Cogn Process (2012) 13:93–110
DOI 10.1007/s10339-011-0430-z

REVIEW

The novel object recognition memory: neurobiology, test procedure, and its modifications

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Received: 23 August 2011 / Accepted: 24 November 2011 / Published online: 9 December 2011

Abstract Animal models of memory have been considered as the subject of many scientific publications at least since the beginning of the twentieth century. In humans, memory is often accessed through spoken or written language, while in animals, cognitive functions must be accessed through different kind of behaviors in many specific, experimental models of memory and learning. Among them, the novel object recognition test can be evaluated by the differences in the exploration time of novel and familiar objects. Its application is not limited to a field of research and enables that various issues can be studied, such as the memory and learning, the preference for novelty, the influence of different brain regions in the process of recognition, and even the study of different drugs and their effects. This paper describes the novel object recognition paradigms in animals, as a valuable measure of cognition. The purpose of this work was to review the neurobiology and methodological modifications of the test commonly used in behavioral pharmacology.

Keywords Novel object recognition · Memory · Learning · Novelty · Animals

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Introduction

Over time, the relationship between novelty and behavior has received much attention from researchers. Novelty is an alteration from expected likelihood of an event on the basis of both previous information and internal estimates of conditional probabilities. More important than a definition of novelty is to know that animals can be affected by a novel stimulus. The novel stimuli can change animals' behavior, provoke stress responses, elicit approach behavior, and cause an increase in corticosterone plasma levels, which is a major index of stress and suggests that confinement in a novel environment is stressful (Bevins et al. 2002).

Behavioral tests that evaluate the ability of recognizing a previously presented stimulus constitute the core of animals' models of human amnesia (Baxter 2010). Among the tests used are the visual paired comparisons task (VPC) in humans, the open-field task, the one-trial novel object recognition (NOR) test, and delayed non-matching to sample (DNMS) in rodents (Ennaceur 2010). These tests normally assess animal's behavior when it is exposed to a novel and a familiar object. In DNMS, animals learn that if they choose the novel object, they will be rewarded. However, in VPC and NOR tests, there are no rewards and animals explore the novel object as their natural propensity to the novelty, and it is possible to evaluate the index of stimulus recognition (Baxter 2010).

Ennaceur and Delacour (1988) studied for the first time the novel object and novel location recognition tests. They concluded that these tests are simple behavioral assays of memory that rely primarily on a rodent's innate exploratory behavior in the absence of externally applied rules or reinforcement. The NOR task has become a widely used model for the investigation into memory alterations. However, it

can be configured to measure working memory, attention, anxiety, and preference for novelty in rodents (Goulart et al. 2010; Silvers et al. 2007). Yet, it has also been used to test the effects of various pharmacological treatments and brain damage (Goulart et al. 2010).

The way how performance of animals is evaluated in the NOR test may also vary. It can be calculated through different indexes, as discrimination index, index of global habituation, or preference index depending on the aim of each study (Ennaceur and Delacour 1988; Gaskin et al. 2010; Hammond et al. 2004). It is important to note that the object recognition in animals may be measured by the difference in the exploration time of novel and familiar objects. The recognition measure is influenced by the interval between time spent with novel object and time spent with sample object as well as the time allowed for rats to explore the sample in a first trial. Thus, a wider range of variables can be sensitive to brain lesions and pharmacological treatments (Ennaceur and Delacour 1988).

The NOR task is particularly attractive because it requires no external motivation, reward, or punishment but a little training or habituation is required, and it can be completed in a relatively short time (Silvers et al. 2007). As previously mentioned, when animals are exposed to a familiar and a novel object, they approach frequently and spend more time exploring the novel than the familiar one (Ennaceur 2010). However, the environment influences the choice of animal as well. The increased preference produced by object-environment pairings reflects a conditioned association between environmental cues and the appetitive effects of receiving access to novel stimuli (Bevins et al. 2002). Like this, we can note that environmental familiarization interferes with novel object interaction. The preference for a novel object means that presentation of the familiar object exists in animals' memory (Ennaceur 2010). The recognition of novelty requires more cognitive skills from the subject, relative to tasks measuring exploration of novel environments or a single novel object (Silvers et al. 2007). This concept is the basis of the classical NOR test which has been used in the study of memory functions in rodents, as already mentioned (Ennaceur 2010). Animal paradigms like the NOR that evaluate recognition memory and object recognition memory in particular have become increasingly useful tools for basic and preclinical research as it allows studying the neural basis of memory.

The NOR task is very useful to study short-term memory, intermediate-term memory, and long-term memory, through manipulation of the retention interval, i.e., the amount of time animals must retain memory of the sample objects presented during the familiarization phase before to the test phase, when one of the familiar objects is replaced by a novel one (Taglialatela et al. 2009). It is commonly accepted that memory of a single episode would be much more vulnerable than that based on the repetition of some conditions, such as responses to a reinforcer or the association of stimulus (Ennaceur and Delacour 1988).

Moreover, results of the NOR paradigm are influenced by both hippocampal and cortical lesions (Buckmaster et al. 2004; Clark et al. 2000). It is widely accepted that in both the monkey and the rat brain, the perirhinal cortex plays an important role in object recognition memory (Aggleton et al. 2010), i.e., the ability to evaluate a previously encountered item as familiar depending on the integrity of the medial temporal lobe (Hammond et al. 2004). This brain structure plays an important role in recognition memory formation, and when some damage exists, the performance in recognition memory tasks is impaired (Albasser et al. 2009). Studies with primates and rodents have shown that for visual object recognition memory, the parahippocampal regions of the temporal lobe (namely the perirhinal, entorhinal, and inferior temporal cortices) are very important (Hammond et al. 2004).

Recent reviews have described the use of the NOR task and its variants in many experimental works. Here, we will examine some modifications of procedures of this task, particularly differences between animals, time and number of observation and localization of objects, apparatus, and different measure indexes used. We will also talk about how and why object recognition has been considered and evaluated. The results obtained after administration of specific drugs are also topics of analysis. We will discuss what kind of memory can be measured and which brain structures are involved in object recognition memory.

Modification of the novel object recognition test

The basic procedure of the NOR and its modifications

The NOR task evaluates the rodents' ability to recognize a novel object in the environment. Basically, in the NOR task, there are no positive or negative reinforcers, and this methodology assesses the natural preference for novel objects displayed by rodents. The task procedure consists of three phases: habituation, familiarization, and test phase. In the habituation phase, each animal is allowed freely exploring the open-field arena in the absence of objects. The animal is then removed from the arena and placed in its holding cage. During the familiarization phase, a single animal is placed in the open-field arena containing two identical sample objects ($A + A$), for a few minutes. To prevent coercion to explore the objects, rodents are released against the center of the opposite wall with its back to the objects. The experimental context is not drastically different during the familiarization and the test

phase. After a retention interval, during the test phase, the animal is returned to the open-field arena with two objects, one is identical to the sample and the other is novel ($A + B$) (Ennaceur 2010; Ennaceur and Delacour 1988; Gaskin et al. 2010; Hammond et al. 2004; Taglialatela et al. 2009). During both the familiarization and the test phase, objects are located in opposite and symmetrical corners of the arena and location of novel versus familiar object is counterbalanced (Hammond et al. 2004). Normal rats spend more time exploring the novel object during the first few minutes of the test phase, and when this bias is observed, the animal could remember the sample object. However, if animal repeats brief exposures to the sample object over a period of a few days, it can discriminate the sample from a novel object after delays of several weeks (Mumby et al. 2002). The strongest novel object preference scores tend to occur early in the test phase; while the novel object is still relatively novel, since in the course of time, the novel object became familiar (Broadbent et al. 2010). Despite animals spent more time exploring the novel object, the recognition performance varies according to the delay between the familiarization and the test phase, as well as the time of exploration of the sample during the familiarization phase (Ennaceur and Delacour 1988).

The procedure described above is the basis of the NOR. However, taking into account the objective of each investigation, some modifications can be made to the original method. In the study of Hale and Good (2005), for a half of rats, the sample object was *A* and the novel object was *B*, while for the other half, the sample object was *B* and the novel object *A*. These modifications were made to reduce object and place preference effects. The objects apparently had no natural significance for rats and had never been associated with reinforcement.

A modification in the number of objects presented in the familiarization and the test phase could be also observed. It is noted in Oliveira et al. (2010), Sarkisyan and Hedlund (2009) and Benice et al. (2006) works, where three distinct objects were presented during the familiarization phase. In the test phase, three objects were also presented, but one of them had a novel spatial location. Also, in Benice and Raber's study (2008), three objects were used. In the test phase, the location of objects did not change, but one of them was replaced by a novel one.

