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The Object Recognition Task: A New Proposal for the Memory Performance Study

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1. Introduction

In the last few decades, there has been extensive research in the cognitive neurophysiology of learning and memory. Most relevant experimental studies were focused on the possible role of neuropeptides on memory performance and the neurobiological bases of their actions. In general, scientists believe that the answers to those questions relies in understanding how the information about new events is acquired and coded by neurons, how this information is modulated and if it is possible to revert age-related or diseases associated cognitive to failures.

Memory is broadly divided into declarative and nondeclarative forms. The formation of declarative memory depends on a neural system anatomically connected in the medial temporal lobe that recruits hippocampus, dentate gyrus, the subicular complex, and the adjacent perirhinal, entorhinal, and parahippocampal cortices (Squire & Zola-Morgan, 1991; Eichenbaum & Cohen, 2001). In both, animals and humans, declarative memory supports the capacity to recollect facts and events and can be contrasted with a collection of nondeclarative memory abilities: habits and skills, simple forms of conditioning, and other ways that the effects of experience can be expressed through performance rather than recollection (Squire, 1992; Schacter & Tulving, 1994).

Numerous tests have been used for studying memory; they differ in several ways other than just the type of information that must be remembered. Other differences include the nature of the motivation or reward, the reinforcement contingencies, and the amount of training required. The behaviors that are measured to assess memory also vary considerably and include conditioned reflexes (e.g., Pavlovian fear conditioning), speed or accuracy of spatial navigation (which can involve either swimming -water maze- or running -radial maze-). The object recognition test (e.g., novel object recognition -NOR- or novel object preference -NOP-), also known as the visual paired comparison task in studies with humans and monkeys, is a non-spatial and non-aversive procedure extensively applied to study neuronanatomical and molecular mechanism involves in recognition memory process, a form of declarative memory (Ennaceur & Delacour, 1988; Puma et al., 1999; Bizot et al., 2005).

Recognition memory is a fundamental facet of our ability to remember. It requires a capacity for both identification and judgment of the prior occurrence of what has been identified (Mandler, 1980). This memory includes two components, a recollective (episodic) component that supports the ability to remember the episode in which an item was encountered, and a familiarity component that supports the ability to know that an item was presented (Mandler,

1980; Tulving, 1985; Quamme et al., 2002; Yonelinas, 2002). An important question concerns whether the brain structures that comprise the medial temporal lobe memory system differ in their contributions to recognition memory, or if they differ in how they support its recollective and familiar components. The first possible interpretation was that recognition memory is supported by the cortical areas along the parahippocampal gyrus (for example, the perirhinal cortex) and that the hippocampus itself is needed only for more complex tasks of declarative memory such as forming associations and conjunctions among stimuli (Aggleton & Shaw, 1996; Vargha-Khadem et al., 1997; Tulving & Markowitsch, 1998; Rich & Shapiro, 2009). Good recognition performance has been described following restricted hippocampal lesions in a case of developmental amnesia (Vargha-Khadem et al., 1997; Baddeley et al., 2001). A second possible interpretation was that the hippocampus is essential for normal recognition memory but that the hippocampus itself supports only the recollective (episodic) component of recognition. Under this view, judgments based on familiarity can be supported by adjacent cortex in the medial temporal lobe or perhaps by other structures important for nondeclarative memory (Yonelinas et al., 1998; Eldridge et al., 2000; Brown & Aggleton, 2001; Verfaellie & Keane, 2002; Yonelinas, 2002).

