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Behavioural Brain Research 167 (2006) 156–164
BEHAVIOURAL BRAIN RESEARCH
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Research report

Supramammillary and adjacent nuclei lesions impair spatial working memory and induce anxiolitic-like behavior

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Received 18 February 2005; received in revised form 31 August 2005; accepted 5 September 2005
Available online 19 October 2005

Abstract

The present study assesses the involvement of the supramammillary and adjacent nuclei in spatial memory and anxiety-like behaviors. Rats with electrolytic lesions in the supramammillary nucleus were pre- and post-operatively trained in two spatial memory tasks and two anxiety tasks. Spatial memory tasks were performed in an open field with seven different goal positions containing the reward. Anxiety-like behaviors were tested in the elevated T-maze. In the spatial reference memory task, neither lesioned nor sham-lesioned groups were impaired. In the working memory task, lesioned animals were permanently impaired in their ability to solve the delayed-matching-to-position task. This working memory deficit is not related to increased proactive interference. It could be related to impairment of the rats ability to reorganize spatial stimuli. Consequently, rats were not able to achieve an optimal performance level to solve spatial tasks with continuous changes in the place location. In the elevated T-maze, lesioned rats reduced passive avoidance response but no changes in the escape response were observed. These results suggest a clear involvement of the supramammillary nucleus in working memory and behavioral inhibition but not in either spatial reference memory or in escape responses.

Keywords: Spatial learning; Mammillary region; Electrolytic lesion; Sprague–Dawley rats

1. Introduction

Previous studies have shown the relevance of the mammillary region (MR) in both memory and emotion [2,12,29].

The mammillary region is located around the mammillary bodies, at the caudal end of the hypothalamus, and comprised of several adjacent nuclei and fibre systems [28]. The main nuclei of this hypothalamic region are the mammillary bodies, premammillary nuclei, supramammillary and ventral tegmental nuclei [27,28]. The principal fibre systems included in the MR are the fornix, mammillary peduncle, mammillothalamic and mammillotegmental tracts [5,6,11,19].

The mammillary bodies (MB) have been mainly associated to learning and memory. In this sense, it has been shown that MB lesions impair memory functions in both animals and humans [32]. Most behavioral studies about the effects of MB lesions in memory functions have involved other MR or adjacent nuclei. The supramammillary nucleus (SuM) is located dorsally to the MB and has been included in the majority of these lesion studies [28,29].

To date, few experiments have studied directly the SuM involvement in memory, independently of MB. Increased c-Fos immunoreactivity in SuM has also been related to spatial memory but no clear relationship was found between SuM and spatial working memory in the study of Santín et al. [22]. Vann et al. also reported an increase of c-Fos immunoreactivity in SuM neuronal nuclei related to radial-arm maze training [33,34]. Notwithstanding, chlordiazepoxide microinfusions in SuM and adjacent regions were found to reduce theta hippocampal frequency and impair spatial memory. However, the spatial memory deficit was very small and was not observed when microinfusions were introduced in the hypothalamic nuclei located 500 μm outside the SuM [16,17,38,39]. More recently, Shahidi et al. have reported a clear involvement of SuM in spatial memory [25].

The role of MR in emotion has been less studied. Moreover, several studies have shown the importance of some MR nuclei such as MMn and SuM in several behaviors related to emotion [16,37]. In general, it has been suggested that anxiety-

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doi:10.1016/j.bbr.2005.09.002

like behaviors decrease in animals with MR lesions and this effect has been reported in both conditioned and spontaneous responses. To our knowledge, Pan and McNaughton research has been the first to directly study the role of SuM and surrounding nuclei in emotional behavior [16]. In this sense, SuM lesion induces anxiolytic-like behaviors such as decreased contextual fear evaluated in the conditioning chamber and an increase in both ambulation in the open field and behavioral inhibition in the operant chamber. These behavioral results have led Pan and McNaughton to suggest that SuM (and adjacent nuclei) would be more involved in emotional behaviors than in cognitive processes [16].

The aim of our study is to clarify the role of the SuM and surrounding nuclei in spatial memory and emotional behavior. This was assessed avoiding the concommitant MMn and MLn involvement in these psychological processes because the lesions did not reach these ventral MR nuclei. Behavioral experiments were designed to study the performance of the rats in two spatial memory tasks and two anxiety-like behaviors pre- and post-surgery.

2. Material and methods

2.1. Animals

Twenty-four male Sprague–Dawley rats (280–320 g) were obtained from Criffa (Barcelona, Spain). All rats were maintained in groups of four at a temperature of $22 \pm 2\,^{\circ}$C and on a constant light–dark cycle (08:00–20:00 h). Water was available ad libitum. Food was available ad libitum at the beginning of the experiments. A week before the behavioral assessment, the food was restricted to achieve a 20% weight loss. During the restriction process, pieces of biscuit were mixed in with the food to accustom animals to the reward that would later be used in the behavioral study. The care and use of the animals were in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC), and were conducted with approval of the Animal Care and Use Committee of the University of Malaga.

