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Research report

Hippocampal-dependent memory in the plus-maze discriminative avoidance task: The role of spatial cues and CA1 activity

Anderson H.F.F. Leão[a], André M. Medeiros[c], Gênedy K.S. Apolinário[a], Alícia Cabral[a], Alessandra M. Ribeiro[d], Flávio F. Barbosa[b], Regina H. Silva[a,c,*]

[a] Memory Studies Laboratory, Department of Physiology, Universidade Federal do Rio Grande do Norte, Natal, RN, Brazil
[b] Memory and Cognition Studies Laboratory, Department of Psychology, Universidade Federal da Paraíba, João Pessoa, PB, Brazil
[c] Laboratory of Behavioral Neuroscience, Department of Pharmacology, Universidade Federal de São Paulo, São Paulo, SP, Brazil
[d] Laboratory of Neuroscience and Bioprospecting of Natural Products, Department of Biosciences, Universidade Federal de São Paulo, Santos, SP, Brazil

HIGHLIGHTS

- Distal and proximal cues are required to retrieve the discriminative avoidance task.
- Proximal cues are more salient than distal cues to spatial discrimination.
- Anxiolytic and amnestic effects of muscimol on CA1 are independent.
- Muscimol dose-dependently impaired the performance in the avoidance task.
- Limited CA1 activity is required to learn the discriminative avoidance task.

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ABSTRACT

The plus-maze discriminative avoidance task (PMDAT) has been used to investigate interactions between aversive memory and an anxiety-like response in rodents. Suitable performance in this task depends on the activity of the basolateral amygdala, similar to other aversive-based memory tasks. However, the role of spatial cues and hippocampal-dependent learning in the performance of PMDAT remains unknown. Here, we investigated the role of proximal and distal cues in the retrieval of this task. Animals tested under misplaced proximal cues had diminished performance, and animals tested under both misplaced proximal cues and absent distal cues could not discriminate the aversive arm. We also assessed the role of the dorsal hippocampus (CA1) in this aversive memory task. Temporary bilateral inactivation of dorsal CA1 was conducted with muscimol (0.05 μg, 0.1 μg, and 0.2 μg) prior to the training session. While the acquisition of the task was not altered, muscimol impaired the performance in the test session and reduced the anxiety-like response in the training session. We also performed a spreading analysis of a fluorophore-conjugated muscimol to confirm selective inhibition of CA1. In conclusion, both distal and proximal cues are required to retrieve the task, with the latter being more relevant to spatial orientation. Dorsal CA1 activity is also required for aversive memory formation in this task, and interfered with the anxiety-like response as well. Importantly, both effects were detected by different parameters in the same paradigm, endorsing the previous findings of independent assessment of aversive memory and anxiety-like behavior in the PMDAT. Taken together, these findings suggest that the PMDAT probably requires an integration of multiple systems for memory formation, resembling an episodic-like memory rather than a pure conditioning behavior. Furthermore, the concomitant and independent assessment of emotionality and memory in rodents is relevant to elucidate how these memory systems interact during aversive memory formation. Thus, the PMDAT can be useful for studying hippocampal-dependent memory when it involves emotional content.

Abbreviations: PMDAT, plus-maze discriminative avoidance task; EPM, elevated plus-maze; %TAV, percent time in aversive arm; %TOA, percent time in open arms; AV, aversive arm; CA1, Region 1 of hippocampus; CA3, Region 3 of hippocampus; DG, Dentate Gyrus; BLA, basolateral amygdala; GABA, gamma-aminobutyric acid.

* Corresponding author at: Departamento de Farmacologia- UNIFESP, Rua Botucatu, 862, Edifício Leal Prado, 1°.andar, São Paulo, SP, CEP 04023062 Brazil.
E-mail addresses: reginahsilva@gmail.com, r.silva@unifesp.br (R.H. Silva).

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1. Introduction

The plus-maze discriminative avoidance task (PMDAT) is a modified elevated plus-maze (EPM) paradigm that can assess aversive memory and anxiety-like behavior concomitantly [1]. In this task, one of the enclosed arms is paired with aversive stimuli (light and noise). In a 10-min training session, rats are placed in the apparatus facing the intercept between the open arms. Every time the animal enters the aversive enclosed arm, an aversive stimulation (100 W light, 1500 lux at the maze floor level; and an 80 dB white noise) is produced until the animal leaves the arm. Upon a second exposition to the maze (24 h later) the aversive stimuli is no longer presented, and the retention of the aversive memory is assessed based on the relative time spent in the non-aversive arm versus the previously aversive arm. Concurrently, anxiety-like behavior is computed based on the time spent in the open arms during the training session.

The PMDAT has been useful to investigate the effects of emotionality on the acquisition of an aversive memory. For instance, both anxiogenic (caffeine) and anxiolytic (chlordiazepoxide) manipulations impair performance in the task [1], suggesting that an optimal level of anxiety is required to perform the task [2]. The paradigm is also useful to dissociate amnestic effects from anxiety parameters, with amnestic [3,4] and memory-enhancing [5,6] manipulations decreasing and increasing, respectively, the avoidance response to the aversive arm. In addition, the PMDAT also allows for the evaluation of motor activity through the measurement of the total distance travelled in the apparatus.

