Non-effects of mammillary body lesions on spontaneous alternation: pre and postoperative study

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Abstract

This study was designed to investigate the potential effects of medial mammillary nucleus (MMn) lesions on spontaneous alternation behavior in rats. Behavioral measurements were made in a water T maze in which the side arms were not differentially reinforced. Spontaneous alternation was tested before and after surgery alone. In both experiments, volumetric estimates of the MMn lesion were made by stereologic calculations. The results obtained do not support the direct participation of the MMn in retrograde or anterograde memory processes related to spontaneous spatial alternation. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

Several studies have shown that animals with mammillary body (MB) lesions present loss of memory for spatial localizations. These impairments have been found in post-lesion performance tasks such as spontaneous alternation (Beracochea and Jaffard, 1987), rewarded spatial alternation (Beracochea and Jaffard, 1990), delayed-non-matching-to-position (Beracochea et al., 1989) in mice, spatial delayed alternation in monkeys (Ridley and Vaker, 1991) and in the 8 arm radial maze in rats (Saravis et al., 1990; Sziklas and Petrides, 1993). However, the relationship between the MB and spatial memory is still not conclusive. Aggleton et al. (1991) did not observe any impairments in the acquisition of spatial tasks such as the delayed-non-matching-to-position in rats, or, when they did detect them, these were only mild (Aggleton et al. 1990).
The aim of this study was to assess the effect of electrolytic lesion of the MB on spontaneous spatial alternation. Spontaneous alternation in rats involves the use of memory (Douglas and Isaacson, 1975; Beracochea and Jaffard, 1985). Working memory processes are required for the animals to remember the T maze branch they have previously visited. This alternation could be understood to be a passive form of memory because it occurs in the absence of reinforcement (Beracochea and Jaffard, 1990). We designed a test with post-training lesions and another with pre-training lesions which enabled us to establish the differences between the effects of learning and performance (Vanderwof and Cain, 1994) by taking measurements before and after lesion induction. Studies of spontaneous alternation in the T maze are preferable to other kinds of tasks in that they do not require use of a reward (Beracochea and Jaffard, 1987; Santín et al., 1996) and also because the animals do not need to be subjected to privation processes that alter their physiological condition. In this work, since rats are semi-aquatic animals, spontaneous alternation was assessed in the water T (Vanderwof and Cain, 1994).

2. Materials and methods

2.1. Subjects and apparatus

For the post-training lesions experiment, 30 male Wistar rats (initial weight 280 ± 18.54 g) from the central vivarium of the University of Oviedo were used. For the pre-training lesions experiment, 37 male Wistar rats, with initial weights of 278 ± 15.3 g were used. All rats were donated by the central vivarium of the University of Oviedo. During the experiment, the animals were kept on a 12-h light/dark schedule (08:00–20:00 h) with free access to food and water.

Behavioral tests were performed in a water T maze made of aluminum with the following dimensions: width: 17.5 cm, height: 51 cm and arm length: 50 cm. The water was maintained at a constant temperature of 20 ± 1°C. The tasks were performed between 11:00 and 13:00 h.

2.2. Behavioral procedure

The rat was placed on the central arm of the maze. The trial was considered to have finished when the rat had reached the end of either the left or right arm of the maze. Immediately afterwards, the animal was returned to the cage for 5 s before the next trial began. Animals carried out five trials per day on 8 consecutive days. The maze arm chosen in each trial by each animal was recorded.

In the post-training lesions study, 24 h after the last trial, the animals were randomly assigned to one of three groups (group 1: submitted to induction of electrolytic lesions (n = 10), group 2: received a similar treatment to group 1 except without emission of electrical discharges (n = 10), group 3: animals were maintained under normal conditions (n = 10)). The post-operative period was 8 days for all animals. After this, the animals were again tested in the water T maze on 2 consecutive days following the same procedure as that described above. In the pre-training lesions’ experiment the animals were randomly allocated to one of the three treatment groups previously described (unoperated group (n = 14), sham-operated group (n = 10), group of animals lesioned in the MMn (n = 13)). A total of 8 days after surgery, all the animals were tested in the water T maze for 8 days and the spontaneous alternation behavior already described was recorded.

2.3. Surgery

For the surgical procedure the rats were anesthetized (Equithesin 3 ml/kg i.p.) and placed in a stereotaxic apparatus (Narishige, Japan) with bregma and lambda at the same level. The stereotaxic coordinates at which the lesion of the medial mammillary nuclei (MMn) was made, following Paxinos and Watson anatomical atlas (Paxinos and Watson, 1986), were: 4.8 mm posterior to the Bregma, on the midline and 9 mm below the skull. An electrical discharge with an intensity of 2 mA was administered for 10 s (Lesion generator: Cibertec. GL-2). After this the rats were intravenously administered benzathine penicillin G (Benzetacil. Antibióticos. LTD.) (3 mg/Kg i.m.).
2.4. Histology and stereology