Single-cell recordings in humans and experimental animals also suggest a role for the hippocampus in recognition memory performance. For example, neurons recorded from the hippocampus during visual or olfactory recognition tasks can convey stimulus-specific information as well as an abstract match-nonmatch signal—that is, a response that signals the outcome of the recognition process rather than a signal about the stimulus itself (Fried et al., 1997; Wood et al., 1999; Suzuki & Eichenbaum, 2000). Perhaps, it should not be surprising that recognition memory, including the component of recognition memory that supports familiarity judgments, depends on the integrity of the hippocampus. The hippocampus is the final stage of convergence within the medial temporal lobe, receiving input from both the perirhinal and parahippocampal cortices, as well as the entorhinal cortex. The entorhinal cortex receives about two-thirds of its cortical input from the perirhinal and parahippocampal cortices and originates the major cortical projections to the hippocampus (Suzuki & Amaral, 1994). Anatomical considerations alone suggest that the hippocampus is positioned to combine and extend the operations of memory formation that are carried out by the more specialized structures that project to it.

The Object Recognition Task and the memory performance study

The capacity for recognition memory has been particularly well documented in mice, rats, and monkeys, as well as in humans. **Object recognition** is the ability to perceive some object's physical properties (such as shape, color and texture) and apply semantic attributes to the object, which includes the understanding of its use, previous experience with the object and how it relates to others (Enns, 2004). One of the models for object recognition, based on neuropsychological evidence, provides information that allows dividing the process into four different stages (Humphreys et al., 1999; Riddoch & Humphreys, 2001; Ward, 2006):

Stage 1 Processing of basic object components, such as colour, depth, and form.

Stage 2 These basic components are then grouped on the basis of similarity, providing information on distinct edges to the visual form. Subsequently, figure-ground segregation is able to take place.

Stage 3 The visual representation is matched with structural descriptions in memory.

Stage 4 Semantic attributes are applied to the visual representation, providing meaning, and thereby recognition.

When a subject sees an object, it knows if the object was seen in a past occasion, this is called recognition memory. Every day we recognize a multitude of familiar and novel objects. We do this with little effort, despite the fact that these objects may vary somewhat in form, color, texture, etc. Objects are recognized from many different vantage points (from the front, side, or back), in many different places, and in different sizes. Objects can even be recognized when they are partially obstructed from view. Not only do abnormalities to the ventral (what) stream of the visual pathway affect our ability to recognize an object but also the way in which an object is presented through the eyes. The ventro-lateral region of the frontal lobe is involved in memory encoding during incidental learning and then later maintaining and retrieving semantic memories (Ward, 2006). Familiarity can induce perceptual processes different to those of unfamiliar objects which mean that our perception of a finite amount of familiar objects is unique. Deviations from typical viewpoints and contexts can affect the efficiency for which an object is recognized most effectively. It is known that not only familiar objects are recognized more efficiently when viewed from a familiar viewpoint opposed to an unfamiliar one, but also this principle applies to novel objects. This deduces to the thought that objects representations in the brain are probably organized in a familiar fashion of the objects observed in the environment (Bulthoff & Newell, 2006). Recognition is not only largely driven by object shape and/or views but also by the dynamic information (Norman & Eacott, 2004). Familiarity then can benefit the perception of dynamic point-light displays, moving objects, the sex of faces, and face recognition (Bulthoff & Newell, 2006). Recollection shares many similarities with familiarity; however it is context dependent, requiring specific information from the inquired incident (Ward, 2006).

The distinction between category and attribute in semantic representation may inform the ability to assess semantic function in aging and disease states affecting semantic memory, such as in Alzheimer's disease (AD) (Hajilou & Done, 2007). The semantic memory is known to be used to retrieve information for naming and categorizing objects (Laatu et al., 2003), individuals suffering from Alzheimer's disease have difficulties in recognizing objects because of semantic memory deficits. In fact, it is highly debated whether the semantic memory deficit in AD reflects the loss of semantic knowledge for particular categories and concepts or the loss of knowledge of perceptual features and attributes (Hajilou & Done, 2007).

It has been widely demonstrated that spontaneous exploratory activity in the rat can be used to provide a valid measure of memory function (Ennaceur & Delacour, 1988; Ennaceur & Meliani, 1992a,b; Ennaceur & Aggleton, 1994; Ennaceur et al., 1996; Hirshman & Master, 1997). In animals the "Object Recognition Task" has been the method more used to measure exploratory activity. It can be conducted on mice and rats, and the recognition memory is assessed by measuring animal's ability to recognize an object previously presented. The novel object recognition task was introduced by Ennaceur & Delacour in 1988, in order to assess the ability of rats to recognize a novel object in an otherwise familiar environment.

