



## Research report

## Spatial task for rats testing position recognition of an object displayed on a computer screen

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## ABSTRACT

We developed two spatial tasks for rats employing computer monitor for stimuli presentation. Both tasks were aimed for testing rats' ability to recognize position of a distant object. In the first task the object was stationary except moments when it jumped from one position to another. In the second task it moved continuously across the screen. Rats were trained in an operant chamber located in front of the monitor. They responded to the object position by pressing a lever for food reward. Responses were reinforced when the object was displayed in a to-be-recognized position in the first task and when it was passing through a to-be-recognized region in the second task. The to-be-recognized position as well as the to-be-recognized region had to be determined with respect to surrounding orientation cues. Responding rate of well trained rats negatively depended on the distance between the object and the to-be-recognized position/region. In the first task this relationship was apparent during a short time after the object changed its position and it held even for newly presented unfamiliar positions of the object. We conclude that in both tasks the rats recognize position of the object by estimating distance between the object and the to-be-recognized position/region. We also analyzed contribution of timing behavior to the solution of the second task.

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## 1. Introduction

Several behavioral tasks are used in research of rodent spatial cognition. In most of them a subject navigates through an environment in order to reach a goal place or to avoid a punished place (e.g. [1,5,24,27]). During the navigation the subject determines its own position with respect to available spatial cues and it executes a goal-directed locomotion.

Animals do not only determine their own position in an environment but they also determine positions of other objects and animals. This ability has been addressed in experimental conditions as well. Some of the tasks exploits natural tendency of rats to preferentially explore objects which changed their location between two subsequent sessions in comparison to objects which remained in the same position [7,11]. In other tasks animals learn association between objects and locations. A subject is rewarded for approaching a familiar object only if the object is presented in a particular position [6,23].

In the above tasks, a subject actively moves through an environment as it navigates toward a hidden goal or approaches an

object. The goal-directed locomotion indicates whether the animal is able to solve the task. The goal-directed locomotion can be replaced by a non-locomotor operant behavior such as lever pressing [16,18,20,28,34]. In these tasks a subject together with an operandum are passively transported through an environment. The subject has to recognize and remember location in which operant responses are reinforced. Such tasks test directly the ability to recognize places because navigation to the places is eliminated by the passive transport. It is possible to modify these tasks in such a way that the environment moves around a subject instead of the subject through the environment [28,34]. This modification eliminates inertial cues generated by passive transport of the subject.

Non-locomotor operant tasks were used in neuroscience research for studying role of hippocampus in recognition of places [19] and for studying activity of hippocampal neurons and theta rhythm under condition in which ambulatory and/or inertial cues were eliminated [16,34].

The non-locomotor operant tasks can be used to study the ability to recognize position of an object. We present two tasks both aimed to test the ability of rats to recognize position of a distant object independently of the position of the rats themselves. Food-deprived rats were trained in an operant chamber to press a lever for food reward. The operant chamber was located in front of a computer screen. Responses of the rats were reinforced when a stationary object was displayed in a particular position in the first task

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or when a moving object was passing through a particular region in the second task.

The present tasks follow our previous study [25] where operant responses were reinforced when a moving object stopped at a particular position marked by another stationary object. The rats could discriminate reward and non-reward periods by means of several strategies some of them were non-spatial. In the present tasks the goal location is not marked by a visual cue and the object does not change its behavior when it arrives to the goal location. This eliminates the non-spatial strategies present in [25].

In the present tasks the subject does not approach the object but it remains in the same place (operant chamber) during the whole experiment. This can be useful for dissociating neural activity representing subject's position from activity representing object's position. In addition, to our knowledge the second task in which the object moves across the screen is the first task in which rats recognize position of a moving object. The continuous movement of the object corresponds to the situation when an animal sees a moving classmate, prey or predator.

## 2. Materials and methods

### 2.1. Subjects

The subjects ( $n = 13$ ) were male Long-Evans rats 3 months old at the beginning of the experiment. The rats were obtained from the breeding colony of the Institute of Physiology, Czech Academy of Sciences and housed in groups of two to three per cage in a temperature-controlled room (21 °C) with a 12:12 light/dark cycle. Water was freely available but access to food was restricted to maintain the rats at 90% of their free feeding weight. All procedures were in accordance with Institutional and NIH guidelines and the directive of the European Communities Council (86/609/EEC).

### 2.2. Apparatus

The rats were trained in two identical apparatuses (A and B) located in one experimental room. The reason for using two apparatuses was to shorten the time necessary for performing the experiment as two rats were trained simultaneously. Each apparatus consisted of an operant chamber, a feeder, an 19"-LCD monitor and a computer (Fig. 1A). The operant chamber (length  $\times$  width  $\times$  height: 24 cm  $\times$  14 cm  $\times$  36 cm) had opaque walls. The front wall was only 4 cm high allowing direct view on the monitor located 37 cm in front of the chamber. The operant chamber and the monitor were standing on edges of two 75 cm high pedestals. This prevented rats from escaping over the front wall. The operant chamber was equipped with a horizontal lever (2.5 cm  $\times$  2.5 cm large, protruding from the left wall 14 cm above the floor, 4.5 cm from the front wall) and with a semicircular hopper (4 cm diameter, located on the right wall 5.5 cm above the floor, 4 cm from the front wall). If activated, the feeder delivered one to three 20 mg-pasta pellets to the hopper. The computer registered lever presses, activated feeder and displayed graphics on the monitor screen. The software was written by the authors in Quick Basic 7 and used 640 pxl  $\times$  480 pxl resolution for the graphical output.

