. This study showed the memory enhancing effects of phosphodiesterase type 5 (PDE5) inhibitors. The same team of researchers, in their subsequent work

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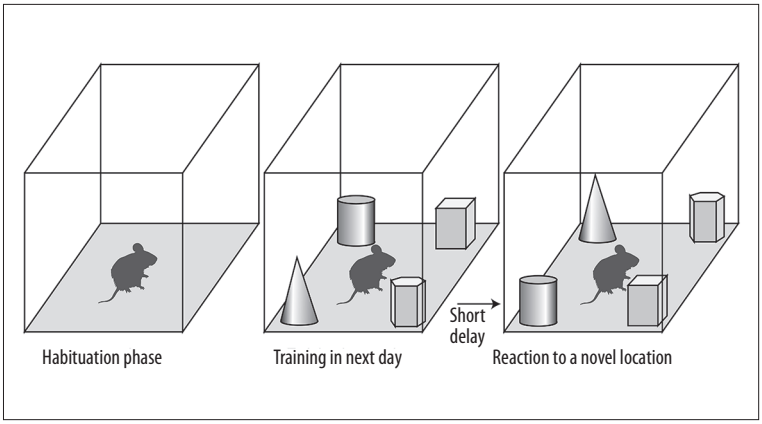

Figure 4. The Object-Location Test. It is used to assesses the ability of rodents for spatial memory and discrimination. The test is based on the spontaneous tendency of rodents to spend more time exploring a novel object than a familiar one, and also to recognize the situation when an object has been relocated. During first-day testing, the animals is habituated in an open field arena. The next day, 4 objects of different shapes are introduced to the arena. In the first trial, the animal is allowed to explore the arena with the 4 objects. Shortly thereafter, the animal again encounters the 4 objects, but 2 of them have switched positions. The trials are recorded using a camera mounted above the arena and the subject isscored for the amount of time spent sniffing the objects. The object-location discrimination index is calculated.

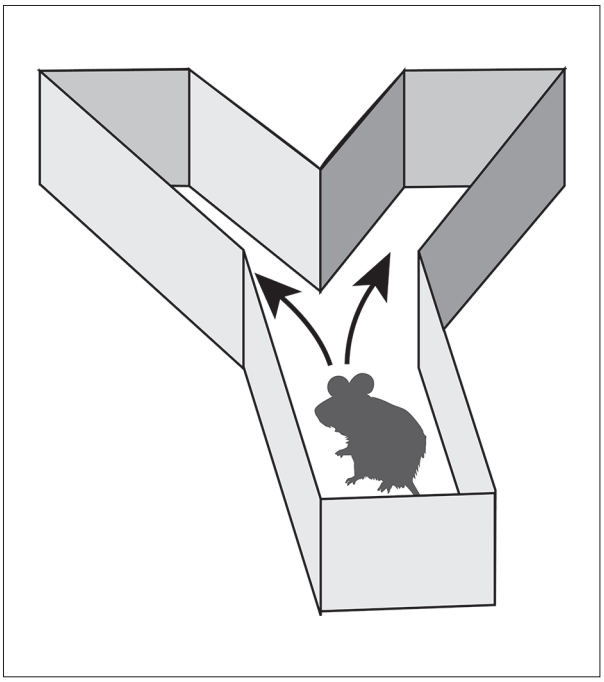

Figure 5. Y (or T) Maze Spontaneous Alternative Test. This is the behavioral test for measuring the willingness of rodents to explore a new environment. Mice prefer typically to to explore a new arm of the maze rather then returning to one previously visited. Intially, an animal is inserted to the center of the maze and allowed to freely explore the 3 arms. Over multiple arm entries, the animal should show a tendency to enter a less-recently visited arm. The number of arm entries and the number of triads are recorded to calculate the percentage of alternation.

performed on mice, demonstrated some differences in nitric oxide (NO)-mediated cyclic GMP (cGMP) signaling in the hippocampus between rats and mice [12]. A postsynaptic role of cGMP could be involved in this respect. Taken together, these studies show that inhibition of PDE5 improves object recognition memory in rats and mice [12].

Levin et al. wrote that in a variety of studies the nicotinic agonists have been shown to improve cognitive function [13] and they experimentally determined the attentional function. Adult female Sprague-Dawley rats were trained on the visual signal detection task. Rats discriminate whether or not a light signal occurred on a trial and respond with a lever press on 1 side after a signal and on the opposite side after the absence of a signal in order to receive a food pellet reinforcer [13].

The authors of a recently published paper reported the impact of zaprinast and rolipram on a model of spacial and emotional memory in mice used for verification of influences of the elevated plus maze test (EPM) and passive avoidance test [14].