Since then, the test has become popular for testing object recognition memory in rodents in general, and the effects of amnesic drugs on exploratory activity in particular (Hammonds et al., 2004). The main advantages of this test are, first: each animal can be tested repeatedly with new stimuli in the same session, thus permitting comparisons between subjects in different conditions; and, second: animals do not require extended training or habituation. Other advantages are that the familiarization phase is identical for all the four versions of the test (with the exception that there are two familiarization phases on the context-memory task); the test does not require external motivation, reward or punishment, and the task can be completed in a relatively short period of time. For these reasons, the "Novel Object

Recognition task" is an excellent option for testing animals which have received previous treatments which might alter the reward system, food and water intake or general stress levels. The object recognition task includes two-trials, the first is an acquisition phase or sample phase, also called training phase, and the second one is known as testing phase. Each of them usually has a duration that can vary between 2 to 5 minutes. In the training phase, in order to get familiarized with the objects, a rodent is placed in an enclosure and exposed for a set length of time to two identical objects that are located in a specified distance from each other (Figure 1 panel a). The animal is then removed from the environment, according to the memory type to assess, and a predetermined amount of time is allowed to pass. The rodent is then retested in the same environment except that one of the two previously used (familiar) objects is replaced with a novel one, that differs from the familiar object in shape (Figure 1 panel b), texture and appearance (e.g., a plastic block is replaced with a metal ball). In each phase, the time spent exploring each of the objects is quantified. Usually, exploration of an object is defined as time spent with the head oriented towards and within two centimeters of the object (Benice & Raber, 2005). Turning around or sitting on the object is not considered as an exploratory behavior. This test gives information on **working, short-term or long term memory** depending on elapsed time between the training and the testing phase. Additionally, this test provides information about the exploratory behavior, which is related to attention; anxiety and preference for novelty in rodents. Memory acquisition occurs when the animal perceive the object's physical properties and apply semantic attributes to the object. During consolidation, which can last from minutes to days, this memory is moved from a labile to a more fixed state. During retrieval, the animal supports the ability to know that an item was presented. Then, this test allows evaluating acquisition, consolidation and retrieval, depending on the time course of the manipulations. Pharmacological or physical manipulations such as drug administration or stress, before the training phase can affect both, the early acquisition stage and the consolidation memory stage. If manipulations are performed immediately after training phase affects the late acquisition and consolidation stage. Oppositely, if manipulations are done before the test phase only the retrieval stage is affected. The different stages of memory can be quite difficult to isolate experimentally, because behavioral techniques potentially affect two or more stages of memory. Short-lived treatments, however, can isolate consolidation stage independently of acquisition or retrieval.

The novel object recognition task depends on preference for novelty and also requires more cognitive skills from the subject to explore of novel environments or a single novel object. In order to discriminate between a novel and a familiar object, two identical objects are presented to the subject and then it has to recall the two objects (process known as working memory). Upon replacement of one of the familiarized objects by a novel object, if the animal can recognize that one object as novel, the animal will typically display differential behavior directed towards the novel object. The task scoring has often involved the experimenter recording of the time spent around a novel object versus time spent with a familiar object, and calculation of a novelty or "discrimination index" is based on these measurements (Haist & Shimanura, 1992; Ennaceur et al., 1997; Donaldson, 1999).

Behavioral observation of each animal in the "Novel Object Recognition Task" is time-consuming, and can hinder the ability to use many subjects, particularly in studies requiring exact timing (such as following a pharmaceutical or lesion treatment or a developmental exposure) or many treatment groups (such as dose-response studies). Then, in order to fully describe behavior and to collect all variables of interest, the test session must be recorded by

using videotaping and/or computer software to assist the experimenter observation (Belcher et al., 2005; Ennaceur et al., 2005; Belcher et al., 2006; He et al., 2006). Also, the novel object preference dependent upon strain, sex, and age.