In the study of Hale and Good (2005), the number of objects was different. They used four different objects placed in the center of the four squares of the arena. In the familiarization phase, animals contacted with four objects. In the test phase, two objects were placed in the same position remaining two objects switched positions. The object position alteration occurred in a diagonal plane. During the familiarization, an object was placed in the top left, while in the test phase, it was placed in the lower right, or vice versa.

Piterkin et al. (2008) who evaluated the role of the hippocampus in the modulation of novel object preference made a modification in the test phase. Animals explored sample objects in one context and, after a retention interval, they returned to either the same context or to a different one, where they encountered sample objects paired with novel objects. However, this different context was also familiar. Only local features proximal to the object changed between sample exposure and test, whereas global features of the context did not change.

In Williams et al.'s study (2007), in the familiarization phase, two identical objects were placed in the open-field arena. After delay, in the test phase, two identical objects used in the familiarization phase were placed in open-field arena, but one of them was displaced 90° from the original position.

Another modification can be observed in the study of Burke et al. (2010). They tested object recognition memory in the aged rats and developed three experimental conditions. The first one was the basis of the NOR. The second experience could be considered as a modification of the NOR and involved the simultaneous presentation of two identical objects during both the familiarization phase and the test phase. As such, in familiarization phase, two identical novel objects (C + C) were simultaneously presented, but during the test phase, either two different novel objects ($D + D$) were presented ("novel condition") or the objects from familiarization phase were presented again ("repeat condition"). Here, each animal executed two trials of recognition testing; one trial was the novel condition, while the other was the repeat condition. In this test, to promote more exploration, the position of the objects in the open-field arena was also changed. The order of trials and stimuli presented were counterbalanced across animal groups. After familiarization phase and retention interval, animals that participated in the repeat condition were exposed to the novel condition, while animals that participated in the novel condition were exposed to the repeat condition. This modification allowed direct comparison of exploration time in the test phase relative to exploration time in the sample phase, because both phases involved exploration of a pair of identical objects. The third experiment could be also considered as a modification of the NOR. It evaluated the NOR test with context change. For this, an open-field arena *A* was used for both familiarization phases, while the both test phases occurred in open-field arena *B*. Here, each animal participated in two object familiarization and test phases.

Dere et al. (2005) assessed the long-term memory for different objects, their spatial location, and their order of presentation in a familiar open field. Authors designed a

three-trial object exploration task in which different versions of the novelty preference paradigm, the memory for spatial locations in which objects were explored, and the temporal order memory for object presentations were combined to examine whether mice could simultaneously encode and subsequently remember the "what,"-"where,"- and -"when" components of a unique episode, during two sample trials separated by 50 min, and remembered during a single test trial applied after another delay of 50 min. According to the behavioral criteria for episodic-like memory in animals, these results showed that during a single test trial the mice were able to recognize previously explored objects, remember the location in which particular objects were previously encountered, as well as to discriminate the relative recency in which different objects were presented.

A different test phase of NOR was made by Weible et al. (2009), as they worked with two sample objects in the first two test sessions. In the third and fourth test sessions, a sample and a novel object were used. And finally, in the fifth and sixth test sessions, objects had a novel location. Each test phase lasted 10 min, 6 min apart.

This way, we can observe an amount of features that could be evaluated with single modifications of a method of the NOR task, although all modifications of this test are always based on three steps: habituation, familiarization, and test phase.

Animals

The NOR test is widely used to evaluate object recognition memory in rodents and lead itself well cross-species generalization (Gaskin et al. 2010; Reger et al. 2009). Thus, it is important to understand what kind of animals has been used in the NOR test and which are their features (details presented in Table 1). Sometimes animals' models with specific modifications were necessary. In Taglialatela et al.'s study (2009), transgenic animals to study Alzheimer's disease (AD) have been used, since these mice suffer from progressive decline in several forms of declarative memory including fear conditioning and novel object recognition. For the same purpose, this kind of mice was also used in Hale and Good research (2005), once they studied the effect of a human amyloid precursor protein mutation that results in an autosomal dominant familial form of AD on processes supporting recognition memory, including object location memory.

Although most of studies have used rats or mice, we found two works where monkeys have been used to recognize objects. The first one was developed by Buckmaster et al. (2004), who applied the DNMS test and the object discrimination acquisition and retention test. They worked with nine feral-born, experimentally naive, male cynomolgus monkeys (*Macaca fascicularis*), weighing 4.2–6.0 kg at the beginning of the experiment, estimated to be about 5–7.5 years old. As rodents, they were maintained on a 12-h light/dark cycle, housed individually and fed with primate chow twice per day in an amount that supported reliable performance, and received a daily multivitamin and water available ad libitum. The second one was developed by Peissig et al. (2007), who distinguished between the effect of long-term object familiarity and that due to short-term repletion, but object familiarity was only measured over the course of several days or even weeks. In their research, they used 2 adult male rhesus macaque monkeys (*Macaca mulatta*), weighing between 9 and 13 kg.

According to the European Community Council Directive for the Care and Use of Laboratory Animals of 24 November 1986 (86/609/EEC), all procedures and the place where animals live must be controlled with respect to variables temperature (19°–23°C ± 1°C), humidity (45–60%), and light (12/12-h light/dark cycle). Regarding the form of animal feeding, during the most of experiments, animals had free access to food (laboratory chow) and water in their home cages. However, sometimes animals received reduced daily ratio. It is important to note that in all studies with animals, the procedures were designed to minimize the potential discomfort during behavioral tests. Depending on the laboratory and country, experiments were performed in a set of standards that researches must respect and comply in order to protect the rights of animals and minimize suffering. It is important to note that in most articles, this issue was approached.

We may notice extensive modifications that were made to the work in respect of animals used, their features, and characteristics. Collectively, the findings cited above suggest some differences in preference for novelty, dependent upon gender, strain, sex, and especially age. It is important to remember that in the first research of Ennaceur and Delacour (1988), these authors used a total of 220 male Wistar rats weighing 200–250 g, housed in individual cages, with 12 h of light–dark cycle (7.00 a.m. to 7.00 p.m.), the ambient temperature 23 ± 1°C, and free access to food and water.

Exploration concept

A concept that needs to be clarified is the "exploration." Ennaceur and Delacour (1988) defined as exploration of an object the directing the nose at a distance ≥2 cm to the object and/or touching it with the nose, while turning around or sitting on the object was not considered as an exploration. For most studies, exploration was defined as the orientation of animal's snout toward the object, sniffing or touching with snout, while running around the object, sitting or climbing on it was not recorded as exploration

Table 1 Animals used in the NOR test

Gender	
Rats	Aggleton et al. 2010; Albasser et al. 2009; Aubele et al. 2008; Bevins et al. 2002; Broadbent et al. 2010; Burke et al. 2010; Clark et al. 2000; Ennaceur and Delacour 1988; Frumberg et al. 2007; Gaskin et al. 2010; Goulart et al. 2010; Herring et al. 2008; Nanfaro et al. 2010; Piterkin et al. 2008; Reger et al. 2009; Silvers et al. 2007
Mice	Benice and Raber 2008; Benice et al. 2006; Bilsland et al. 2008; Botton et al. 2010; Clarke et al. 2010; Dere et al. 2005; Hale and Good 2005; Hammond et al. 2004; Oliveira et al. 2010; Schindler et al. 2010; Wang et al. 2007; Weible et al. 2009
Sex	
Males	Aggleton et al. 2010; Aubele et al. 2008; Bevins et al. 2002; Botton et al. 2010; Burke et al. 2010; Clark et al. 2000; Dere et al. 2005; Ennaceur and Delacour 1988; Frumberg et al. 2007; Gaskin et al. 2010; Goulart et al. 2010; Hammond et al. 2004; Herring et al. 2008; Nanfaro et al. 2010; Oliveira et al. 2010; Piterkin et al. 2008; Reger et al. 2009; Silvers et al. 2007; Schindler et al. 2010; Wang et al. 2007
Both males and females	Bilsland et al. 2008; Hale and Good 2005
Age	
Animals 2–4 months old	Clark et al. 2000; Clarke et al. 2010; Dere et al. 2005; Frumberg et al. 2007; Goulart et al. 2010; Hammond et al. 2004; Mumby et al. 2002; Oliveira et al. 2010; Nanfaro et al. 2010; Piterkin et al. 2008; Walf et al. 2009
Immature, i.e., 20–23 days (weanling), 29–40 days (juvenile), and more than 50 days (young adulthood) old	Reger et al. 2009
Aged, i.e., 7–9 months and 24–25 months old	Burke et al. 2010
Housing	
Individual cage	Broadbent et al. 2010; Burke et al. 2010; Ennaceur and Delacour 1988; Gaskin et al. 2010; Mumby et al. 2002; Oliveira et al. 2010; Piterkin et al. 2008
In groups of 2–5/cage	Aggleton et al. 2010; Botton et al. 2010; Goulart et al. 2010; Herring et al. 2008; Nanfaro et al. 2010
Feeding	
Ad libitum	Aggleton et al. 2010; Albasser et al. 2009; Aubele et al. 2008; Bevins et al. 2002; Bilsland et al. 2008; Botton et al. 2010; Broadbent et al. 2010; Burke et al. 2010; Clark et al. 2000; Clarke et al. 2010; Goulart et al. 2010; Hammond et al. 2004; Herring et al. 2008; Nanfaro et al. 2010; Oliveira et al. 2010; Reger et al. 2009; Sarkisyan and Hedlund 2009; Silvers et al. 2007; Schindler et al. 2010; Wang et al. 2007
Access restricted, 25–30 g/day	Benice et al. 2006; Gaskin et al. 2010; Piterkin et al. 2008 Mumby et al. 2002