At the end of the experiment, the animals were anesthetized by inhalation of ethyllic ether (Probus. Spain) and intravascularly perfused with buffered formaldehyde 10% (0.1 M and pH 7.4). Histological analyses were done on coronal sections embedded in paraffin and 10 μm thick sections were stained with toluidine blue. The lesion volume was estimated using Cavalieri’s principle (Gundersen and Jensen, 1987). Serial sections were made of the mammillary complex and five equidistant sections were chosen. The outline of the lesioned zone was sketched on the sections and the number of dots within the outline were counted with a dotted template. Then, an unbiased volumetric estimator was applied: \[ \text{EST}(v) = T \times \left[ \frac{1}{M^2} \right] \times \frac{[a/p]}{S_p} \] where: \( T \) is the mean distance between sections; \( M \) corresponds to the total magnification; \( [a/p] \) is the reference area of the points test; \( S_p \) corresponds to the total number of dots counted in one animal.

Only those animals in which the lesion of the MMn included over 70% of the structure were used for the behavioral analysis. Animals with smaller lesion volumes or lesions in adjacent structures were rejected.

2.5. Data treatment and statistics

In the post-training lesions study, the spontaneous alternation was calculated for each individual and the mean value was calculated for each group on each day. A 2-way ANOVA with one repeated measure was applied (groups × days) and post-hoc comparisons were done (Tukey’s honest significant difference [HSD] test) to analyze the evolution of the alternation over the 10 day period. The same ANOVA was applied to the escape latencies. Data analysis was applied a second time to determine the influence in each group of certain trials on subsequent trials. Thus, the alternation percentages were calculated individually between consecutive tests, on the last two preoperative days and the two postoperative days (i.e. the alternation percentages between trials 1 and 2, trials 2 and 3, trials 3 and 4 and trials 4 and 5, thus obtaining four rates of alternation on the two preoperative days). A 2-way ANOVA with one repeated measure was applied (groups × rates). The term alternation rate was used to refer to the mean of the alternation percentages between consecutive trials over a specific number of days, which was two in this case. In the pre-training lesions study, the alternation rates were analyzed in the first 2 days when the rats exhibited the highest alternation rates. The escape latencies and the spontaneous alternation were calculated as explained above. Similarly, a 2-way ANOVA with one repeated measure was applied to these parameters.

3. Results

3.1. Histological results

In the post-training lesions experiment, the volume of the MMn lesions ranged from a maximum of 94.59% to a minimum value of 73.1%. Fig. 1 shows a reconstruction of the lesion. In the pre-training experiment, the volume of the MMn lesion in the ten animals ranged from 100 to 70.9%. The maximum and minimum area of the lesion are recorded in Fig. 2.
3.2. Behavioral results

3.2.1. Post-training lesions experiment

There was no statistically significant difference between the alternation percentages of the three groups \( F_{(2, 18)} = 1.41052; P ≥ 0.269 \). Significant differences were found between the results obtained on the different days \( F_{(9, 162)} = 14.43; P ≤ 0.000 \). The post hoc comparisons (HSD) detected differences between mean values on the following days: D1 compared to all the other days, D2 compared to D6, D7, D8, D9 and D10, D3 compared to D6, D8 and D10, and D4 compared to D8; \( P ≤ 0.05 \) (Fig. 3). The escape latencies were not statistically different among the three groups, but differences between the latencies at 10 days \( F_{(9, 162)} = 9.639; P ≤ 0.000 \) were observed. Post hoc comparisons revealed statistically significant differences \( P ≤ 0.05 \) between the following days: D1/D3 and the following days; and D2/D4 and the following days (Fig. 3).

The 2-way ANOVA applied to the alternation rate did not detect any differences among the three groups \( P ≥ 0.118 \) or among the alternation rates \( P ≥ 0.94 \) or any interaction between the two variables \( P ≥ 0.845 \).

3.2.2. Pre-training lesions experiment

Analysis of the alternation did not reveal any statistically significant differences between the groups \( F_{(2, 30)} = 0.1301; P ≥ 0.878 \) although there were significant differences in these values on different days \( F_{(7, 210)} = 5.935; P ≤ 0.000 \). Post hoc comparisons (Stoline test) detected statistically significant differences \( P ≤ 0.05 \) between the following pairs of days: D1 compared to all the other days except D2; D2 compared to D6, D7 and D8 (Fig. 4). Analysis of the...
escape latencies did not reveal any differences among the three groups ($P \geq 0.104$), on the different days ($P \geq 0.084$) or with the interaction of both these variables ($P \geq 0.364$).

Analysis of the alternation rates did not reflect differences among the groups ($P \geq 0.936$), or among the alternation rates ($P \geq 0.676$) or of the interaction effect between the variables ($P \geq 0.193$).