The analysis of the results obtained indicates that non-amnesic animals will spend more time exploring the novel object than the familiar one. An absence of any difference in the exploration of the two objects during the second phase can be interpreted as a memory deficit or, in case of testing an amnesic drug, a non-functioning drug.

The preference for novelty is also influenced by factors such as training phase duration and the inclusion of common features in the familiar object and the novel object (Ennaceur & Delacour, 1988; Ennaceur & Aggleton, 1994; Ennaceur et al., 1996). Advantages associated with this class of measure include the fact that performance does not depend on the retention of a rule, nor is it influenced by changes in response to reward. Furthermore, because the test uses a forced choice design it is less likely to be affected by changes in impulsivity or activity. As a consequence such tasks can provide a relatively pure measure of "working memory" (Honig, 1978; Olton & Feustle, 1981).

The experimental conditions are crucial when this behavioral protocol is applied to aged animals. It has been known that aging is associated with memory impairments. The recognition memory has been recently investigated in aged rats using the object recognition task (Platano et al., 2008). In this regard, it has been demonstrated that the object recognition did not occur in rats older than 18 months (Bartolini et al., 1996). However, in different experimental conditions aged rats (25-27 months old) showed a good object recognition memory performance. Since no effective tasks are reported in the literature for aged rats, a new training protocol was developed (Platano et al., 2008), in this protocol a combination of the repetition of five training sessions in 3 days and smaller area exploration resulted in the establishment of the object recognition memory that persisted for at least 24 h for in both adult and aged rats. On the other hand, the older animals training the small area showed a higher synaptic density and a lower synaptic average area, indicating that the use of the smaller area is very important, because this non-anxiogenic environment induced a good plastic reactivity and memory performance. The authors suggested that this new protocol may be useful to compare functional and structural change associated with the memory formation in adulthood and the physiopathological aging (Platano D et al, 2008).

Apparatus

The apparatus consisted of an open box (100×100×50 cm high) made of aluminum with the inside painted in matt grey. The floor was covered with woodchip bedding which was moved around between trials/days to stop build-up of odor in certain places. The objects to be discriminated were available in four copies and made of an inert material such as glass; plastic or metal Figure 1. The weight of the objects ensured that they could not be displaced by the rats. To achieve this, some objects were filled with water or sand.

Behavioral Testing procedure

Pre-training. The animals are handled for 1 week and then all animals are given one habituation session in which they are allowed 5 min to explore the arena without stimuli (without objects). This habituation is especially important for the animal to become familiar with the environment, increasing the interest of the animal by the objects presented in the training phase.

Training Phase: the animals are placed into the arena facing the center of the opposite wall and exposed for a set length of time to two identical objects (A1 and A2) that are located in

the corner a specified distance from each other (15 cm from each adjacent wall) and allowed to explore for 3 min (Figure 1panel a). The time that the animal explored each object is measured. The rats are then removed to its home cage.

Test phase: this phase is different depending on the NOR variant. The same is done 5 min, 2 h or 24 h after the training phase in order to measure working memory, short-term memory or long-term memory.

There are four versions of this task, Novel object preference task, Object location task, Temporal order task and Object-in-place task. The following describes this phase in the different NOR:

Novel object preference task. The procedure comprised a training phase (acquisition), followed by a test phase (consolidation). In test phase the animal is re-placed in the arena, presented with two objects in the same positions: one object (A1) that is used in the training phase and the other object is a novel object (B) (Figure 2A). The positions of the objects in the test and the objects used as novel or familiar are counterbalanced between the animals.

Object location task. In this test, the rat's ability to recognize that an object that it had experienced before had changed location is assessed. In the test phase, one object (A1) is placed in the same position had occupied in the training phase. Object A2 is placed in the corner adjacent to the original position, so that the two objects A1 and A2 are in diagonal corners. Thus, both objects in the test phase are equally familiar, but one is in a new location (Figure 2B). The position of the moved object is counterbalanced between rats.