(Aggleton et al. 2010; Aubele et al. 2008; Bilsland et al. 2008; Broadbent et al. 2010; Clark et al. 2000; Clarke et al. 2010; Goulart et al. 2010; Hale and Good 2005; Nanfaro et al. 2010; Reger et al. 2009; Silvers et al. 2007; Schindler et al. 2010). Sometimes, when animal's head was oriented within 45° of the object, it can also be viewed as exploration (Gaskin et al. 2010; Mumby et al. 2002; Piterkin et al. 2008). However, the major difference between studies was the distance from the snout to the object that each one considered as exploration, basically within 1–4 cm (Aggleton et al.

2010; Botton et al. 2010; Broadbent et al. 2010; Burke et al. 2010; Clark et al. 2000; Ennaceur and Delacour 1988; Gaskin et al. 2010; Hale and Good 2005; Mumby et al. 2002; Nanfaro et al. 2010; Piterkin et al. 2008; Sarkisyan and Hedlund 2009; Silvers et al. 2007; Wang et al. 2007; Williams et al. 2007).

However, in Aubele et al.'s research (2008), three behaviors were evaluated during the experiment. They were categorized as ambulating, rearing, or remaining stationary; times spent on these three activities were measured separately. Thus, they defined "Ambulation" as the crossing of at least 1 floor grid line within a 3-s period; "Stationary" was when the animal remained unmoving at least during 3 s; "Rearing" was defined as a lifting of the forelimbs and sitting back upon the haunches. The behaviors were quantified from digital recordings. In several studies, when animals showed lack of exploration activity, they were excluded from the experiment (Bilsland et al. 2008; Clarke et al. 2010; Ennaceur and Delacour 1988; Reger et al. 2009; Taglialatela et al. 2009).

Habituation, familiarization, and test delays

The NOR test consists of the habituation phase, the familiarization phase, and finally the test phase. The time that animal spent during each of these phases as well as the delay between them can differ from study to study (for details, see Table 2).

Starting from the first study that used the NOR test (Ennaceur and Delacour 1988), five experiments were developed. Animals were randomly allocated to four groups. In experiment 1, animal explored, during 2 min,

Table 2 Habituation, familiarization, and test phase in the NOR paradigm

Habituation phase	
1 day, with different duration and number of sessions	*One session*: 3 min (Aubele et al. 2008), 5 min (Goulart et al. 2010; Oliveira et al. 2010; Walf et al. 2009), 6 min (Silvers et al. 2007), 10 min (Bevins et al. 2002; Botton et al. 2010; Gaskin et al. 2010; Hale and Good 2005; Wang et al. 2009); *two sessions*: 10 min (Taglialatela et al. 2009), *four sessions*: ~20–30 min (Piterkin et al. 2008)
2–5 consecutive days with different duration	*2 days*: 5 min (Albasser et al. 2009; Hammond et al. 2004), 10 min (Bevins et al., Gaskin et al. 2010; Burke et al. 2010), *3 days*: 5, 10 or 30 min (Benice and Raber 2006; Herring et al. 2008; Reger et al. 2009; Sarkisyan and Hedlund 2009), *4 days*, 20 min (Clarke et al. 2010), 10 min (Williams et al. 2007), *5 days*, 5 min (Clark et al. 2000; Oliveira et al. 2010)
Familiarization phase	
1 day, with different duration and number of sessions	3 or 5 min (Ennaceur and Delacour 1988), 3 min (Aubele et al. 2008; Ennaceur and Delacour 1988; Reger et al. 2009; Nanfaro et al. 2010; Walf et al. 2009), 4 min (Burke et al. 2010), 5 min (Clarke et al. 2010; Ennaceur and Delacour 1988; Reger et al. 2009), 10 min (Botton et al. 2010; Frumberg et al. 2007; Hale and Good 2005; Taglialatela et al. 2009; Wang et al. 2007), 15 min (Nanfaro et al. 2010), three consecutive 10-min trials (Benice and Raber 2008)
2–5 consecutive days with different duration	2 days (Ennaceur 2010, Silvers et al. 2007), 3 days (Benice et al. 2006—5 min; Sarkisyan and Hedlund 2009—5 min; Schindler et al. 2010—6 min), 5 days (Weible et al. 2009—10 min)
Time of contact with an object	20 s (Ennaceur and Delacour 1988; Stemmelin et al. 2008), 30 s (Buckmaster et al. 2004; Clark et al. 2010, Herring et al. 2008; Goulart et al. 2010; Williams et al. 2007), 38 s (Hammond et al. 2004), 5 min (Stemmelin et al. 2008), 10 min (Hammond et al. 2004; Williams et al. 2007), 20 min (Goulart et al. 2010)
Delay between the familiarization and the test phase	10 s (Clark et al. 2000), 1 min (Ennaceur 2010; Ennaceur and Delacour 1988), 2 min (Hale and Good 2005; Taglialatela et al. 2009), 5 min (Hammond et al. 2004), 15 min (Gaskin et al. 2010; Reger et al. 2009; Piterkin et al. 2008), 10 min (Clark et al. 2000), 30 min (Hale and Good 2005), 1 h (Clark et al. 2000; Piterkin et al. 2008; Reger et al. 2009; Stemmelin et al. 2008; Williams et al. 2007), 3 h (Gaskin et al. 2010), 4 h (Aubele et al. 2008; Frumberg et al. 2007; Taglialatela et al. 2009; Walf et al. 2009), 24 h (Albasser et al. 2009; Bevins et al. 2002; Botton et al. 2010; Burke et al. 2010; Clark et al. 2000; Clarke et al. 2010; Ennaceur and Delacour 1988; Gaskin et al. 2010; Goulart et al. 2010; Hale and Good 2005; Herring et al. 2008; Nanfaro et al. 2010; Reger et al. 2009; Wang et al. 2007), 48 h (Ennaceur and Delacour 1988)
Test phase	
1 day, with different duration and number of sessions	3 min (Aubele et al. 2008; Clarke et al. 2010; Ennaceur 2010; Ennaceur and Delacour 1988; Reger et al. 2009; Nanfaro et al. 2010; Stemmelin et al. 2008), 4 min (Burke et al. 2010), 5 min (Clarke et al. 2010; Frumberg et al. 2007; Gaskin et al. 2010; Goulart et al. 2010; Mumby et al. 2002), 6 min (Silvers et al. 2007; Schindler et al. 2010), 10 min (Bevins et al. 2002; Hale and Good 2005; Taglialatela et al. 2009; Wang et al. 2007), 15 min (Oliveira et al. 2010), two consecutive 10-min trials (Benice and Raber 2008, Benice et al. 2006)
2–6 consecutive days with different duration	2 days: 3 or 5 min (Ennaceur and Delacour 1988; Sarkisyan and Hedlund 2009), 6 days: 5 min (Weible et al. 2009)

the empty open-field arena. Then, two testing sessions that comprised two trials were performed. In the first trial, i.e., a familiarization phase (T1), rats explored only one object during 5 min. After 1-min (group 1), 1-h (group 2), 4-h (group 3), and 24-h (group 4) delay, a test phase (T2) occured. Here, animals explored the familiar object and a novel one during 3 min. A repetition of this procedure was performed after 48-h delay. In experiment 2, authors fixed the time spent exploring object during T1, in order to make the test more sensitive to retention duration. Thus, animals remained in the apparatus until they explored the object during 20 s. In experiment 3, during T1, two identical objects were presented instead of one, in order to make T1 and T2 more comparable. Animals explored the empty open-field arena during 2 min. On the next day, rats had a familiarization phase (T1) when they explored two identical objects during 3 min. After 1-min (group 1) or 1-h (group 2) delay, during the test phase, they also explored two objects, the familiar and novel one. A repetition of this procedure was developed after 48-h delay. In experiment 4, experimental conditions and behavioral testing were similar to those described in experiment 3; however, all animals were submitted in a random sequence to 3 different intertrial delays (1 min, 1 h, and 24 h), one session per delay. The intersession interval was 48 h. This experiment had a purpose to understand the influence of the retention time. Lastly, in experiment 5, animals were exposed to experimental conditions for a 2-min session by day for 2 days. On the third day, only one session of the test began. Familiarization and test phase lasted 3 min, and the intertrial delay 1 min. There were three animal groups that were exposed to pair of objects identical to the sample ($A + A$), a pair of two identical new objects ($B + B$), or the sample and a new object ($A + B$) according to the groups. The aim of this experiment was to control the performance of rats by exposing them to a pair of familiar objects or a pair of new objects in T2 session.

