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Research report

Effects of scopolamine and L-NAME on rats' performance in the object location test

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Abstract

The object location task is a new procedure evaluating spatial memory abilities in the rat. The aim of the present study was to characterize this behavioural paradigm by pharmacologic means. For this purpose, the effects of the muscarinic receptor antagonist scopolamine and the inhibitor of the nitric oxide synthase L-NAME on object location were assessed in the rat. In a first study, object location was impaired when the delay condition of 60-min was utilized. Subsequently, pre-training administration of scopolamine (0.2 mg/kg but not 0.07 mg/kg) induced delay-dependent performance deficits in this test. These impairments seem to be centrally mediated since the peripheral muscarinic receptor antagonist methylscopolamine (0.2 mg/kg) did not affect object location under the same conditions. Finally, pre-training treatment with L-NAME (30 mg/kg but not 10 mg/kg) also induced delay-dependent performance deficits in the object location task. These results indicate that the object location test is sensitive to pharmacological treatment and could be used for assessing the therapeutic potential of promnesic compounds.

Keywords: Spatial memory; Object location; Scopolamine; L-NAME; Rat

1. Introduction

Object recognition is a non-spatial working memory task, does not involve at all, the learning of a rule since it is based on the spontaneous exploratory behaviour of rats towards objects [8]. The standard form of this test involves exposing a rat to two identical copies of an object (sample trial) for 2–3 min. After a delay, the rat is then exposed to a novel object and an identical copy of the familiar object (choice trial). Successful recognition is displayed by the rat spending a greater amount of time exploring the novel object during the choice trial.

The same authors developed a novel version of this procedure, named object location test, aiming to evaluate spatial working memory in rodents [9]. Spatial memory is the ability of an organism to acquire a cognitive representation of location in space and the ability to effectively navigate the environment [1]. During the sample trial of this new paradigm, similarly to object recognition task, rats are exposed to two identical objects. After a certain delay, animals are re-exposed to the same two objects, one of which has been displaced to a new location within the apparatus. This task assesses the ability to discriminate the novelty of the object location, but not the object itself and the test arena already familiar to the animal [9]. Successful recognition is displayed by the rat spending a greater amount of time exploring the object in the new location during the choice trial.

Spatial memory tasks use positive or negative reinforcers, such as food (radial arm maze test) or water immersions (water maze task). Object location test lacks of a reinforcer and of learning rules. It is entirely based on the spontaneous exploratory activity of the rat and thus, can be considered as a "pure" working memory test, completely free of reference memory component which is present in other spatial memory tasks [9]. The strong involvement of the aforementioned reinforcers in spatial tasks used in animals is probably one of the causes of difficulty in reproducing experimentally the amnesic syndromes, since human learning and memory capacities are not usually tested under strong reinforcers [9].

At the moment, there is no experimental evidence whether or not object location is sensitive to pharmacological manipulation in the rat. The aim of the present study was to assess the sensitivity of this spatial memory task to effects of pharmacological treatment. For this purpose, the effects of the muscarinic receptor

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antagonist scopolamine and the NO synthase (NOS) inhibitor L-NAME on spatial recognition memory were evaluated in the object location test. It is well known that cholinergic system and nitric oxide (NO) play a consistent role in cognition [3,13]. Reciprocally, behavioural investigations have demonstrated that scopolamine and L-NAME disrupted rodents' performance in memory tasks including object recognition [2,9–12].

2. Materials and methods

2.1. Animals

Male, 3-month-old Wistar rats (Hellenic Pasteur Institute, Athens, Greece) weighing 250–300 g were used in this study. The animals were housed in Makrolon cages (45 cm long × 35 cm high × 20 cm wide) three per cage, in a regulated environment (21 ± 1 °C; 50–55% relative humidity; 12-h light/dark cycle, lights on at 07:00 h) with free access to food and water. Experiments were conducted in the room where only these animals were housed, and took place between 09:00 h and 13:00 h. Behavioural observations and evaluations were performed by an experimenter who was unaware of the pharmacological treatment.

Procedures involving animals and their care were conducted in conformity with the international guidelines, in compliance with National and International laws and policies (EEC Council Directive 86/609, JL 358, 1, 12 December, 1987; *NIH Guide for Care and Use of Laboratory Animals*, NIH publication no. 85–23, 1985).

2.2. Behaviour

2.2.1. Object location test

The test apparatus consisted of an open box made of Plexiglas (80 cm long × 50 cm high × 60 cm wide) which was illuminated by a 60 W lamp suspended 60 cm above the box. In the different parts of the apparatus the light intensity was equal. The apparatus was located in a large observation room with external cues (large and distinctive objects) surrounding the experimental arena to help rats to resolve this spatial memory task. These cues were kept in a constant location throughout the period of testing.

The objects were in three different shapes: cubes, pyramids and cylinders 7 cm high; they could not be displaced by rats. The cubes were from metal, the pyramids were from glass and the cylinders were plastic.

