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Research report

Standard object recognition memory and "what" and "where" components: Improvement by post-training epinephrine in highly habituated rats

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ABSTRACT

The present work examined whether post-training systemic epinephrine (EPI) is able to modulate short-term (3 h) and long-term (24 h and 48 h) memory of standard object recognition, as well as long-term (24 h) memory of separate "what" (object identity) and "where" (object location) components of object recognition. Although object recognition training is associated to low arousal levels, all the animals received habituation to the training box in order to further reduce emotional arousal. Post-training EPI improved long-term (24 h and 48 h), but not short-term (3 h), memory in the standard object recognition task, as well as 24 h memory for both object identity and object location. These data indicate that post-training epinephrine: (1) facilitates long-term memory for standard object recognition; (2) exerts separate facilitatory effects on "what" (object identity) and "where" (object location) components of object recognition; and (3) is capable of improving memory for a low arousing task even in highly habituated rats.

1. Introduction

There is extensive evidence that emotional experiences tend to be remembered better than neutral ones. This effect is due, at least in part, to the endogenous release of stress-related hormones, such as epinephrine (EPI). Animal research has shown that post-training administration of EPI can improve memory for several learning tasks in a dose-dependent manner [6–8,20–23,25,42]. Not only does EPI enhance memory, but it can also accelerate the process of acquisition in tasks that require multiple trials [36]. The effects on memory of post-training manipulations of EPI levels (both pharmacologically or behaviourally, for example by presentation of arousal-enhancing material or by inducing muscle tension), as well as the effect of blocking β-adrenergic receptors, have also been found in humans [3,4,26,33,35,45]. EPI appears to modulate memory via several brain mechanisms, especially by noradrenergic activation of the basolateral amygdala [27].

While there is a large body of evidence that EPI modulates memory for highly arousing aversively motivated tasks, the influence of this hormone on memory for non-arousing or at least low arousing tasks is less clear. Object recognition memory based on spontaneous exploration is one memory task that seems devoid of highly aversive stimuli, since it does not require food or water deprivation and is not aversively motivated. To our knowledge, there is only one work that has examined the effect of post-training EPI on novel object recognition memory. Specifically, Dornelles et al. [12] found that post-training EPI improved object recognition memory under certain conditions, an effect that was prevented by pre-training systemic administration of the β-adrenoceptor antagonist propranolol. There are also reports of improved object recognition memory by post-training infusion of norepinephrine into the basolateral amygdala [41], and by post-training glucose [29]. Taking into account that peripheral EPI may induce its memory modulating effects mainly by increasing noradrenergic activity in the basolateral amygdala, and that glucose release may contribute to the memory-enhancing effects of EPI [19], the latter data give additional support to the view that EPI may modulate recognition memory.

The procedures used to test object recognition memory based on the spontaneous tendency of rodents to explore novel stimuli can be modified in order to examine different components of this kind of memory. In the test trial of the standard procedures of novel object recognition memory tasks [14], both the novel and the familiar objects are placed in one of the two positions occupied by the copies of the familiar object during the training trial (and never in different positions). Thus, the discrimination between novel and familiar objects may be based on the features of the individual objects combined with those of their position in the box. In

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contrast, when both the novel and the familiar objects are placed in unfamiliar positions, object recognition memory must be based solely on the specific features of the object itself (object identity memory or "what" condition). To test the influence of position in object recognition procedures, two copies of the familiar object can be used during the test trial, one of them placed in a familiar position, and the other one in a novel position (object location memory, or "where" condition) [11]. The neural substrates involved in object recognition memory may be different depending on the specific procedures used. Thus, while the perirhinal cortex appears to play a crucial role in standard object recognition memory, the hippocampus and related structures seem to be mainly required when spatial or contextual cues are relevant during the encoding of the object information (as in object location procedures), but there are also evidences of an involvement of the hippocampus in standard object recognition under certain circumstances [1,10,47].

The modulatory effects of EPI on memory seem to be more marked in the long-term than at shorter intervals, especially when a single training session is carried out. For example, post-training EPI improved the retention of two-way active avoidance conditioning when tested 20 or 45 days later, but not when the retention test was carried out 24 h or 11 days after the acquisition session [7,43,44]. Using the standard object recognition memory procedures, Dornelles et al. [12] found that EPI enhanced long-term (96 h) memory in rats submitted to repeated memory tests. However, the use of repeated tests implies that the amount of exposure to the familiar object is increased, and this may modify the mnesic gradient. Therefore, the influence of post-training EPI on the mnesic gradient of standard object recognition memory tasks after a single exposure to the familiar object remains to be examined.

In view of the former considerations, the present work was aimed at determining whether post-training systemic administration of EPI can improve memory for low arousing novel object recognition, as well as for the separate "what" and "where" elements of this kind of memory, indicating an influence of this hormone on the separate components that jointly shape episodic-like memories. A second aim of this work was to examine whether the effects of post-training EPI on novel object recognition are more marked in tests of long-term memory than in short-term memory tests. Since the usual duration of memory in untreated rats in this task ranges from several minutes to several hours, the effects of EPI on recognition memory were measured 3 h, 24 h, and 48 h after the training trial. To minimize the degree of emotional arousal induced by training, all the rats were submitted to several sessions of habituation to the training box.