We can note that both the habituation and the familiarization phases occurred during only 1 day with different duration and number of sessions or during 2–5 consecutive days from experiment to experiment. As in the study of Ennaceur and Delacour (1988), some researchers fixed the time that animals should contact with the object, in order to make the test more sensitive to retention duration. In the most studies, there was a delay between the familiarization and the test phase which varied from study to study and allowed checking the retention capacity of animals (Table 2). It is important to note that in some studies, researchers often used different retention intervals from assay to assay or from animal group to animal group, in the same work.

Concerning the test phase, we also observe different contact times. As for the familiarization phase, authors defined in different manner the time that animal should contact directly with the object.

However, there are studies that are very special and deserve a different approach. It is the case of the study of Piterkin et al. (2008), where they used a circular track apparatus divided into multiple compartments with the use of modular walls. During the first experiment, animals were habituated to the apparatus during four daily sessions of 20–30 min each. Here, the panel that separated the start and the end compartment was removed, and animal could explore the entire track freely. However, a door in each divider wall opened in only one direction; so after the rat left a compartment, it could not return. Traveling around the track in that direction only, the rat became familiar with a different pair of matching sample objects in different compartments. For the test, the two objects in each compartment were replaced by a novel object and a copy of the sample, and the rat once again traveled around the track. These experiments assessed the performance of rats with hippocampal lesions, when the learning and test contexts were the same or when the contexts were different. In the first experiment, the context shift involved conducting the test phase in a second circular track that was located in a different room. Thus, rats with lesions and control groups were allowed exploring sample objects in one context, and after a retention interval, they returned to either the same context or to a different, but familiar context, where they encountered sample objects paired with novel objects. In the second experiment, there was only one circular track and one room, and objects were removed from one compartment of the apparatus during the sample exposure phase, to a different compartment for the test phase. Moreover, only local features proximal to the object changed between sample exposure and test, while global features of the context did not.

In the study of Bevins et al. (2002), they associated the NOR test with place conditioning. They used an apparatus that had three compartments, one black, one white, and one, small, gray, each with different kind of flooring. In one of them, during the first 2 days, animals were placed in the center gray compartment for 10 min, and the non-preferred compartment, in which the least amount of time was spent, was defined. On the third day, 1 h before going to the apparatus, both Same and Novel groups contacted with an object in their home cage. Then, animals were placed in the non-preferred compartment, but while Novel group had accessed to a novel object, the Same group had accessed to a sample object, the same as it had in the home cage. Animals explored the place for 10 min. This procedure was repeated for 8 days. After 24-h delay, on day 11, each animal was placed in the center gray compartment

and allowed free access to both end compartments for 10 min without objects. Authors concluded that animals that contacted a novel object, repeatedly paired with an environment (Group Novel), displayed a significant increase in preference for that environment while this shift was not seen if the object was familiar (Group Same). This way, they concluded that object novelty is required condition of an increase in preference for the non-preferred compartment.

At each stage of the NOR test (habituation, familiarization, and test phase), there were studies with particular characteristics. For example, in the study of Frumberg et al. (2007), it is possible to observe a modification in the habituation phase. Here, animals were placed in the test room for at least 45 min to adjust to conditions. We can note that this phase was performed in relation to the room and not to the open-field arena.

In Broadbent et al.'s (2010) study, it is possible to note a modification not only in the habituation phase but also in the design of all study. During the familiarization phase, each rat was allowed to explore the objects for 5 min on 12 different sessions (three times each day for 4 days). The authors calculated the total amount of time each rat spent exploring the objects during these 5-min periods. As there was a huge reduction in object exploration across the days of familiarization, authors aimed to further explore the relationship between amount of exploration during the familiarization phase and subsequent object recognition memory. Interestingly, these findings indicated that the less time animals spent exploring the objects during the familiarization phase, the stronger was the novel object preference during the test phase. The implication of these data was that animals that learned about the familiar objects more effectively became less interested in the objects across the multiple familiarization episodes than animals that learned about the objects less efficiently.

In one experiment from the Gaskin et al.'s study (2010), animals were habituated to the open-field arena for 10 min, on 2 consecutive days. However, it was possible to observe a modification in familiarization and test phase, as they occurred in the form of intersession during 5 days. That is, on day 1, animals were familiarized with two identical copies of a sample object for 5 min in the open-field arena. Then, there were 2 h of retention interval. After that, animals were placed back in the open-field arena for 5 min, now with the third copy of the novel object. However, on day 2 to day 5, the same procedure was repeated, but the retention interval increased to 24 h.

This analysis allows us to understand that each study can differ in respect to time that each phase i.e., habituation, familiarization, and testing phase, takes and to the number of trials or duration of sessions.

Apparatus

It is important to understand what kind of apparatus is used in the NOR task, i.e., its size, shape, colors, materials, and how these parameters may differ.

Ennaceur and Delacour (1988) used an open box made of wood 65 × 45 × 65 cm. However, in the course of time, other materials have appeared, and their size or shape also varied from experiments to experiments (for details, see Table 3). Concerning their shapes, most of them had a rectangular or quadrangular form, with different dimensions. Less common were the circular arenas, as for example in Piterkin et al.'s study (2008) which is worth to be mentioned in details. These authors used a circular track divided into multiple compartments through the use of modular walls (more detailed procedure was already described in the previous paragraph). Concerning the size, this apparatus formed a circle with an extern diameter of 270 cm for one track, or 300 cm for the other, extended

Table 3 Apparatus used in the NOR paradigm

Material	Plywood (Hale and Good 2005; Goulart et al. 2010), acrylic (Botton et al. 2010), plastic (Aubele et al. 2008; Clarke et al. 2010; Hammond et al. 2004), plexiglas (Benice and Raber 2008; Clark et al. 2000; Reger et al. 2009; Sarkisyan and Hedlund 2009; Williams et al. 2007), acrylonitrile butadiene styrene (ABS) (Hammond et al. 2004), polyvinyl chloride plastic (PVC), (Clarke et al. 2010; Gaskin et al. 2010; Mumby et al. 2002), and wood (Albasser et al. 2009; Burke et al. 2010; Oliveira et al. 2010; Nanfaro et al. 2010)
Shape	Rectangular (Aubele et al. 2008; Benice and Raber 2008; Botton et al. 2010; Broadbent et al. 2010; Clarke et al. 2010; Gaskin et al. 2010; Goulart et al. 2010; Oliveira et al. 2010; Nanfaro et al. 2010; Stemmelin et al. 2008; Walf et al. 2009)
	Quadrangular (Albasser et al. 2009; Benice and Raber 2008; Burke et al. 2010; Clark et al. 2000; Hale and Good 2005; Schindler et al. 2010; Taglialatela et al. 2009)
	Circular (Weible et al. 2009 (60 cm in diameter and 45 cm height), Williams et al. 2007 (91 cm in diameter and 51 cm height), Piterkin et al. 2008)
Color	Black (Burke et al. 2010; Clark et al. 2000; Weible et al. 2009), opaque (Broadbent et al. 2010; Taglialatela et al. 2009), gray (Albasser et al. 2009; Bilsland et al. 2008; Gaskin et al. 2010; Mumby et al. 2002), white (Hale and Good 2005), and transparent (Benice and Raber 2008)

from the floor to a height of 40 cm. Both intern and extern walls had a slight concave curvature to give the animal inside the apparatus greater visual access to extra maze room cues. Divider walls separated the track into nine compartments, i.e., seven test compartments and a start and an end compartment. Each divider wall had a swinging door at the bottom center, 10 cm in diameter, that could be set to open in only one direction. Thus, when rat passed through it and into the adjacent compartment, it could not return to the previous one. These researchers used two apparatus with different visual, tactile, and olfactory properties of walls, where in one track animal explored in clockwise direction and in the other in counterclockwise direction.

