Behavioural Brain Research 250 (2013) 62–73

Contents lists available at SciVerse ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

One-trial object recognition memory in the domestic rabbit (*Oryctolagus cuniculus*) is disrupted by NMDA receptor antagonists

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HIGHLIGHTS

- We describe an object recognition memory (ORM) task for rabbits based on scent-marking.
- NMDA antagonists disrupt ORM when administered prior to the sample phase.
- Short-term habituation and sensitization underlie ORM in the present paradigm.
- Tactile, but not olfactory novelty sensitizes rabbit scent-marking behavior.
- Habituation and sensitization modulate the expression of scent-marking.

ARTICLE INFO

Article history:
Received 7 October 2012
Received in revised form 24 April 2013
Accepted 28 April 2013
Available online 4 May 2013

Keywords:
Object recognition memory
Habituation
Sensitization
Scent marking
Rabbit
Short term memory

ABSTRACT

The spontaneous response to novelty is the basis of one-trial object recognition tests for the study of object recognition memory (ORM) in rodents. We describe an object recognition task for the rabbit, based on its natural tendency to scent-mark ("chin") novel objects. The object recognition task comprised a 15 min sample phase in which the rabbit was placed into an open field arena containing two similar objects, then removed for a 5–360 min delay, and then returned to the same arena that contained one object similar to the original ones ("Familiar") and one that differed from the original ones ("Novel"), for a 15 min test phase. Chin-marks directed at each of the objects were registered. Some animals received injections (sc) of saline, ketamine (1 mg/kg), or MK-801 (37 μg/kg), 5 or 20 min before the sample phase. We found that chinning decreased across the sample phase, and that this response showed stimulus specificity, a defining characteristic of habituation: in the test phase, chinning directed at the Novel, but not Familiar, object was increased. Chinning directed preferentially at the novel object, which we interpret as novelty-induced sensitization and the behavioral correlate of ORM, was promoted by tactile/visual and spatial novelty. ORM deficits were induced by pre-treatment with MK-801 and, to a lesser extent, ketamine. Novel object discrimination was not observed after delays longer than 5 min. These results suggest that short-term habituation and sensitization, not long-term memory, underlie novel object discrimination in this test paradigm.

1. Introduction

In rodents, tests to assess object recognition memory (ORM) are based on the animal's natural tendency to explore novelty [19,21,71]. In its most basic form, the novel object recognition (NOR) test comprises a *sample phase*, in which the animal is allowed to explore a set of 2 or more objects, after which it is removed from the testing arena for a fixed delay period. During the delay, one of the objects is replaced with a different one that has novel characteristics. The animal is subsequently returned to the arena for a *test phase*. The rodent's natural tendency is to investigate the novel object more than the familiar one, a response that necessarily requires representation of the original objects in memory. The proportion of time the animal spends investigating the novel object compared to the familiar one is considered to be an index of novel object discrimination, and therefore a quantitative measure of ORM. Most studies have investigated the capacity to discriminate novel from familiar based on visual and/or spatial cues, but specific experimental designs can probe tactile and olfactory novelty as well [2,43].

Similar tests based on the subject's spontaneous response to novelty, such as the visual paired-comparison task, have been applied in studies carried out on human subjects and non-human primates [13,15,39,44,50,74]. It is important to emphasize that, in NOR tasks, the experimentally-measured behavioral response to novelty (gaze direction in humans, and, in rodents, directing the

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http://dx.doi.org/10.1016/j.bbr.2013.04.049

snout close to the object and/or sniffing it), is spontaneous and untrained. Since the subject is not required by previous training to hold a mental representation "online" across the delay period, the NOR test should not be considered a measure of working memory, even when the delay is very short. Based on what is known of the neuroanatomical substrates associated with test performance, the NOR test is proposed to represent short- to long-term declarative/episodic-like memory, depending on the length of the imposed delay. In the context of object recognition, working memory can be distinguished from declarative/episodic memory by the specific demands of the task: working memory is used when the subject is explicitly required to retain a mental representation of an object across a delay, in order to compare it with a second object (which may or may not be novel with respect to the first), whereas declarative/episodic memory is used when object recognition is spontaneous and based solely on novelty [21,39].

In rodents, an extensive line of evidence from lesion studies has implicated the perirhinal cortex as a brain region necessary for novel object recognition based on visual cues, whereas detection of object novelty based on spatial cues relies more on the function of the hippocampus, parahippocampal cortex, and entorhinal cortex [1,8,9,70], although some studies challenge this view [17]. The results of at least one study indicate that object recognition based on haptic and olfactory cues might rely on regions other than the perirhinal cortex [3]. Inactivation of the perirhinal cortex prior to the sample phase by lidocaine infusion disrupted ORM, even when tested after a delay of just seconds, implicating this structure in ORM encoding [68]. Studies in monkeys present a more complicated picture, with both hippocampal and perirhinal cortex lesions clearly disrupting performance in visual object recognition tasks [74], but with some studies suggesting that deficits induced by hippocampus lesions may depend on specific methodological variables, such as extent of lesion, encoding time, and visual clarity of stimuli [30,50,73]. Similarly, functional brain imaging studies of human subjects have associated activity of perirhinal cortex and parahippocampal cortex, respectively, with novel object recognition and spatial memory [14,54].