Temporal order task. This task comprised two training phases and one test trial. In each training phase, the subjects are allowed to explore two copies of an identical object. Different objects are used for training phases 1 (A1 and A2) and 2 (B1 and B2), with a delay between the training phases of 1 h. The test trial is given 3 h after training phase 2. During the test trial, an objects from training phase 1 (A1) and an objects from training phase 2 (B1) are used (Figure 3). The positions of the objects in the test and the objects used in training phase 1 and 2 are counterbalanced between the animals. If temporal order memory is intact, the subjects will spend more time exploring the object from training 1 (i.e., the object presented less recently) compared with the object from training 2 (i.e., the "new" object).

Object-in-place task. This task comprised a training phase and a test phase separated by a 5 min delay. In the training phase, the subjects are presented with four different objects (A, B, C, D). These objects are placed in the corners of the arena 15 cm from the walls. Each subject is placed in the center of the arena and allowed to explore the objects for 5 min. During the delay period, all of the objects are cleaned with alcohol to remove olfactory cues and any sawdust that had stuck to the object. In the test phase, two of the objects (e.g., B and D, which were both on the left or right of the arena), exchanged positions, and the subjects are allowed to explore the objects for 3 min (Figure 2C). The time spent exploring the two objects that had changed position is compared with the time spent exploring the two objects that had remained in the same position. The objects moved (i.e., those on the left or right), and the position of the objects in the sample phase are counterbalanced between rats. If object-in-place memory is intact, the subject will spend more time exploring the two objects that are in different locations compared with the two objects that are in the same locations.

The following parameters are analyzed: the time spent exploring each objects A1 and A2 in the training phase, the time spent exploring each objects B and A2 (object recognition) or objects A1n (A1in its new location) and A2 (object location) in the test phase. The data are expressed as the percentage (%) of time that the animals explore identical objects ($t_{A2}/[t_{A1} + t_{A2}] \times 100$) during training and the % of time that the animals explore the novel object ($t_B/[t_B$

+ t_{A2}] $\times 100$) in the retention test (Novel Object Exploration -% Time) and total exploration time. The time percentage used for the novel object exploration is considered as an index of memory retention.

In publications by Carlini et al, it was represent the treatment or peptide effect on memory performance in the object recognition test, in a graph in which the percentage time exploration of one object in the training phase and the novel object in the test phase were indicated. As it can be seen, the Figure 4 shows that the animals explore 50% of time in each object in the training phase (these not show preference for one object), while the control animals explore 70 - 80 % of time in the novel object in the test phase under normal experimental condition. In this case it was measured the peptide effect upon short and long-term memory retention. It should be noted that two novel objects were used, and two tests were carried out. The first test was performed one hour after training, the animal was placed in the box for the retention test and allowed to explored for 3 min the objects: one of them was the same as the one used for training (1-familiar object) and the other one was a novel object (3-novel object), then the animal returned to its home cage. The second test was carried out twenty four hours later, the animal was tested in the box but object 3 was changed for another novel object (4-novel object) that the rat had never encountered before (Carlini et al., 2008).

In addition, it can also analyzed: $d1$ the index of discrimination, i.e. the difference in time spent exploring the two objects in the training phase (e.g. B-A2 in the object recognition task and A1n-A2 in the object location task), $d2$ the discrimination ratio, i.e. the difference in exploration time (i.e. $d1$) divided by the total time spent exploring the two objects in the training phase (e.g. B-A2/B+A2) in the object recognition task and A1n-A2/A1n+A2 in the object location task) (see Table 1).

The data are evaluated with repeated measures analysis of variance, including training and test phase.