However, an apparatus should take into account the objective of work and be adapted to the features of animals. It the study of Reger et al. (2009), they had three sizes of arenas according to animals' ages. The weanling arena that measured $32 \times 52 \times 30$ cm^3 accommodated for animals that weighed at least 50 g, the juvenile arena that measured $52 \times 52 \times 30$ cm^3 for animals that weighed at least 100 g, and adult arena that measured $70 \times 70 \times 30$ cm^3 for animals of 200 g and more.

As it can be seen from Table 3, the color of apparatus was also a particularity of each study. The floor of apparatus can be covered with sawdust (Albasser et al. 2009; Gaskin et al. 2010; Goulart et al. 2010) or paper beddings (Wang et al. 2007). This cover could be agitated between trials or regularly replaced. In Gaskin et al. (2010), a stainless steel tray served as floor and was covered with sawdust; however, the floor was removed through a slot at the bottom of one wall to facilitate changing the sawdust between each trial.

An adaptation in the apparatus was made by Bevins et al. (2002), as they used two place condition chambers to evaluate the NOR. Here, each chamber had rectangular dimensions of $31 \times 24 \times 45.5$ cm; one of them had walls painted flat black, flooring made of 13 metal rods, and newspaper lining the litter tray, while the other had walls painted flat white, flooring made of hardware cloth, and pine wood chips lining the litter tray. Between these two chambers, there was a small chamber with inside dimensions of $15 \times 24 \times 45.5$ cm, gray walls, and an aluminum floor. The walls of this compartment were raised 11 cm during preference test to allow an animal to move freely between compartments.

Yet, Aggleton et al. (2010) used a bow tie–shaped maze to develop the NOR task. This maze was made of opaque Perspex, it was 120 cm long, 50 cm wide and 50 cm high, and both ends were triangular. There was an opaque door in the center of the corridor that could be raised by the researcher. The far wall of each triangle contained two recessed food wells, 3.5 cm in diameter and 2 cm depth. The food wells were divided by a short, opaque dividing wall that protruded 15 cm from the center of the end wall. These food wells were covered by objects in the experiment proper.

Kind of objects

Objects that have been used in the NOR test vary widely in shapes, sizes, textures, materials, colors, and appearance. From the familiarization to the test phase, object features change when a novel object that is somehow different from the familiar one is presented. For instance, it can be observed in Nanfaro et al.'s study (2010), where during the familiarization phase, animal contacted with two pink truncated pyramids (familiar object) while in the test phase with a gray opaque candlestick (novel, unfamiliar object) and a pink truncated pyramid. Thus, novel and familiar objects had different colors, shape, and size which allowed recognizing them as novelty. It is also important to know whether object eliciting abnormally high levels of spontaneous investigation does not influence the outcome of experiments. Thus, Gaskin et al. (2010) preselected novel/sample object pairs on the basis that each object in the pairs elicited the same amount of spontaneous investigation.

Many objects have been used in this test. For instance, cans, bottles, tins, glasses, pots, pyramids, candlestick, tower, cylinder, box, Playmobil toys (man, woman, monkey, horse, and cow), Lego toys, coffee mugs, teacups, socks, PVC pipe, a sheet of newspaper wadded into a ball, Styrofoam dome, tennis ball, bath loofah, shuttlecock, pet toys, and glass vase have been used (Albasser et al. 2009; Benice and Raber 2008; Bevins et al. 2002; Botton et al. 2010; Nanfaro et al. 2010; Sarkisyan and Hedlund 2009). The objects can be made of metal, glass, porcelain, glazed ceramic, rubber, durable nontoxic plastic, aluminum, or wood (Benice and Raber 2008; Broadbent et al. 2010; Burke et al. 2010; Clark et al. 2000; Ennaceur and Delacour 1988; Goulart et al. 2010; Hale and Good 2005; Oliveira et al. 2010; Piterkin et al. 2008; Reger et al. 2009; Mumby et al. 2002; Sarkisyan and Hedlund 2009; Schindler et al. 2010; Walf et al. 2009), i.e., materials that cannot be easily gnawed by animals and that can be easily cleaned. Concerning the object height, this was influenced by kind of object and varied between 4.5 and 24 cm (Aubele et al. 2008; Gaskin et al. 2010; Goulart et al. 2010; Mumby et al. 2002; Piterkin et al. 2008; Sarkisyan and Hedlund 2009; Silvers et al. 2007). However, concerning the weight, the object should be heavy enough that animals cannot move it, as well as height enough to unable animals climbing or resting on it during trials (Clark et al. 2000; Ennaceur and Delacour 1988; Hale and Good 2005; Gaskin et al. 2010).

However, as safeguard in some studies, Velcro (Broadbent et al. 2010; Clark et al. 2000; Reger et al. 2009) or glue (Clarke et al. 2010) to fix object to the arena floor was used. The object copy number differed from work to work. While there were researchers who used three identical copies (Burke et al. 2010; Gaskin et al. 2010; Nanfaro et al. 2010; Piterkin et al. 2008), others used four copies of each object (Mumby et al. 2002). These copies were used interchangeably.

The aim of each research influenced the object choice. It can be observed in Reger et al.'s work (2009), where they studied the developmental aspects of memory in weanling, juvenile, and adult rats. Here, object size needed to be age-appropriate, objects were no taller than twice the size of an animal, and they did not resemble living stimuli. Sometimes objects were carefully selected. Aubele et al. (2008) placed an animal in the open-field arena with four objects belonging to four categories that were differentiated by size and shape. Thus, they defined criteria for different categories such as large (>18 cm tall), small (<12 cm tall), smooth (having a regular, cylindrical shape), and complex (having sharp angles, curves, or extending features). Then, the object categories were as follows: small/smooth objects (e.g., small bowl); large/smooth objects (e.g., soda can); small/complex objects (e.g., teacup); and large/complex objects (e.g., coffee mug).

The kind of familiar or novel object as well as the relative position should be counterbalanced and randomly permuted for each experimental animal.

Object position

Objects are usually placed in the extreme of the experimental apparatus. However, distance between objects or objects and apparatus corner is different depending on experimental work conditions. It is important to exchange the position of the objects (familiar and novel) for each experimental animal to avoid the use of potential confounding spatial clues (Nanfaro et al. 2010). In the test phase, the novel object should be placed in 50% trials in the right side and 50% in the left side of the open-field arena (Goulart et al. 2010).

Many differences were observed in the object position. It is possible to find studies where the objects were placed equidistantly from each other and from arena corners (Sarkisyan and Hedlund 2009); where the objects were positioned in two adjacent corners 9 cm from walls (Botton et al. 2010); and where the objects were in opposite corners approximately 2 cm (Hammond et al. 2004), 10 cm (Aubele et al. 2008), 23 cm (Albasser et al. 2009), or 27 cm (Gaskin et al. 2010) from the wall. Animals can be placed at the center or at the opposite end of the open-field arena to start the experiment.

Cleaning

It is important to note that after each session of the NOR, the arena and objects have to be cleaned to ensure that behavior of animals was not guided by odor cues. However, the cleaning solution varied from study to study. While some researchers used 10% ethanol solution (Botton et al. 2010; Broadbent et al. 2010; Hammond et al. 2004; Goulart et al. 2010; Reger et al. 2009), others used 70–75% (Aubele et al. 2008; Burke et al. 2010; Clarke et al. 2010; Dere et al. 2005; Hale and Good 2005; Gaskin et al. 2010; Sarkisyan and Hedlund 2009) or 95% ethanol solution (Clark et al. 2000). In other studies, 5% acetic acid (Benice and Raber 2008; Benice et al. 2006), 70% isopropanol (Taglialatela et al. 2009), or solution of diluted chlorine bleach (Mumby et al. 2002) has been also used.

However, it should be noted that in the study of Ennaceur and Delacour (1988), throughout the experiment no cleaning of the arena was done, in order to saturate it with olfactory stimuli.