The cognitive map theory proposed by Tolman [7] states that spatial discrimination and recognition rely on the relative configuration of spatial cues. Thus, to succeed, the rat most likely uses a map-like representation of the positions of cues in the environment. Further investigation on the relevance of distal and proximal cues (performed on dry and wet mazes) indicated that distal cues have a predominant role in setting the animal's orientation in the apparatus frame of reference and in providing a distinction between spatially similar locations [8]. That is, once the apparatus frame of reference is established by distal cues orientation, animals can navigate using interoceptive and proximal cues.

As a maze-based learning paradigm, and in spite of the engagement of aversively motivated learning, the PMDAT rationale postulates that spatial cues are important to navigate in the apparatus and acquire a spatial configuration. Nevertheless, the relevance of such cues has never been investigated in the PMDAT paradigm. For this reason, we designed an experiment to test the relevance of proximal (light bulb and speaker in the aversive arm) and distal (posters and window in the wall) cues to retrieve an aversive memory in the PMDAT.

Furthermore, O'Keefe and Dostrovsky [9] first described a neurobiological correlation for Tolman's cognitive maps, demonstrating that hippocampal place cells fired selectively to the exploration of defined areas in a maze. Later, studies revealed that these cells fired according to the configuration of distal cues, changing its place field relative to distal cue rotation [10,11]. Nonetheless, studies employing more salient intra-maze proximal cues or proximal and distal cues with equivalent salience suggest that these cues can exert a strong control over place cell firing rate. These studies report split place field ensembles, with populations of place cells firing to local and distal cues, or ambiguously to both types of cues [12–15]. Thus, both proximal and distal cues can exert influence over the hippocampal place cell firing rate.

Previously, we have shown that the basolateral amygdala (BLA) is a key structure for aversive memory formation in the PMDAT [16]. When administered into the BLA before training, muscimol and anandamide impaired aversive memory in the task, but did not affect anxiety [16,17]. BLA activity has also been implicated in modulating aversive memory storage in other brain regions, such as the hippocampus [18]. This observation is true for aversive conditioning tasks [19,20] such as contextual fear conditioning [21,22]. Nonetheless, whether the hippocampus is required to encode aversive memory in the PMDAT is not clear. Based on the current rationale for spatial navigation in maze-based tasks, we postulate that the dorsal hippocampus is required for spatial configuration and aversive memory acquisition in the PMDAT. To address whether the PMDAT is a hippocampus-dependent paradigm, we temporarily inactivated the dorsal hippocampus before a training session with muscimol, a reversible $GABA_A$ agonist. Addressing these issues is relevant for further characterization of the PMDAT paradigm and will support future investigations of anxiety and learned fear-related behaviors, as well as their involvement with hippocampus function in aversive memory acquisition.

2. Materials and methods

2.1. Animals

Three-month-old male Wistar rats (300–350 g) were housed in cages (45 × 35 × 15 cm; 4–5 animals per cage) with water and food *ad libitum*, under controlled conditions of temperature (23–24 °C) and light/dark cycle (12–12 h, lights on 6:30 am). The rats were handled according to the Brazilian law for the use of animals in scientific research (Law Number 11.794) and all procedures described received the approval of the local ethical committee (#047-2012). Animals were handled 10 min/day for 3 days before behavioral testing.

2.2. Surgery and histology

The rats were anaesthetized with intramuscular ketamine (100 mg/kg) and xylazine (10 mg/kg). Next, the animals were positioned in a stereotaxic apparatus (Insight, Brazil), and the skull was exposed. Stainless guide cannulas (25 gauge, 12 mm) were implanted bilaterally, and aimed at the dorsal CA1 hippocampal subregion (CA1: AP −3.4 mm from bregma; ML ± 1.9 mm; and DV −1.9 mm) according to the Paxinos and Watson atlas [23]. The guide cannula tips were placed 1 mm above the injection site. The guide cannulas were anchored to the skull with steel screws and dental acrylic. At the end of the surgery, each cannula was temporarily sealed with a stainless steel wire to protect it from obstruction. After surgery, the animals received anti-inflammatory (diclofenac sodium 75 mg/ml, i.m.) and antibiotic (penicillin 60.000 UI/ml, i.m) treatment. The animals were given 7 days for post-operative recovery prior to the start of the behavioral procedures.

After the experiments, the rats were deeply anaesthetized with 1 ml of thiopental sodium (25 mg/ml) and perfused intracardially with 0.9% saline followed by a 10% formol-saline solution. In experiment III, fluorophore-conjugated muscimol (0.2 μl) was injected into the guide cannulas 15 min before perfusion to assess muscimol diffusion. The brains were removed and stored in 10% formol-saline for 4 h, and placed in 30% sucrose at 4 °C for at least 72 h before being frozen and sectioned to a 50 μm thickness using a cryostat (Leica, Germany). The sections were mounted onto glass slides, stained with DAPI (1 mg/ml diluted in PBS IX 10 mM, pH 7.4, at a proportion of 1:1000), and mounted in Aqua Polymount (Polysciences Inc., USA). The slides were examined under fluorescence microscopy to verify of the exact placement of the cannula, infusion needle tip and the spreading of the fluorophore-conjugated muscimol. Only data from animals with correct cannulae placement were computed for statistical analysis. To analyze muscimol spreading, images that centered in the tip of the injection site were acquired and averaged