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Object, spatial and social recognition testing in a single test paradigm

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ABSTRACT

Animals have the ability to process information about an object or a conspecific's physical features and location, and alter its behavior when such information is updated. In the laboratory, the object, spatial and social recognition are often studied in separate tasks, making them unsuitable to study the potential dissociations and interactions among various types of recognition memories. The present study introduced a single paradigm to detect the object and spatial recognition, and social recognition of a familiar and novel conspecific. Specifically, male and female Sprague-Dawley adult (> 75 days old) or preadolescent (25–28 days old) rats were tested with two objects and one social partner in an open-field arena for four 10-min sessions with a 20-min inter-session interval. After the first sample session, a new object replaced one of the sampled objects in the second session, and the location of one of the old objects was changed in the third session. Finally, a new social partner was introduced in the fourth session and replaced the familiar one. Exploration time with each stimulus was recorded and measures for the three recognitions were calculated based on the discrimination ratio. Overall results show that adult and preadolescent male and female rats spent more time exploring the social partner than the objects, showing a clear preference for social stimulus over nonsocial one. They also did not differ in their abilities to discriminate a new object, a new location and a new social partner from a familiar one, and to recognize a familiar conspecific. Acute administration of MK-801 (a NMDA receptor antagonist, 0.025 and 0.10 mg/kg, i.p.) after the sample session dose-dependently reduced the total time spent on exploring the social partner and objects in the adult rats, and had a significantly larger effect in the females than in the males. MK-801 also dose-dependently increased motor activity. However, it did not alter the object, spatial and social recognitions. These findings indicate that the new triple recognition paradigm is capable of recording the object, spatial location and social recognition together and revealing potential sex and age differences. This paradigm is also useful for the study of object and social exploration concurrently and can be used to evaluate cognition-altering drugs in various stages of recognition memories.

1. Introduction

The object recognition test is a widely used behavioral paradigm for the study of short-term and long-term memory in rodents (Bevins and Besheer, 2006; Ennaceur and Delacour, 1988). It relies on the innate tendency of rodents to explore novel stimuli in a familiar environment and tend to spend more time exploring a novel stimulus than with a familiar one. The object recognition test can also be modified to measure spatial recognition memory, the ability of an animal to recognize change in spatial location of a familiar object which has been explored in the sample phase. A spatial recognition is observed if the rodent spends more time exploring the object encountered in the novel location (Dix and Aggleton, 1999).

In the social domain, rodents also have the abilities to recognize individuals of the same species and to distinguish them from other individuals. These abilities, termed social recognition, can be examined in rodents using the similar procedure to that of object and spatial recognition (Thor and Holloway, 1982). A typical test involves two short (2–5 min) encounters of a subject animal with the same conspecific, separated by a brief interval. A social recognition is demonstrated if the subject reduces its social exploration time (e.g., sniffing, chasing, grooming, etc.) with the testing partner from the first encounter to the second (Li, He, and Munro, 2012; Thor and Holloway, 1982). A more commonly used variant of this test employs the social

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habituation–dishabituation process to ascertain that the reduction in social investigation is not due to fatigue or lack of social motivation (Ferguson, Young, and Insel, 2002; Gao and Li, 2014; Li et al., 2012; Sekiguchi, Wolterink, and van Ree, 1991b). The social recognition memory is evidenced by the findings that a normal rat or mouse displays a continuous decrease in its time investigating the same testing partner and increases its investigation time if a novel partner is introduced (Akers, et al., 2006; Holloway and Thor, 1988; Prediger, Batista, Miyoshi, and Takahashi, 2004). This paradigm is useful for the study of the basic neural and neuroendocrine mechanisms of social behavior (Ferguson et al., 2002; Sekiguchi, Wolterink, and van Ree, 1991a), and effects of psychoactive drugs on social interaction, social recognition and anxiety (Gao and Li, 2014; Li et al., 2012).

The common denominator of all three paradigms is their use of the natural behavioral tendency of rodents to investigate novel social and nonsocial stimuli more persistently than they investigate familiar ones, thus affording high ecological relevance. These tasks are easy to implement and do not require lengthy training and testing. Animals are also not food or water deprived, thus, avoiding the introduction of an additional aversive motivation while making the results easy to interpret. Because of these features, these paradigms are attractive to researchers in behavioral neuroscience as important tools to study learning and memory (Blaser and Heyser, 2015; Ennaceur and Delacour, 1988). One thing to note is that each paradigm measures different types or aspects of learning and memory, targeting different memory systems and they are often used separately. To the best of our knowledge, there is no paradigm that incorporates all three recognitions into a single paradigm. However, such a combined paradigm might be ecologically more valid, as animals often have to face multiple stimuli simultaneously in their environment and have to make a decision which one to spend more time exploring. Its advantages over a single test are apparent. For example, it would allow a simultaneous recording of multiple memory systems (object, spatial and social recognition), as well as their interaction (McDonald, Devan, and Hong, 2004), which a single paradigm is unable to do. Furthermore, the integrated paradigm is useful for the identification of the shared and distinct neural substrates underlying different recognition memories (e.g., hippocampus, perirhinal cortex, prefrontal cortex, etc.). Finally, such an integrated paradigm can be efficiently used to model certain cognitive deficits associated with autism spectrum disorders (ASD) or schizophrenia. For example, because both social and nonsocial stimuli are used in the same test sessions, a presumably valid animal model of ASD should show a deficit in social exploration and recognition, but enhanced “object obsession”, as children with ASD generally have social cognitive deficits and “object obsession” (Williams, Costall, and Reddy, 1999). This integrated paradigm is also useful in screening novel compounds targeting different recognitions in a single test.