To achieve the stated aims, five experiments were carried out. Experiments 1, 2, and 3 tested whether post-training systemic EPI is capable of improving spontaneous object recognition memory in its standard form at different retention delays: 3 h (Experiment 1), 24 h (Experiment 2), and 48 h (Experiment 3). Experiments 4 and 5 tested whether post-training EPI is capable of improving two different components of recognition memory: memory of the object itself, i.e. whether the animals distinguished a new from a familiar object when both objects were placed in locations not occupied during training ("what" condition; Experiment 4); and memory for the location of familiar objects, i.e. whether the animals recognised that an object was moved to a new location ("where" condition; Experiment 5).

2. Materials and methods

All the experiments were carried out with male Wistar rats obtained from our laboratory breeding stock. The number of animals used in each experiment, as well as their mean age and weight at the beginning of the experiment, were the following: Experiment 1 ($n = 16$; mean weight 437.60 g, SE 9.85; mean age 98.00 days, SE 0.53); Experiment 2 ($n = 20$; mean weight 449.22 g, SE 7.85; mean age 97.17 days, SE 0.81); Experiment 3 ($n = 20$; mean weight 443 g, SE 9.13; mean age 96.37 days, SE 0.93); Experiment 4 ($n = 18$; mean weight 437.89 g, SE 10.62; mean age 99.11 days, SE 0.70); Experiment 5 ($n = 16$; mean weight 427.54 g, SE 9.09; mean age 92.69 days, SE 1.00). All rats were singly housed in standard plastic-bottomed cages with sawdust bedding, kept under conditions of controlled temperature (20–22 °C) and humidity (40–70%), and maintained on a 12-h light–dark cycle (lights on at 8:00 a.m.). Experiments were performed during the first half of the light phase of the cycle. Rat-chow pellets (Panlab S.L, A04) and water were provided *ad libitum*. All procedures were carried out in compliance with the European Community Council directive for care and use of laboratory animals (86/609/EEC) and with the related directive of the Autonomous Government of Catalonia (DOGC 2073 10/7/1995).

2.1. Apparatus

The apparatus consisted of an open box (50 cm width × 50 cm length × 35 cm height) made of chipboard covered with dark brown melamine. A 12 cm diameter white disk was placed hanging from the upper edge of one wall of the box, midway between the two adjacent corners, to facilitate spatial orientation. The open box was enclosed in a sound-attenuating cage (72 cm width × 72 cm length × 157 cm height) made from white melamine and ventilated by an extractor fan. The illumination on the floor of the box apparatus was 50–60 lux. Objects varying in shape, colour, and size were constructed from Lego. Since the objects were made of the same material, they could not be distinguished by olfactory cues. The objects were weighted so that the animals could not move them around the arena. They were not known to have any ethological significance for the rats and they had never been paired with a reinforcer. All behavioural sessions were recorded by a video camera (Canon MVX10i). Tapes were analysed off-line by an observer who was unaware of the treatment conditions.

2.2. Procedures

2.2.1. Handling and habituation

The animals were handled for approximately 5 min on the two consecutive days after being housed singly. Two days after the last handling session, the rats received three sessions of habituation to the experimental apparatus on two consecutive days (two sessions the first day, 2 h delay, one session the second day). Each habituation session consisted of 12 min of exploration in the absence of objects.

2.2.2. Neophobia test

Two hours after the last habituation session, a test for measuring anxiety induced by the presentation of novel objects (similar to that described in [16] was conducted (neophobia test)). An unfamiliar object was exposed in the centre of the open box. The animals were placed in the box facing away from the object and allowed to explore it for 5 min. Throughout the experiment, exploration of an object was defined as directing the nose towards the object at a distance of ≤2 cm or touching it with the nose. Turning around or sitting on the object was not considered as exploratory behaviour.

2.2.3. Recognition memory task: training trial

Object recognition training began 24 h after the neophobia test. In the training trial, the objects were placed as shown in Fig. 1, at 3 cm from the surrounding walls. For Experiments 1, 2, 3 and 4, the objects were placed in the corner of the wall with the white disk, while in Experiment 5 they were located in the corners of the wall adjacent to the one with the white disk. The rat was placed in the experimental apparatus facing the centre of the wall opposite the objects, and was allowed to explore for 15 min. The time spent exploring each object was recorded. To avoid the presence of olfactory cues, the apparatus and objects were thoroughly cleaned with a vinegar solution (20%) and dried before the first rat and after each animal.

2.2.4. Recognition memory task: retention session

A retention session was carried out at 3 h (Experiment 1), 24 h (Experiment 2) or 48 h (Experiment 3) for the standard object recognition memory, and at 24 h for Experiment 4 ("what") and Experiment 5 ("where"). In the retention session, the objects were placed as shown in Fig. 1, at 3 cm from the surrounding walls. For Experiments 1, 2 and 3, the objects (one familiar and one novel) were placed in the same locations used during the training trial. In Experiment 4, the objects (one familiar and one novel) were located in the corners of the wall opposite the one with the white disk. In Experiment 5, the objects were identical to the ones used in the training trial, but one of them was moved to a new location. In all the experiments, the rat was placed in the experimental apparatus facing the centre of the wall opposite the objects, and was allowed to explore for 5 min. The time spent exploring each object was recorded. To analyse cognitive performance, a discrimination index was calculated as follows: (time exploring the new object or location – time exploring the familiar object or location) × 100/total time exploring both objects or locations. This kind of ratio makes it possible to adjust for any differences in total exploration time [30]. The tasks used in the present work are based on the natural tendency of rats to explore novelty, so that a ratio significantly higher than zero (i.e. animals