Several studies have shown that antagonists to the NMDA receptor (ketamine, MK-801, AP-5) disrupt performance in the NOR test, apparently interfering with the encoding, consolidation and/or retrieval of ORM. Thus, MK-801 administered systemically 20 or 30 min before the sample phase impaired novel object recognition in the test phase carried out after a delay of 1.5–24 h, thereby implicating NMDA receptors in the encoding process [18,51]. When administered immediately after the sample phase, or 30 min before the test phase, MK-801 either had no effect [51] or impaired object recognition [18]; the latter study suggesting that NMDA receptor antagonism might also have effects on consolidation and/or retrieval processes. When infused directly into the perirhinal cortex 15 min before sample phase, AP-5 disrupted ORM when tested 3 h later (long-term ORM), but not 5 min later (short-term ORM). When AP-5 was infused into the perirhinal cortex immediately after the sample phase (but not when infused 40 min later) long-term ORM was similarly disrupted. By contrast, when CNQX (an AMPA receptor antagonist) was infused into this same region before the sample phase, both short- and long-term ORM were disrupted. Taken together, these results indicate that NMDA receptor activation in the perirhinal cortex mediates the consolidation of long-term, but not short-term ORM, whereas AMPA receptor activation within this region is involved in encoding as well as consolidation processes [69]. Similarly, MK-801 infused directly into the hippocampus 15 min before the encoding phase decreased performance in a one-trial spatial memory task, when the delay between sample and test phases was 20 min [10].

In a previous study [33], we described the display of "chinning" (a scent marking behavior displayed by domestic rabbits) during a prolonged test periods (up to 90 min) in an open field arena containing "markable" objects (bricks). Chinning is a behavior in which the rabbit rubs the undersurface of its chin across the surface of the object being marked, thereby depositing secretions derived from the animal's submandibular scent glands ([48], and Fig. 1 of the present paper]. An advantage of studying this behavior is that a single "chin-mark" is a discrete and unambiguous behavioral event that is easily registered and quantified. In a previous study, we found that chinning, along with ambulatory behavior within the arena, habituated across a 30 min test session, that is, the expression of these behaviors steadily declined. After this test session, the rabbit was returned to its cage for a 5 min delay, during which the original bricks were replaced with bricks having a novel form, or bricks having the same form as the original ones. When the rabbit was then returned to the arena for a second 30 min test session, chinning frequency was increased only in response to the novel bricks, showing that the habituation response was specific to the previously encountered "familiar" stimulus (i.e., the "stimulus-specificity" characteristic of habituation) [57], and ruling out fatigue as a possible explanation for the decline in chinning across the first session. Moreover, we found a double dissociation between chinning and ambulation with respect to novel stimuli that reliably increased behavior: chinning – but not ambulatory behavior – was increased by novel objects, whereas ambulatory behavior, but not chinning, was increased by changing the location of the test arena. In the case of chinning, results of this study suggested that novel visual and/or textural cues were those most likely responsible for stimulating this behavior, as changes in the olfactory characteristics or spatial configuration of the objects had no effect [33].

For simplicity, in the present article we refer to the novelty-induced increase in chinning as "sensitization". We use this term in a general descriptive sense, in order to refer to a stimulus-induced increase in behavioral response [23,56]; in the present case, the relevant stimulus is novelty. By using this term we also wish to distinguish the present phenomenon from "dishabituation", which properly refers to a stimulus-induced increase in responsiveness to the *original* habituating stimulus [57,63], a response that clearly *was not* observed in the present studies. However, it is important to emphasize that the neural mechanisms that underlie the novelty-induced increase in chinning have not been characterized and could involve either the *cellular processes* of sensitization (e.g., facilitation of synaptic transmission) and/or reversal of habituation (e.g., reversal of synaptic depression; the latter process is also referred to as "dishabituation") [29,63].

In the present study, we wanted to design an object memory task for male and female rabbits, using chin marking frequency as an indicator of novelty discrimination. The motivation behind this objective was threefold. First, we believe that the rabbit represents a convenient model with which to study in the neurobiology of ORM in a non-rodent species. Complementary investigations of ORM in rabbits should be useful order to determine whether neural mechanisms underlying ORM in the rodent can be generalized to other species. A second, long-term goal is to establish a rabbit model for cognitive symptoms of schizophrenia, based on ORM deficits induced by acute NMDA receptor antagonism [37]. Therefore, in the present study we also tested the effect of two NMDA antagonists, ketamine and MK-801, on the rabbits' performance in this task. Finally, the results of a previous study suggested that tactile cues (as opposed to visual, spatial or olfactory cues) might be particularly important for object recognition in the present experimental paradigm; therefore, in the present study, we wanted to further characterize the specific object attributes that are recognized as novel and which provoke an increase in chin marking.