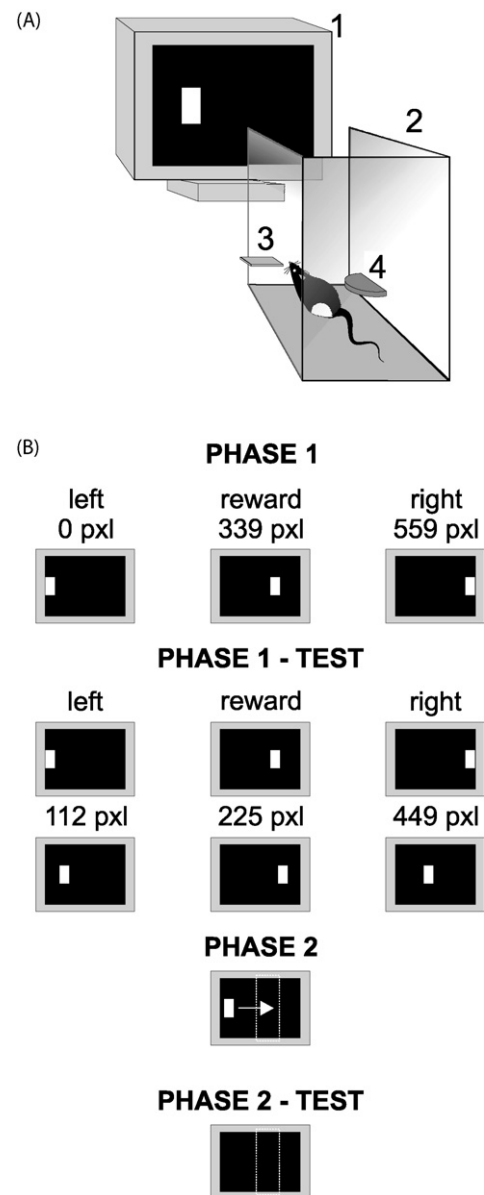
### 2.3. Behavioral training

#### 2.3.1. Pretraining

Food-deprived rats were first habituated to the rat chamber and then trained to press the lever for food under continuous reinforcement schedule. The rats required from three to seven sessions lasting approximately 30 min to learn the operant behavior. During the training a white rectangle (width  $\times$  height: 80 pxl  $\times$  150 pxl) was displayed in position 339 pxl which was approximately in 3/5 of the screen from the left side. We call this position "reward position". Each rat was trained in only one apparatus during the whole experiment (Pretraining, Phase 1 and Phase 2). The rats were assigned to the apparatuses randomly.

#### 2.3.2. Phase 1

Rats were trained to discriminate the reward position (339 pxl) of the object on the screen from two other positions: left (0 pxl) and right (559 pxl) (Fig. 1B, Phase 1). The rats were rewarded only if they pressed the lever when the object was in the reward position. At the beginning of a session the object was displayed in the reward position. The session started after a rat pressed the lever. Since this moment the object changed its position every 135 s in a pseudorandom order. The sequence was: Rew, L, R, L, Rew, R, L, Rew, R, L, R, Rew, L, R, Rew, L for the apparatus A and Rew, L, Rew, R, L, Rew, R, L, Rew, R, L, Rew, L, R, Rew, L, R for the apparatus B, where Rew denotes the reward position, L the left position and R the right position. These sequences repeated three times during the session. We used different sequences for each apparatus to prevent possible synchronization of the reward periods between the apparatuses. If the reward periods were synchronized then a rat in one apparatus



**Fig. 1.** (A) Experimental apparatus: 19"-LCD monitor (1), operant chamber (2) with lever (3) and hopper where food pellets were delivered (4). (B) Stimuli presented on the computer screen in Phase 1 and in Phase 2 including stimuli presented in the test sessions in both phases.

could detect reward periods by hearing the sound of activated feeder from the other apparatus.

The rats were rewarded for each correct response in the beginning of the training. This continuous reinforcement schedule was changed to variable ratio schedule after the rats had started to preferentially respond during the reward periods. A subject should emit several responses to get a single reward and this number changed randomly after each reward. The average number of responses necessary for activating the feeder gradually increased during the training. Individual rats reached different values. They were between 2.5 and 5.5.

After the rats reached asymptotic performance we carried out a test session. In the test session the object was presented in six positions: in three familiar positions (0 pxl, 339 pxl and 559 pxl) and in three new positions (112 pxl, 225 pxl and 449 pxl) (Fig. 1B, Phase 1—Test). Each new position was presented nine times for 15 s (three times after each familiar position). Responses in the new positions were not rewarded. The test session was carried out four times. These sessions were interspersed among 41 standard sessions. Two test sessions were carried out shortly after the rats reached stable level of performance and two before the beginning of Phase 2.

#### 2.3.3. Phase 2

Twelve rats trained in Phase 1 continued in Phase 2. In Phase 2 the object moved continuously across the screen. It shuttled between the left and the right sides of

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