Light and sound conditions

Most of the NOR test occurred in sound-isolated room and under certain light condition. The sound insulation was specified in Aubele et al.'s paper (2008), where the test was conducted in a low level of background white noise, almost 50 dB. Ennaceur and Delacour (1988) used a room with a masking white noise of 70 dB above the human threshold. In the upper part of the room, a light bulb was fastened which provided a constant illumination of about 40 lux at the level of the test apparatus. It is important to note that from study to study and from laboratory to laboratory, working conditions varied widely. Some differences were observed in light condition; although tests were made with constant illumination, its intensity varied, and it can range from <10 lux (Silvers et al. 2007) to 30–40 lux (Clarke et al. 2010; Ennaceur and Delacour 1988; Weible et al. 2009). Generally, the light bulb was suspended over the box (Clark et al. 2000; Ennaceur and Delacour 1988; Nanfaro et al. 2010; Weible et al. 2009). Thus, both Nanfaro et al. (2010) and Clark et al. (2000) used 25- and 60-W light bulbs, respectively. Fluorescent lights have also been used (Bevins et al. 2002; Broadbent et al. 2010).

Result analyses and indexes

The relationship between amount of exploration during the familiarization phase and subsequent object recognition memory can be evaluated with the NOR test. Time spent by the animal in exploring individual objects during familiarization phase; total time spent by the animal in

exploring both objects during the test and training phase; and discrimination index, i.e., the difference between time spent exploring novel and familiar objects, during test phase can be considered.

There are two measures of discrimination behavior according to Ennaceur and Delacour (1988). The first measure (D_1) for the habituation phase is the difference in exploration time for novel versus familiar objects, i.e., the exploration time devoted to the novel object (T_N) minus the time devoted to the familiar object (T_F), [$D_1 = (T_N - T_F)$]. The second measure, *Discrimination Index* (DI), allows discrimination between the novel and familiar objects, i.e., it uses the difference in exploration time for familiar object, but then dividing this value by the total amount of exploration of the novel and familiar objects [$DI = (T_N - T_F)/(T_N + T_F)$]. This result can vary between +1 and −1, where a positive score indicates more time spent with the novel object, a negative score indicates more time spent with the familiar object, and a zero score indicates a null preference (Aggleton et al. 2010; Aubele et al. 2008; Burke et al. 2010; Oliveira et al. 2010; Silvers et al. 2007). The DI is also expressed as the ratio of the total time spent exploring both objects, making it possible to adjust for any difference in total exploration time (Broadbent et al. 2010). Similarly, this measure can be applied when both objects are identical, in the familiarization phase, but here the mathematic formula will be $DI = (T_R - T_L)/(T_R + T_L)$, where T_R represents the exploration time devoted to the right sample and T_L represents the exploration time devoted to the left sample (Aubele et al. 2008). These two measures (D_1 and DI) are not independent; however, they cannot be considered as equivalent and they are combined as two estimations of recognition process.

According to Ennaceur and Delacour (1988), an *Index of global habituation* can be also determined, by comparing the total time spent in exploring the two objects during the familiarization phase to that spent in the test phase. As control measures, it is possible to determine the overall level of exploration as well as the side and object preferences. These authors also showed that there were no significant differences in the global index of habituation according to the delay, and that the index of discrimination is not affected by length of the intertrial delay.

Over the time, other measures of the NOR were developed. A percent of time spent exploring the novel object relative to the total time spent exploring both objects can be a measure of novel object recognition (Benice et al. 2006; Broadbent et al. 2010; Oliveira et al. 2010; Sarkisyan and Hedlund 2009). This concept can be represented by *Recognition Index* (RI), i.e., the time spent investigating the novel object relative to the total object investigation [$RI = T_N/(T_N + T_F)$], and it is the main index of retention (Botton et al. 2010; Gaskin et al. 2010; Mumby et al. 2002; Piterkin et al. 2008; Schindler et al. 2010).

In Wang et al.'s research (2007), they applied a measure of cognitive function through the *Preference Index*. This is a ratio of the amount of time spent exploring any one of the two objects in training phase (*A* or *B*) or the novel one in test phase (*C*) over the total time spent exploring both objects, i.e., *A* or $B/(B + A) \times 100$ (%) in the training session and *B* or $C/(B + C) \times 100$ (%) in the test phase. Therefore, a preference index above 50% indicates novel object preference, below 50% familiar object preference, and 50% no preference (Hammond et al. 2004). A distance traveled can be also a measure of exploration. During the habituation phase, animals gradually decreased the total distance traveled across days of exposure to the experimental environment, which means an increased familiarization (Oliveira et al. 2010).

The total amount of familiar object exposure as well as the amount of time that animals spent exploring this object would be related to the magnitude of IRs, as already mentioned. However, in Gaskin et al.'s study (2010), they observed that no significant correlation between the amount of time animals spent exploring the familiar object during the familiarization phase and the magnitude of IRs obtained during the test phase. This way, in the NOR test, a correlation between familiar object exploration time during the familiarization phase and the amount of novel object preference in the test phase is not a necessary condition for novelty preference.

Measuring devices

Nowadays, several devices are used to automatically record the data and there are many experiments that are made under video recording, in off-line or in online mode. In any experiment that studies learning and memory, particularly those related to locomotor and exploratory activity of animals, it is important to exclude the potential effect of confounding variables. This way, it is increasingly important to use automatic equipments (Hale and Good 2005). With this kind of technology, the behavior of animals is scored in real time and data can be analyzed when needed. One of them is the EthoVision tracking software that was used to manually score exploratory behavior. Each object is assigned a zone and a keyboard button to be identified. The researcher only needs to press the key at the beginning or the end of experiment (Albasser et al. 2009; Hale and Good 2005; Hammond et al. 2004; Schindler et al. 2010).

However, more important than to determine how long each animal interacts with novel or sample object is to allow quantifying various locomotor parameters, like total distance traveled, time spent moving, or number of rears

(Taglialatela et al. 2009). Opto-Varimex and TopScan have been used in some experiments which can measure the features described above (Broadbent et al. 2010; Taglialatela et al. 2009; Oliveira et al. 2010; Nanfaro et al. 2010).

Another automatic video recording was used in Reger et al. study (2009). Here, a black permanent marker was used to shade from the tip of the rat's nose to between the ears, which allowed the tracking system to measure the time spent interacting with the objects. Around each object, a circular zone was created digitally, so that movement $\leq$5 cm from the objects' center could be detected as object interaction by the system. The observer carefully watched the test or if warrant reviewed the NOR run via backup video footage for such tracking errors, which were subtracted from the interaction time.

A modification in the computer-assisted scoring was made in Clark et al.'s (2000) experiments. To collect and analyze the data during both familiarization and test phase, a specially designed software and button press device were used. There were two buttons, one for each object. They were depressed when rat explored each object and released when rat stopped exploring the object. When 30 s of object exploration was accumulated, the computer automatically beeped and terminated that phase of the trial. This way, it was possible to ensure whether animals had the same amount of contact time with objects. Thus, it allowed evaluating preference for the novel object at all points during the test phase and more detailed behavior analysis than could have been obtained by manual stopwatches.

In spite of all new technologies, many researchers still observe the animals directly. For this, they use manual stopwatches to record the time that an animal spends around the novel and familiar objects, and trials are recorded by a trained observer (Benice et al. 2006; Burke et al. 2010; Clarke et al. 2010; Sarkisyan and Hedlund 2009; Schindler et al. 2010; Williams et al. 2007).

Neuronal processes and brain structures involved in the NOR test

Memory consolidation but not persistence seems to be hippocampus-dependent. During the NOR task, memory is consolidated and spatial or contextual characteristics of objects could be relocated in different parts of the brain (Oliveira et al. 2010). However, when a given memory is recovered in the presence of novelty, it is set into a labile phase and requires stabilization to persist. This processing memory is called reconsolidation, and it is involved in reorganization of the already formed memories, allowing incorporation of new information (Clarke et al. 2010). It is known that rate of neurogenesis in the hippocampus is linked with spatial memory consolidation (Sarkisyan and Hedlund 2009).

In the medial temporal lobe, there are a set of structures, particularly the hippocampus and adjacent cortical areas including entorhinal, perirhinal, and parahippocampal cortex that are involved in normal memory function (Baxter 2010). These structures are highly integrated, but while the perirhinal cortex is involved in object recognition after short retention intervals, the hippocampus is responsible for long-term object recognition (Reger et al. 2009). The hippocampus receives inputs from the perirhinal cortex, which is itself the site of several information entrances as visual, olfactory, and somatosensory stimulus, all of them involved in object recognition (Clarke et al. 2010). In the NOR memory formation, dorsal hippocampus plays an important role, especially when spatial or contextual information is a relevant factor (Goulart et al. 2010). When rats are placed into a particular environment, the hippocampus-based system rapidly gets contextual information. However, extrahippocampal systems obtain contextual information more slowly, which leads a longer duration of exposure or multiple exposure in an environment (Piterkin et al. 2008).