The object location test was performed as described elsewhere [9]. This test consisted of a period of habituation, a sample trial and a choice trial. During habituation, the animals were allowed to freely explore the apparatus without objects for 2 min, once a day (10:00 h) for three consecutive days before testing. On the testing day, a session of two 2-min trials was given. During the "sample" trial (T1), two identical samples (objects) (e.g., two plastic cylinders) were placed in two opposite corners of the apparatus 10 cm from the sidewall. A rat was placed in the middle of the apparatus and was left to explore these two identical objects. After T1, the rat was put back in its home cage and an intertrial interval (ITI) was given. Subsequently, the "choice" trial (T2) was conducted. The rat was re-introduced to the apparatus. During T2, one of the two similar objects was moved to a different location (new location, NL) while the other object remained in the same position (familiar location, FL) as in the T1. All locations of objects were used in a balanced manner to reduce potential biases due to preferences for particular locations. To avoid the presence of olfactory trails, the apparatus and the objects after each trial were thoroughly cleaned.

Exploration was defined as follows: directing the nose toward the object at a distance of no more than 2 cm and/or touching the object with the nose. Turning around or sitting on the object was not considered as exploratory behaviour. The times spent by rats in exploring each object during T1 and T2 were recorded manually by using a stopwatch. From this measure, a series of variables was then calculated: the total time spent in exploring the two identical objects in T1 and that spent in exploring the two objects in the two different locations (FL and NL) in T2. The discrimination between FL and NL during T2 was measured by comparing the time spent in exploring the object in FL with that spent in exploring the object in NL. As this time may be biased by differences in overall levels of exploration [5] a discrimination index (D) was then calculated; $D = \mathrm{NL} - \mathrm{FL}/\mathrm{NL} + \mathrm{FL}$. D is the discrimination ratio and represents the difference in exploration time expressed as a proportion of the total time spent exploring the two objects in T2 [5].

2.3. Drugs

Scopolamine HBr and methylscopolamine HBr (Sigma, St. Louis, MO, U.S.A.) were dissolved in saline (NaCl, 0.9%) and injected subcutaneously (s.c.). L-NAME (N^{ω}-nitro-L-argininemethylester) (Sigma, St. Louis, MO, U.S.A.) was dissolved in saline and injected intraperitoneally (i.p.). Control animals received the vehicle (NaCl, 0.9%).

2.4. Experiment 1: effects of different ITIs on object location memory

The aim of this study was to evaluate at which ITI (5 min, 20 min or 60 min) spatial recognition memory is extinguished in the 3-month-old rat. Rats were randomly divided into three experimental groups (eight rats per group) as follows: 5 min; 20 min; and 60 min.

2.5. Experiment 2: effects of scopolamine on the object location task tested at different ITIs

The aim of the study was to assess whether or not and at which ITI (5-min or 20-min) scopolamine affected acquisition of the object location test. Rats were randomly divided into six experimental groups (10 rats per group) as follows: vehicle-5 min; vehicle-20 min; scopolamine 0.07 mg/kg-5 min; scopolamine 0.07 mg/kg-20 min; scopolamine 0.2 mg/kg-5 min and scopolamine 0.2 mg/kg-20 min. Control rats were given s.c., the vehicle 60 min before starting T1. Scopolamine was injected 60 min before T1.

2.6. Experiment 3: effects of methylscopolamine on the object location task

The aim of the study was to investigate whether or not the performance deficits produced by scopolamine in the object location test were centrally mediated. For this purpose, the effects of the peripheral muscarinic receptor antagonist methylscopolamine on the object location were evaluated at the same conditions (dose and ITI) at which scopolamine impaired animals' performance in this task. Rats were randomly divided into three experimental groups (eight rats per group) as follows: vehicle; scopolamine 0.2 mg/kg and methylscopolamine 0.2 mg/kg. For this experiment, the 20-min ITI has been selected. Control rats were given s.c., the vehicle 60 min before starting T1. Scopolamine and methylscopolamine were injected 60 min before T1.

2.7. Experiment 4: effects of L-NAME on the object location task tested at different ITIs

The aim of the study was to investigate whether or not and at which ITI (5-min or 20-min) L-NAME affected acquisition of the object location test. Rats were randomly divided into six experimental groups (10 rats per group) as follows: vehicle-5 min; vehicle-20 min; L-NAME 10 mg/kg-5 min; L-NAME 10 mg/kg-20 min; L-NAME 30 mg/kg-5 min and L-NAME 30 mg/kg-20 min. Control rats were given i.p., the vehicle 60 min before starting T1. L-NAME was administered 60 min before T1.

2.8. Statistical analyses

Data are expressed as mean ± S.E.M. In experiments 1 and 3, total exploration times during T1 and T2 were evaluated by the two-way analysis of variance (ANOVA) test with a split-plot design (between-within subjects). Post hoc comparisons were made using the Duncan's test. For experiment 1, the factors were delay and trials. For experiment 3, the factors were treatment and trials. Discrimination index D data were assessed using the one-way ANOVA test followed by the Duncan's post hoc test. For the experiment 1, the factor was delay and for the experiment 3, the factor was treatment.