Numerous authors have long been interested in the factors and neuropeptides that modulate the memory retention, particularly in different conditions such as stress, depression, chronic food restriction and undernutrition and examine these issues in an experimental paradigm commonly known as NOR or NOP (Souza, 1992; O'Dell & Marshall, 2005; Hopkins & Bucci, 2010; Kertész, S et al., 2010). In this paradigm, the animal is first allowed to explore a matching set of two identical objects. At some point later, the animal encounters one of the original objects and a novel object with which it has had no prior experience. Berlyne (1950) first demonstrated that animals will spend more time exploring the novel object than the original (i.e., familiar) one when given equal access to both, thus displaying a preference for novel stimulation. If a delay is added between training and test, NOR can also become a useful measure of retention across time (e.g., Ennaceur & Delacour, 1988; Anderson et al., 2004; Anderson, 2006a,b).

It should be noted that object recognition and object location tasks are based on spontaneous exploratory activity, and as a consequence they do not exclude the possibility of individual animals having a preference for a specific object or place that is independent of the familiarity/novelty of that item. In our lab, we use the NOR to examine the different neuropeptide effects on memory performance (Carlini et al., 2007; 2008) and we also examined the motivational behavior in this test, because it is reasonable to believe that the diminished performance in the object recognition test induced by a memory impairment drug, could be a consequence of a motivational deficit; i.e. animals would be not interested in exploring novel objects. In order to explore the above mentioned hypothesis, we quantified during each

experiment (1) t1, the total time spent in exploring the two identical objects in the training phase; (2) t2, the total time spent exploring the two objects in the test phase.

Particularly, the object recognition test is very interesting because it is a non aversive test. It has been demonstrated that the effects of pharmacological agents that impair or enhance memory retention for an aversive stimulus as the footshock can be investigated using the step down test (Barros et al., 2001).

In publications by Carlini et al., it was reported that the obestatin peptide caused increase in a dose-related manner in the latency time in the step down test, when given immediately post training (0 h), suggesting an increase in memory retention. It is known that in several memory aversive paradigms (as step down), the amygdala has also a critical role. Thus, the results from the step-down experiments, showing memory facilitation, suggest that the amygdala may also play an important role in the central effects of this peptide. However, the obestatin effect on memory retention using other behavioral paradigm, the object recognition task, also was studied. It is reasonable to believe that enhanced memory performance induced by obestatin in the step down paradigm could be test dependent. Nevertheless, the result shown that obestatin affects the performance of the animals in the two memory paradigm used (step down and object recognition test) indicates that these effects were not test dependent. Furthermore, in both memory tests, the hippocampus seems to be the principal structure involved (Carlini et al., 2007).

In the paper by Carlini et al, the step down paradigm was not used because the animals were under chronic food restriction. In this experimental condition the animal exhibit anxiety-like behavior and is more sensitive to footshock, showing decreased latency to escape from footshock. It has been demonstrated that undernutrition during suckling caused hyperreactivity to 0.2 mA footshocks in the step down test (Vendite et al., 1987) and that in the shock threshold test the malnourished animals are more sensitive to electric shock (Rocinho et al., 1997). Footshock escape latency for undernourished rats was less than for well-nourished rats (Souza et al., 1992). Maybe the hyperreactivity or anxiety-like behavior observed in this nutritional condition could be the cause for the improved memory performance showed by these animals, and this can explain some of the interpretations reported in the literature (Souza et al., 1992). Thus, by using the step down test the chronic food restricted animals may show an apparent increase in memory performance. In consequence, under this experimental condition, the object recognition task is more abdicated than the step down, because in it there are not punishment o pain threshold.

Actually, there are some companies that offer several features designed to automate the test procedure and automatically measure and analyze behavioral parameters related to the novel object recognition test. These computational programs automatically record the position of the rodent's nose, relative to spatial zones or objects, measuring the time the animal spends with its nose towards and within a short distance of these zones and, thus, the time the animal spends exploring the separate objects. In addition, the system can also automatically measure elongation of the rodent's body, a parameter frequently used to investigate exploratory behavior. By setting elongation thresholds, you can define different degrees of this behavior as 'stretched', 'normal', or 'contracted' (Benice & Raber, 2005)

Without a doubt, this test, which is frequently applied, could provide new and original insights about physiological processes of learning and memory.