The hippocampus is important for object recognition memory, and if there are lesions on this structure, moderate and reliable anterograde memory impairment will occur, but the task could sometimes be acquired using alternative strategies that involve other brain regions (Broadbent et al. 2010). In Oliveira et al.'s study (2010), if animals with hippocampal inactivation were exposed to shorter periods of habituation in an experimental environment, long-term NOR memory was enhanced. In a different way, after longer periods of contextual habituation, long-term NOR memory was unchanged by hippocampal inactivation. Thus, when familiarization takes place in a stage in which the contextual environment is relatively novel, the hippocampus plays an inhibitory role in the consolidation of object recognition memory. This way, object recognition memory is unaltered by hippocampal inactivation when initial exploration of the objects occurred in a familiar environment. Therefore, this theory was not confirmed by de Lima et al. (2006) as this study, by using reversible hippocampal inactivation technique, reported that the dorsal hippocampus is essential for early and delayed consolidation of the NOR memory, up to 3 h after training. Although the hippocampus could not play a direct role in discriminating the different features of each object, it is fundamental as a novelty detector because of its role in comparing previously stored information with new incoming aspects of one particular situation (Clarke et al. 2010). Hippocampal system has a pivotal role in memory formation, but if it is inactive, it does not induce a generalized amnesia, but rather it would cause impairment in specific types of memory.

This conclusion is possible due to demonstrations of dissociations following inactivation of distinct brain regions, giving strength to the multiple memory systems hypothesis (Oliveira et al. 2010).

Hippocampal formation also plays an important role in memory for contextual information. This way, Piterkin et al. (2008) showed that hippocampus is not critical for encoding or retrieving a representation of the sample exposure context, as the performance of animals with its lesions was sensitive to the context change. Animals with pretraining lesions showed a normal novel object preference when the sample exposure context matched the test context. When animal was reexposed to a familiar context on the test phase, a configural representation of the context that was encoded during the familiarization phase was reactivated and allowed the discrimination between the novel and familiar objects.

The functions of structures of the medial temporal lobe are closely related, especially the hippocampus that extends and combines functions performed by the adjacent cortex (Clark et al. 2000). The perirhinal cortex plays an important role in perceptual processing and, as noted, is involved in object recognition memory over short retention intervals once it is sufficient to support short-term object recognition memory (Baxter 2010; Hammond et al. 2004). When lesions in this brain region exist, the impairment in object recognition memory could be observed (Aggleton et al. 2010). It is possible to suggest that lesions in perirhinal cortex could contribute to some aspects of retrograde amnesia following large temporal lobe lesions (Mumby et al. 2002).

The perirhinal cortex plays a noncritical role in encoding information that underlies accurate object discrimination performance, but when there are lesions in this region, its role could be critical. In the study of Albasser et al. (2009), the authors showed that damage in this brain region was significantly correlated with object recognition, as greater damage was associated with poorer recognition. The time of contact with the objects influenced the performance in the NOR test, and animals with perirhinal lesions had an ability of discriminating novel objects after short intervals. An increase in familiarization phase led to an almost doubling of close-proximity exploration of the familiar object. However, after 24 h, it did not contribute to discrimination between novel and familiar objects in the test phase in animals with perirhinal cortex lesions.

In summary, the hippocampus and the perirhinal cortex play different roles in object recognition memory. While the perirhinal cortex is involved in object recognition once it is necessary to representing basic information about familiarity or novelty of an object, the hippocampus is involved in object memorization by encoding information about the experience of object. The perirhinal cortex codes object recognition decays fast and is not sufficient for maintaining information about object during longer retention intervals, while the hippocampus, by coding object memory, maintains strong novel object preference after long but not short delays (Hammond et al. 2004).

The NOR test applications

As already stated, the hippocampus plays a role in memory processing, recognition, acquisition, and storage of the contextual details and temporal order of previous experiences. Additionally, to the data cited above, some more detailed information has been provided. Since hippocampal serotonin (5-HT) neurotransmission contributed to memory processing, Sarkisyan and Hedlund (2009) studied the possible involvement of 5-HT_7 receptors in hippocampal function using NOR models to assess hippocampus-dependent learning and memory. In Rampon et al.'s study (2000), authors created CA1-specific NMDA receptor 1 subunit–knockout mice to determine the influence of this kind of receptor in nonspatial memory formation as well as in experience-induced synaptic plasticity in the CA1 region of the hippocampus. These data revealed that CA1-KO mice exhibited impairment in object recognition, but this deficit could be abolished by enriching experience which, in turn, increased the synapse density in the CA1 region.

Additionally, the NOR test is also used to test the influence of animals' age in the object recognition, as well as its dependence in relation to changes in hippocampal functions, or even to understand the developmental aspects of cerebral maturation in memory system using immature animals (Baxter 2010; Burke et al. 2010; Reger et al. 2009). Within the Sprague–Dawley strain, comparisons among males have shown adolescents to be more reactive to novelty than male adults. Specifically, adolescents displayed higher activity levels in a novel environment, more rapidly approached a novel object in a familiar environment, and spent more time with a novel object relative to adults (Silvers et al. 2007). Object recognition deficits but not spatial learning in the water maze in aged rats were also described by Baxter (2010). In this study, aged rats behaved as if novel objects were familiar, rather than familiar objects being treated as novel. Interestingly, a similar pattern of behavior has been observed in young rats with perirhinal cortex lesions.

It is commonly accepted that AD is characterized by a progressive decline in several forms of declarative memory including contextual fear conditioning and novel object recognition. This way, studies have used transgenic animals in the NOR test to evaluate whether object recognition memory processing causes changes in hippocampal synaptic efficacy (Clarke et al. 2010; Taglialatela

et al. 2009). There are also studies where a variety of trials were made to understand the generality of the novel object place conditioning effect or whether novelty of objects was important to produce a conditioned increase in environmental preference (Bevins et al. 2002).

We can note that the NOR task has a large field of application not only to test the pharmacological effects of a drug, but also to characterize which brain regions are involved in memory and learning, as well as to understand the profile of diseases like AD with the aim of developing a targeted therapeutic.

Drugs evaluated with the NOR test

The NOR test is sensitive enough to detect alteration in animal behavior. This test has been used to evaluate the influence of several drugs in animals' memory and recognition.

In this variety of drugs, it is possible to mention the effects of psychostimulants. Animals that were exposed to cocaine in prenatal period displayed a preference for the novel object when tested after 20 min, but no preference for the novel object after either 1 or 24 h indicating a deficit in short-term memory in the task (Schindler et al. 2010). Herring et al. (2008) described the role of methamphetamine which decreased the novelty index significantly but not dramatically in adult rats. Authors revealed that methamphetamine treatment induced an effect on path integration learning while dosing regimen had no differential effects on behavior or neurotoxicity. Another finding that further indicated that the NOR task can constitute an important tool to assess long-lasting memory impairments in animals exposed to psychostimulant drugs was provided by Schröder et al. (2003). These data revealed that administration of methamphetamine restricted to a single day can produce a profound, persistent, and selective deficit in a nonspatial hippocampus-dependent memory by impairing both short- and long-term retention in the NOR task, but not acquisition or retention of spatial memory in the Morris water maze in rats.

An association between the administration of a drug and the hippocampus function was possible in the study of Hammond et al. (2004). Here, an acute lidocaine administration was used to temporarily inactivate the hippocampus before training in the NOR test. No effect of intrahippocampal lidocaine on the time needed for animal to accumulate sample object exploration was observed, and this lack of effect suggested that hippocampus inactivation did not affect the familiarization phase activity or the motivation to explore objects. However, after 24 h, animals exhibited impaired object recognition memory which supported a delay-dependent role of the hippocampus in object recognition memory.

The difference between two chemical presentations of the same drug was compared by Nanfaro et al. (2010), specifically the pharmacological effects of Pregnolone (Preg) and Pregnolone sulfate (Preg-S), when injected intracerebroventricularly (icv) into the lateral septum (LS). Preg-S is considered an excitatory neuroactive steroid which can influence cognitive functions, particularly memory processes. Authors showed that Preg-S injected into the LS prior to training session caused an impaired effect, but when it was administered shortly after the training or before the test phase did not. It is important to note that Preg alone impaired neither learning nor memory, which can explain a possible dual role for this neuroactive steroid depending on its chemical presentation.

Botton et al. (2010) used the NOR test to evaluate learning and memory after caffeine administration. Caffeine has a positive effect on cognition in which cholinergic system seems to be involved. Thus, the way how dose and schedule of its administration can influence the memory recognition was studied by these authors. They concluded that pretreatment with caffeine prevented the disruption of both short-term and long-term memory caused by scopolamine. As such, the acute treatment with caffeine followed by its withdrawal may be effective against cholinergic-induced disruption of memory and could prevent cognitive decline associated with AD, since degeneration of the cholinergic neurons of Nucleus basalis of Meynert (NBM) was associated with declined functions observed in this disease.