Celikyurt et al. investigated the effects of a potent nitric oxide-guanylate cyclase activator on learning and memory functions in aged rats, using the Morris Water Maze (MWM) and Passive Avoidance (PA) Test [15]. The same team of researchers used a mouse model to investigate the anxiolytic and antidepressive impacts of Exenatide, a potent agonist for the glucagon-like peptide-1 (GLP-1), which is used in the treatment of type II of diabetes mellitus [16]. They used a modified elevated plus-maze test for evaluation of anxiolytic behavior and a forced swimming test for evaluation of depressive behavior [16].

The Elevated Plus Maze Test (EPM) mentioned in several of the above-cited papers has been described in detail by Komad et al. [17]. Their open access paper includes visualization available online, showing how this test is administered [*http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2762911/*].

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An Attempt to Formulate a Set of Memory Tests Equivalent for Experiments on Animals and Humans

Careful analysis of the above-mentioned investigations performed on humans and animals leads us to propose that the most appropriate candidate for testing memory in both humans and animals is the Novel Object Recognition Test (NORT), which was used by Akar et al. [1]. We conclude this based on the description of this test by Ennaceur and Delacour [34]. The inventors of this method write that the test is based on the differential exploration of familiar and new objects by animals. In a first application of the test, the rats are exposed to 2 identical objects, and in a second trial to 2 dissimilar objects – a familiar and a new one. The authors found that the test is similar to visual recognition tests widely used in primates, and that it is "entirely based on the spontaneous behavior of rats and can be considered as a 'pure' working-memory test completely free of reference memory component and it does not involve primary reinforcement such as food or electric shocks; this makes it comparable to memory tests currently used in man" [34]. Ennaceur and Delacour took advantages of features of their elaborated method for testing in experiments on rats the efficacy of the medicament "piracetam" used in human therapy [24]. There are currently some exhaustive discussions of all aspects of the utilization of this method and some of its modifications [35–38].

In search of a test that would be equivalent for experiments on animals and humans in verification of the efficiency of working memory system, we should consider the Sternberg Item Recognition Paradigm, which is a generalized procedure for reproducing the above-discussed NOR test. This generalization enables it to also assess WMS capacity..

We emphasized in our previous paper that capacity and proper functioning of WMS in experiments on animals usually is done by means of the Delayed Response Task (DRT) [25]. Performing this kind of test, the location of a reward is shown to the animal. The animal must retain information about this location across a delay.

The tests based on the Delayed Response Task also evaluate the visual and spatial function of working memory. This aspect of working memory is also evaluated by the Object-Location Test.

Among the whole range of tests designed to evaluate the capacity of human memory, we should look for the simplest tests used to assess the ability to memorize spatial data.

The best-known and utilized tests evaluating visual-spatial working memory are the Corsi Block-Tapping Test (CBT) and Visual Pattern Span [39–41]. The tool used during the performance of the classic CBT are 9 wooden blocks (3×3 cm) that are randomly distributed on a 25×30 cm board, requiring the subject to press on a series of blocks. The subject should remember which blocks have been tapped on. Subsequently, the subject must recall the sequence and immediately repeat it in the same order. There are several computerized versions of the Corsi test, including the called "walking" version of the test [39–41]. Perrchon et al. clearly illustrated the protocol of the CBT performance in their paper, which is accessible online [41]. It is possible to become familiar with the essence of this test through the readily available websites presenting the popular versions of this test [42].

The Visual Pattern Span Test is simpler. A series of matrix patterns are presented to the subject. The presented matrix has half of the cells colored and half blank. The matrix patterns force the participant to rely on visual spatial memory. The test begins with a small 2×2 matrix. The subject is asked to copy the matrix pattern from memory into an empty matrix. The matrix patterns are increased in size and complexity until the participant's ability to replicate them collapses. On average, participants' performance tends to break down at 16 cells. Analogous functions in humans can be checked by the Bief Visuospatial Memory Test [33,43].

Careful consideration of the data presented above, related to the most well-known tests, is the basis for forming proposals to solve the problem set as the goal of this paper. We propose that 2 pairs of tests – NORT – SIRP and OLT – CBT (BVSMT) be used as the basic tools for interdisciplinary memory assessment in humans and animals, particularly in studies on efficacy of drugs. Moreover, it seems to us that in the many recent software tools available to assess and improve memory capacity, we should always try to find out the implemented rules of NORT – SIRP and OLT – CBT (BVSMT) [22,32].

Conclusions

1. Some of the memory tests already in use are characterized by simplicity and do not require reinforcement techniques, so they are suitable for the assessment of the memory capacity in both animals and humans, which is important for trials of new drugs.
2. Further efforts are needed to establish equivalent memory tests for both animal and human experiments.
3. Two pairs of tests NORT – SIRP and OLT – CBT (BVSMT) constitute the basic tools for interdisciplinary memory assessment, and its essential rules can be found in recently commercialized software for memory training.

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