(a)

(b)

Fig. 1. Mouse exposed to two identical objects (A1 and A2) in training phase (panel a) and mouse exposed to novel and familiar object (B and A2) of Novel object preference task (panel b).
Mouse exploring novel object in test phase. It should be noted that exploration of an object is defined as time spent with the head oriented towards and within two centimeters of the object.

Fig. 2. Diagram of the three object recognition memory tasks. **A-** Novel object preference task: Left (training phase), animals are exposed to two identical objects (A1 and A2). Right (test phase), animal are exposed to two different objects, the sample previously explored in the training phase which is now familiar (A1) and a new object (B), never seen before. **B-** Object location task: Left (training phase), animals are exposed to two identical objects (A1 and A2). Right (test phase), animals are exposed to two objects, previously explored in the training phase which are now familiar (A1 and A2), but one object (A1) is re-localized in relation to training phase. **C-** Object-in-place task. Left (training phase), animals are exposed to four different objects (A, B, C and D). Right (test phase), animals are exposed to four objects, previously explored in the training phase which are now familiar, but two object (A and B) are re-localized in relation to training phase.

Fig. 3. Diagram of the *Temporal order task*. This task comprised two training phases: Left (first training phase), animals are exposed to two identical objects (A1 and A2); middle (second training phase), animals are exposed to two identical objects (B1 and B2) but these are different to first training phase; right (test phase), animal are exposed to two different objects, both previously explored, one object of the first training phase and other of the second training phase.

Fig. 4. Representative graphic of the parameters showed about the object recognition test. The figure show the Obestatin effect on memory performance in object recognition test. The animals received Obestatin (Ob) or ACSF (Control). The results are expressed as percentage of novel object exploration (time percentage = $t_{\text{novel}} / [t_{\text{novel}} + t_{\text{training}}] \cdot 100$) $\pm$ SEM. Training: the rat was placed with two identical objects (1 and 2). Test 1 and 24 h after training the rat was placed in the box with the object 1-3 and 1-4 respectively. *Significant differences with control animals, $p \leq 0.05$ (The graphic correspond to the figure shown in the paper published by Carlini et al. in *Biochem Biophys Res Commun.*, 2007).

Test	Variable measured					
	% Time Training		% Time Nobel		t1	t2
	Object	Exploration	Object	Exploration		
A. Novel Object Preference	$t_{A2}/[t_{A1} + t_{A2}] \times 100$	$t_B/[t_B + t_{A2}] \times 100$			A1 + A2	B + A2
B. Object location	$t_{A2}/[t_{A1} + t_{A2}] \times 100$	$t_{A1n}/[t_{A1n} + t_{A2}] \times 100$			A1 + A2	A1n + A2

Table 1. Index of the different measures involved in the spontaneous recognition memory task for objects and location of objects.
t1 the total time spent exploring two objects A1 and A2 in the sample phase, *t2* the total time spent exploring objects B and A2 (object recognition) or objects A1n (A1 in its new location) and A2 (object location) in the test phase, *d1* the index of discrimination, i.e. the difference in time spent exploring the two objects in the training phase (e.g. B–A2 in the object recognition task and A1n–A2 in the object location task), *d2* the discrimination ratio, i.e. the difference in exploration time (i.e. *d1*) divided by the total time spent exploring the two objects in the training phase (e.g. B–A2/B+A2 in the object recognition task and A1n–A2/A1n+A2 in the object location task).

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Vision-based object recognition tasks are very familiar in our everyday activities, such as driving our car in the correct lane. We do these tasks effortlessly in real-time. In the last decades, with the advancement of computer technology, researchers and application developers are trying to mimic the human's capability of visually recognising. Such capability will allow machine to free human from boring or dangerous jobs.

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