Goulart et al. (2010), in turn, used the NOR test to evaluate the effects of ketamine on consolidation phase of memory, when it was administrated systemically and acutely. They showed that after training, the impaired effect of this drug on long-term retention of memory in animals was dose-dependent. As NOR learning induced a production of hippocampal brain-derived neurotrophic factor (BDNF), the authors showed that ketamine prevented its increase. The consolidation phase of long-term recognition memory was impaired by ketamine, probably by preventing learning-induced increase in BDNF levels in the hippocampus.

Effects of hormones can also be evaluated in the NOR test and can be observed in Walf et al.'s (2009) research. Here, they showed that 17β-estradiol (E2) influenced cognitive and/or affective behavior mainly by contact with β-isoform of the estrogen receptor (ERβ). Animals with higher E2 levels showed better cognitive performance in object recognition and object placement. These authors concluded that endogenous variations of steroids may alter performance in object recognition tasks of young female mice.

The NOR test was also used by Aubele et al. (2008) in gonadectomized and hormone-replaced adult male

rats. The gonadectomized rats that received testosterone exhibited an increase in exploration of novel objects, but gonadectomized and gonadectomized estradiol-supplemented groups explored the novel and familiar objects equally. The results showed that gonadal hormones influenced performance on certain working memory and mnemonic functions related not only to medial and orbital, but also to the perirhinal division of the prefrontal cortex.

It was possible to get an idea about which drugs have been evaluated through this test. It should be noted that each investigation has its own objectives and conclusions in different therapeutic fields which make the NOR test a capital gain because of a broad scope.

Summary

Object recognition memory

The one-trial object recognition task has raised major interest on memory study. With this test, it is possible to analyze cognitive and neuropsychological issues in rodents. Animals are capable of differentiating between objects and of recognizing a previously viewed object from a novel one. However, little is known about animals' perceptual capabilities and how this discrimination and memory performance is obtained upon identification of familiarity and novelty. During the test phase, a novel object needs to be detected and encoded while a familiar object needs to be updated and reconsolidated after long delays. The delay-dependent decrease in memory recognition results from a decay in memory of the familiar object (Ennaceur 2010). It is worth mentioning that in the study of Dere et al. (2005) already cited, the object location memory can be also investigated in the NOR task, as a simultaneous assessment of spatial object memory in addition to temporal order memory during the test trial was achieved. Authors further proved that the mice spent significantly more time exploring the spatially displaced "old familiar" object relative to the stationary "old familiar" object, whereas the two "recent familiar" objects should be explored to similar extents. Such a response pattern would reflect spatial object memory or memory for "what," "when," and "where," i.e., the pivotal components of human episodic memory.

According to Ennaceur and Delacour (1988), when the global amnesic syndromes are analyzed, at least two types of memory could be distinguished. Memory type I or spared is the semantic, the reference or the procedural memory; Memory type II or disturbed is the episodic, the working or the declarative memory. However, there is no general consensus about this type of classification. Working memory is the process that maintains a representation of information for a short period of time, and it is available for posterior use. It describes complex cognitive processes involving rapid processing of ongoing events and is mostly related to spatial tasks (Albasser et al. 2009; Ennaceur 2010). The formation of working memory depends on a system of anatomically related structures in the medial temporal lobe, particularly the hippocampal region (the CA fields, dentate gyrus, and subicular complex) and the adjacent entorhinal, perirhinal, and parahippocampal cortices, as already described. Yet, the semantic memory is defined as a record of facts and concepts, and it is independent of the temporal context in which it was acquired. It is a part of long-term memory and it depends on the values (emotional or motivational) that an individual attributes to an event (Ennaceur 2010). It is important to note that the one-trial object recognition task is sometimes not appropriate to evaluate the novelty, as a lack of discrimination between novel and familiar objects can be interpreted in two opposite ways, i.e., animals spent equal amount of time on both objects because they are both recognized as familiar or because they are both explored as novel (Barker et al. 2007).

In summary, this task is limited to memory of an object, its localization, and its context. It cannot provide measure of memory of when such encounter with an object, a place, and/or a context took place. Thus, the difficulty in detecting the strength of memory of an event remains inaccessible to the experimenter.

Influence of novelty and familiarity on memory

In novelty, detection, attention, and motivation processes are involved. When something new is present, animals stay alerting and need to examine it closely or distally, depending on the risk. On the other hand, if something familiar is present, it will require attention and reevaluation. However, when novel and familiar stimuli are present together, the novel stimulus will be more explored until loses its novelty. This decrease in novelty means that the object becomes familiar which is directly related to the delay. At longer delays, the memory of the familiar objects becomes weaker, while at short delays, it becomes almost intact. When there are contacts with a novel object, the intensity of its exploration depends on the amount of residual memory of the familiar stimulus, at a particular delay interval, which needs updating and reconsolidation (Ennaceur 2010).

Furthermore, subject will explore the familiar object because of remains of some residues of past experience. Yet, novelty preference is only observed when memory is highly accessible, which is called the recent memory phase. If delay between the familiarization phase and the test phase is increased, a familiar preference will occur.

This is called remote memory phase. Intermediate phase is between these two phases when equal attention is given to the novel and familiar stimuli. Moreover, a null preference can occur, which is not a result of forgetting but a shifting preference, when memory is of intermediate accessibility (Bahrick et al. 1997, 2002; Ennaceur 2010).

Conclusion

Despite an ample variety of methods for assessing the ability of recognizing objects, the NOR test has been used quite consistently in different experimental works. Its application is not limited to a field of research and enables that various issues can be studied, such as the memory and learning, the preference for novelty, the influence of different brain regions in the process of recognition, and even the study of different drugs and their effects.

It is consistent through all works that each research team adapts the NOR test taking into account their aims, which means that there is a wide variation in patterns of work as well as in the apparatus adopted. Despite all modifications, every experiment is constituted by three phases: habituation, familiarization, and test phase. Each phase has its own duration and number of trials which are also characteristics of each research. Basically, animals spent more time exploring the novel object than the familiar one. This preferential exploration of novelty has been used to test the effect of several changes in object recognition memory in rodents, not only the novelty object but also the place that influences the animal recognition process. Despite animals have a greater propensity for novelty, it is important to consider the amount of time allocated to each phase of a trial as well as the number of trials and the length of delays between trials. After prolonged exposure, a reduced preference for the novelty takes place, and it means that objects became familiar.

Several animal strains have been used in the NOR test, and a large difference can be observed not only in conditions in which animals are kept, but also how they are used throughout the experience, particularly regarding the feeding, temperature, cleaning, cycles of light/dark, and sound conditions. Another large difference observed in all analyzed studies concerns the apparatus. That parameter varies not only in terms of size and shape, but also in manufactured materials. The same can be observed in the objects used, where their size, shape, material, and location within the apparatus significantly varied.

The object recognition in rodents can be evaluated by the difference in the exploration time of novel and familiar objects; however, the indexes used to obtain the results of an experiment can differ. Over time, the automatic recording devices are being increasingly used, not only because of the easy registration, but also because it permits later viewing of the animal's behavior in a given session. However, some investigators still use stopwatches as a way to collect data.

Different types of memory can be measured with the NOR test. Moreover, different brain structures can be involved in the process of recognition and memorization. Among them, it is important to emphasize the hippocampus and the perirhinal cortex that have distinct roles, though interrelated.

The NOR test is a simple method that does not need external motivation reward or punishment, but a little training or habituation is required, it can be completed in short time so animals do not feel stressed, and it can assess the recognition memory after only one trial, which gives it an advantage over other methods. Despite the NOR test has a wide range of manipulations, it also has its limitations. The level of exploration sometimes can be low or inconsistent. The exploratory activity can increase through the use of large open-field arenas, elevated open-space platform, or mild food deprivation. In some modifications, differences between objects become irrelevant, and it would be difficult to observe a specific effect of an experimental manipulation. The latter is also necessary to be taken into account carefully during the analysis and interpretation of results from the NOR test or the potential of automated protocols.

To summing up, we can note that the NOR test has a large field of application where each research has its own modifications, which makes each experiment unique.

Acknowledgments This work was supported by the Statutory Funds of the Medical University of Lublin (DS 23/11) and received no special grant from any funding agency in the public, commercial, or not-for-profit sectors. M.A. was supported by a fellowship from Socrates/Erasmus student program of the Faculty of Pharmacy, University of Lisbon. Both authors declare that they have no conflicts of interest to disclose.

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