2.2. Surgery

The day after finishing the pre-surgery behavioral tasks, the animals were assigned randomly to one of two groups: supramammillary and adjacent (SuM-ADJ) nuclei lesioned group ($n = 14$) and sham-lesioned group ($n = 10$). During surgery one animal died from each group.

The electrolytic lesions were done stereotaxically (Kopf, USA) under anaesthesia equitexin (3 ml/kg). The coordinates were AP −4.6 mm, DV −8 mm and at midline from the skull [18] with bregma and lambda at the same level. A 2 mA direct current was applied for 10 s. The lesion was made by passing a direct current through an electrode that had a non-insulated end 1 mm in length. Sham-lesioned rats were operated but were not administered the electrical discharge. Post-operative behavioral tests were carried out 10 days after surgery. Animals received paracetamol in the drinking water for 3 days after surgery.

2.3. Apparatus and behavioral procedures

2.3.1. Spatial memory

A 75 cm × 150 cm × 75 cm open field was used to train and test the rats in the memory tasks. In the open field, seven containers of equal size, shape and color were used. All of them contained sand and the reward was placed on top. The animals were introduced manually in the maze from seven different starting locations (around the edge of the maze). When the rat obtained the reward from one of the containers, it was manually removed from the maze and returned to its homecage.

All the animals were trained in the following behavioral tasks:

Habituation: All the animals explored the maze for 3 consecutive days. Each day, four trials were performed each of which lasted for 5 min. In the habituation, the animals acquired the reward from each of the seven containers in the maze. The habituation phase finished when each of the animals had eaten the reward from inside the seven containers.

Spatial reference memory task: Training was carried out for 4 consecutive days with 10 trials per day (both pre- and post-operatively). There was an intertrial interval of 5 min. The reward was always placed in the same container and the rats were both manually and randomly released in the maze, from one of the seven start positions. Solution of this task requires application of a reference memory rule since the information presented remains constant over the training period [9,23]. The time the animals took to reach the reward (escape latencies) and the containers they visited before reaching the reward (errors) were recorded.

Spatial working memory task: Training in the spatial working memory task was carried out on 3 consecutive days. The task is based on the delayed-matching-to-position paradigm (DMTP). This task requires the use of working memory and the information available to solve the task is only valid for one trial [9,23]. Every day, 10 training sessions were carried out with a 5 min interval between sessions. Each session was comprised of two trials: an acquisition trial and a retention trial with an intertrial interval of 30 s. During the acquisition trial, the start position and the location of the rewarded container were randomized. During the retention trial, the start position and the location of the rewarded container were the same as those used for the acquisition trial. This procedure was repeated over the 10 training sessions for the 3 days of the experiment.

The rats carried out these tasks using spatial cues in the experimental room. Previous data obtained in our laboratory, using this maze and the same spatial reference memory task, showed that when distal cues were changed by rearranging the experimental room, the errors increased (data not shown). These previous results, suggested that the rats were not able to use stereotyped behavior such as pattern of turns, to solve the spatial task. The escape latencies and the number of errors were recorded.

2.3.2. Anxiety-like behavior

An elevated T-maze was used to test the rats in the emotional tasks. The T-maze was made of wood and had three similar arms (50 cm × 10 cm). One arm was enclosed by walls 40 cm high and another two open arms were surrounded by a wooden rim 1 cm high. The apparatus was elevated 50 cm above the floor. Illumination was provided by a 60 W lamp located in the ceiling of the room. Environmental temperature was kept at $21 \pm 2\,^{\circ}$C and an air conditioner provided a background noise.

Passive avoidance and escape behaviors were assessed in the elevated T-maze. In the passive avoidance task, the rats are placed at the end of the enclosed arm and cannot see the open arm until they explore it. Exploration of the open arm is an aversive experience, because the rats show an innate fear of elevated, lighted and opened places. In a second training trial, the rats avoid the open arm and spend more time in the closed arm. In the escape task, the rats are placed at the end of one of the open arms. In this task, the animals escape from the open arm towards the closed arm [10].

The training in the passive avoidance task was divided into two trials, with an intertrial interval of 30 s. The rats were kept in the room for 30 min and handled by the researcher before the behavioral test. Briefly, in the passive avoidance task, the rats were placed in the bottom of the closed arm and the time spent to reach the open arm was recorded. The register finished when the rat was in the open arm with its four limbs. The register was cut short if the animal did not leave the closed arm in 120 s, and the rat was placed in its home cage. The escape behavior was studied 30 s after passive avoidance training. The experimenter registered the time spent by the animal to reach the closed arm from the end of the open arm (escape latencies).

2.4. Experimental design

Before surgery, all rats sequentially experienced the following tasks: habituation (days 1–3), spatial learning with reference memory demands (days 4–7), spatial learning with working memory demands (days 8–10) and anxiety tasks