The present study reports our attempt to record the object, spatial and social recognition in an integrated triple recognition test protocol. The key difference between our protocol and others is that we used both objects and social stimuli in every test session (there are four sessions). To validate this paradigm and see whether it yields similar results as traditional object, spatial and social recognition tests do (Hlinak and Krejci, 2003; Nilsson, Hansson, Carlsson, and Carlsson, 2007; van der Staay, Rutten, Erb, and Blokland, 2011), we also tested adolescent rats and evaluated the psychomotor and mnemonic effects of systemic administration of the N-methyl-d-aspartate (NMDA) receptor antagonist MK-801. Previous work suggests that MK-801 primarily disrupt the encoding (acquisition process), but not the consolidation or retrieval of various recognitions (Williams et al., 1999), thus we chose to administer MK-801 after the sample phase. This arrangement would also allow us to see whether we could still record the recognitions even when the total exploration time is reduced by MK-801.

2. Materials and methods

2.1. Animals

Male and female Sprague-Dawley rats (170–200 g upon arrival) were purchased from Experiment Animal Center, Chongqing Medical University, China and used in all experiments except in Experiment 2, which used the preadolescent offspring of nine pregnant female rats. The virgin females were originally obtained from the Experiment Animal center, Chongqing Medical University (China) and mated in our colony. Only one male and one female were used as subjects from each litter to avoid the litter effect (Lazic and Essioux, 2013). All animals were housed two per cage in transparent polycarbonate cages (47 cm L × 32 cm W × 21 cm H) with food and tap water available *ad lib*. Animals were maintained on a day-night cycle of 12:12 (light on between 8:00 a.m. and 8:00 p.m.). The room temperature was maintained at 22 ± 2 °C and the humidity was controlled at 45–60%. All experiments were started around 8:30 am and finished before 17:30 on each experiment day. All animal procedures were approved by the local animal care and use committee at the Southwest University, China.

2.2. Apparatus

Behavioral tests were conducted in a room which was illuminated by diffuse incandescent lighting (15 lx). The apparatus was an open field-like arena with Plexiglas walls (black in Experiment 1 and 2, and white in Experiment 3 and 4) and black bottom (50 cm L × 50 cm W × 40 cm H). During each test, a single sex-matched testing partner serves as a social stimulus. It was confined in a small cage made of metal wire mesh through which the subject rats could see, hear, and smell the testing partner. The objects used in the experiments were two Rubik’s Cubes and two clay figurines. In Experiments 1, 2 and 4, each object was placed 15 cm from the wall, while the cage was 8 cm from the wall. In Experiment 3, the cage was placed 8 cm from the wall, close to one of the corners. Subject rats were videotaped from above, and the amount of time each animal spent with the testing partner (social target) and objects (non-social target) was measured offline. Between each test, the walls and bottom of the arena and objects were cleaned with 75% alcohol.

2.3. Experiment 1: basic procedure of the object, spatial and social triple recognition paradigm in adult rats

All subject rats (n = 16, 8 males and 8 females) and testing partners (n = 16, 8 males and 8 females) were first handled individually for three days for approximately two min each day to minimize stress before behavioral testing. On the first handling day, the subjects and partners were also marked with different colors. On the second and third handling days, all subjects were habituated to the test arena for 10 min, during which a small metal enclosure (for the confinement of testing partners) was also placed in the arena so that it was no longer novel when the formal tests began. The testing partners were also habituated to the test arena for 10 min during the two handling days. They were confined in the small enclosure in the arena during this period.

On the test day, the subject rats and testing partners were brought into the experimental room 30 min before testing and tested individually for a total of four sessions separated by a 20-min interval. In the first test session (sample phase, T1), a subject rat was allowed to freely explore two identical objects (O1 and O2, a Rubik’s Cube) and a partner (coded as “A”) confined in a rectangular enclosure for 10 min. In the second test session (novel object recognition, T2), one of the objects (O1) was replaced with a new one (O3, clay figurines), the rest (O2 and A) remained the same. In the third test session (novel location recognition, T3), the location of the first object (O2) was moved across the arena, while the rest stimuli remained (O3 and A) the same. In the final test session (T4), the partner was replaced with a new one (B). The