4. Discussion

The results show that MMn lesions do not impair performance of this behavioral task. Neither the decrease in spontaneous alternation behavior nor the escape latencies were affected by induction of an electrolytic lesion in the MMn. This work supports previous findings which demonstrate little or no effect of brain lesions on consolidated behaviors (Vanderwof and Cain, 1994). This preservation of retrograde memory after MB lesions has been described in animals. For example, the retention of delayed spatial alternation in monkeys, with 5 s delay intervals (and other visual-spatial tasks) are not affected (Ridley and Vaker, 1991). Similarly, Thompson sustains that the MBs are not important for long-term retention of visual-spatial information acquired in rats (Thompson et al., 1964).

Moreover, we did not detect any proactive interference of the first trials on the following ones in any of the three groups. No differences were found in the alternation rates throughout the study. These results support those obtained by Beracochea and Jaffard (1987) who did not find an increased sensitivity to the proactive interference in animals with lesions in the MB in a study with short intertrial intervals (although there were some differences in the procedures used since Beracochea and Jaffard analyzed the alternation rates exhibited by animals in trials after perseveration).

The results of the experiment on pre-training lesions show that MB lesions are not impaired in post-operative records of spontaneous alternation, escape latencies or alternation rates. These data do not support other studies in which memory impairments were recorded in spatial tasks such as spontaneous and rewarded alternation in animals tested post-operatively (Beracochea and Jaffard, 1990). However, this impairment in task performance could depend on the intertrial interval such as these authors suggested (Beracochea and Jaffard, 1987, 1990). However, there are some differences between the experimental design used by these authors and that used in our study especially with regards to the quantification of alternation since Beracochea and Jaffard (1987) analyzed the effect the MB lesion has on alternation recorded after perseveration by the rats, while we determined alternation in a global sense.

The results of different studies on the involvement of MB in learning and memory tasks are variable. Lesion of this hypothalamic structure was found not to affect the acquisition of non-spatial recognition tasks and to exert a mild effect on the acquisition of reinforced spatial tasks (Aggleton et al., 1990). Our results also reflect that the MMn is not necessary for the normal functioning of some memory functions. In fact, research which used another kind of behavioral measurement (Aggleton et al., 1991) [delayed nonmatching-to-position] showed that acquisition becomes worse in rats with lesions in either the fimbria/fornix or the anterior thalamic nuclei while it is not altered after lesions in the MB.

On the other hand, the rats in the T maze not only use spatial strategies but can also display other kinds of strategies such as the response or cue strategy (Barnes, 1988). We used an opaque water T maze in which the animals use egocentric or response strategies. Neave et al. (1997) have recently emphasized that deficits induced by MB lesions are selective for tasks which involve the use of allocentric and not egocentric information. Our results support the findings of these authors and verify that animals with MB lesions can correctly perform tasks that require the use of egocentric information. Moreover, spontaneous alternation could be considered as a task that requires a passive form of memory. According to Beracochea and Jaffard (1990), this is an automatic task which requires forms of implicit memory. This could, at least partially, explain why no memory impairments were found, if, as many
authors suggest, the MB are critical when spatial information is required (Saravis et al., 1990; Sziklas et al., 1996).

However, this task requires the use of working memory (since the animals must remember which arm they visited in the previous trial in order to alternate). Our results suggest that the MMn lesion does not interfere with acquisition or retention of the information required to perform the tasks that involve implicit memory and working memory forms. Thus, the MMn do not participate in the implicit memory processes that require working memory processes, at least when the intertrial intervals are short. When the intervals between the trails are longer the task becomes more difficult because the rats have to retain the information in their working memory for a longer time. In this case, as many authors have suggested (Beracochea and Jaffard, 1987, 1990), the MB lesions may induce memory impairments.

On the other hand, Neave et al. (1997) demonstrated that lesion of the MB impairs the use of allocentric cues. However, the deficit found may not be exclusive to the use of allocentric information and as Sziklas et al. (1996) suggested, the MB are important in spatial working memory. Thus, closer analysis of the tasks used by Neave et al. (1997) reveal that these require working memory processes (rewarded alternation recorded in the T maze, alternation recorded in a cross-maze and radial maze). Thus, it could be suggested that the MB are important in working memory tasks when spatial information is processed with allocentric and not with egocentric cues (Neave et al., 1997). From this perspective, one can understand why the experiments by Sutherland and Rodriguez (1989) did not show a clear relationship between MB lesion and impaired place learning in the water maze. Sutherland and Rodriguez (1989) only found a mild impairment in the onset of place learning acquisition, but did not detect later deficits in the transfer test or in the reversal test. The place learning task used by Sutherland and Rodriguez (1989) involves reference memory processes because the position of the escape platform is constant during the trials.

It is not a working memory task because the relationship between the stimulus and response is not transitory (Frick et al., 1995). Taken together, these data suggest that MB lesions provoke mild impairments in reference memory (Sutherland and Rodriguez, 1989) and severe deficits in working memory tasks which involve allocentric orientation (Sziklas et al., 1996).

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References


