

IMPLICATIONS OF MAMMILLARY BODIES IN SPATIAL MEMORY IN RATS

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RESUMEN

En este trabajo se realiza una tarea de memoria espacial, laberinto de agua en T y registro de una serie de pautas comportamentales (conducta exploratoria, acicalamiento, pasividad, actividad oral, enriquecimiento) en rata blanca. A continuación se lesiona electrolíticamente el núcleo mamilar lateral, mediante la administración de una corriente continua de 1 mA y 1.3 mA, siendo posteriormente cuantificado el volumen del tejido lesionado.

Los resultados conductuales y fisiológicos registrados son discutidos en relación con el síndrome de Korsakoff.

Palabras clave: Cuerpos mamilares. Memoria espacial. Laberinto de agua.

ABSTRACT

In this work an spatial memory task was made, as well as a water maze T and a registry of some behaviour models (exploratory conduct, grooming, passivity, oral activity, enrichment) in white rat. We after lesioned by electrolytic method the lateral mammillary nucleus (1 mA and 1.3 mA) and we quantificated the volume of the lesion.

We studied the relationship between the conductual-physiological results and the Korsakoff's syndrome.

Key words: Mammillary bodies. Spatial memory. Water-maze.

INTRODUCTION

In the last years, the Korsakoff's disease has been studied in patients which had some alcoholic encephalopathy (Tako, A., Beracochea, D. and Jaffard, R., 1988; Beracochea, D. and Jaffard, R.,

1987). This syndrome is often characterized by memory impairment, plus more variable features such as confabulation, apathy state and loss of insight (Kolb, B. and Wishaw, 1986; Mair, W. G. P., Warrington, E. K. and Weiskrantz, 1979). The reason of these deficits, would reported in thiamine deficits because a excessive intake of alcohol and a decrease intake of

food. This thiamine decrease affect at brain structures for example thalamic and mammillary bodies (MB) considering the close anatomical conexions between the structures of the hippocampal system such as thalamic area and (MB) linkend by fornix (Kolb, B. and Wishaw, 1986).

The diencephalics lesions in both areas could be considered important to cause the most memory impairment (Irle, E., 1987; Greene and Naranjo, 1986) on the other hand, some authors have studied the relationships between structures of hippocampal system which provide the circulation of information as long as it is necessary for its integration in neuronal circuits between the amnesic state and their conexions (Becker, J. T., Walker, J. A. and Olton, D., 1980; Staines, W. A., Daddona, P. E. and Nagy, J. I., 1987; Allen, G. V. and Hopkins, 1989; Shibata, H., 1989; Hayakawa, T. and Zyo, K., 1989). Although, the researchs have attemped these amnesics states with the Korsakoff's syndrome, the anterograde amnesic is the most carактерistic in this pathology, because the patients with this syndrome cannot retain some familiar events and situations.

We have studied the relations between LM and anterograde amnesics states.

The present experiment was designed to determine the relative importance of the LM in this deficits and it is participation in other behavioral patterns.

The animals were tested in the water-maze and many behavioral patterns for example: grooming, passivity, oral activity which registred in the training phase and retention periods.

The MB have been implicated in some different activities: in the regulation of body temperature, motor activity and neuroendocrines processes (thirst, control of appetite and sexual cycles)

(Shibata, K. and Furukawa, T., 1988; Brooks, Ch., 1988).

MATERIALS AND METHODS

Animals

Twenty-four males and females *Rattus norvegicus*, Wistar strain were used for this experiment with weighing about 290-330 g. at the beginning of the experiment.

All experimental animals were housed individually with free access to food and water in a constant environmental temperature ($20 \pm 2^\circ \text{C}$) maintained on 12 h light-dark cycle (8.00 a.m. to 20.00 p.m.).

All tests were conducted between 9.00-14.00 hours.

The twenty-four subjects were assigned to six groups according to the electrolytic lesion intensity:

- G1.- Four males with 1 mA for 10 secs.
- G2.- Four females with 1 mA for 10 secs.
- G3.- Four males with 1,3 mA for 10 secs.
- G4.- Four females with 1,3 mA for 10 secs.
- G5.- Control group, n = 4.
- G6.- Sham-operated group, n = 4 received the same surgical procedure without the electrolytic lesion current.

Apparatus and procedure

The testing was done in a water «T» maze constructed of Plexiglas. It alleys were 10 cms. wide, 50 cms. long and 40 cms. high and was covered of black paper to evit extralaberint-cues presencie. The water maintained at 22°C temperature and the escape plataforms extended 1 cm. below the surface of water.

Procedure

All experimental animals were tes-

ting in different tasks:

a) *Neurological test*: For reported the neurological normality of the animals, they were testing in various and different (Bures and Buresova, 1973):

Grasping reflex: Hold the rat in a suspended position and touch the palm with a stiff wire (1 mm. in diameter). Grasping is accomplished by flexion of fingers around the wire.

Planing reactions: Retrain the rat at the edge of the table and displace one foreleg or hindleg so that its hands over the edge. The leg is immediately withdrawn to the surface of table. And the rats is held by the tail and slowly moved from the edge so that wibrissal estimation is prevented and only visual cues are used. The rat attempts to seize the edge as soon as it is within reach.

Equilibrium tests: The animal was placed on a horizontal wooden bar which is 2 cms. in the diameter and 30 cms. long and suspended 50 cms. above the floor.

Its ability to stay on the bar usually exceeds 30 mins.

b) *Behavioral patterns*: Useful informations about the animals behavior can be obtained by quantitative observation (15 min.) of some activities emitted in the home cage. For the observational periods, different activities were quantitatives for example: grooming, oral activity, passivity, exploratory behavioral.

These observations were made by three judges simultaneously.

c) *Spatial task*: The experimental situations carried out with the water maze «T», in its arms, the escape platforms (10 cms. and 10 cms.) were placed 1 cm. below the surface of water.

The spatial learning task carried out in 11 days. The pretaining phase, in the first day, the animals would go a one arm,

because the escape platform was placed in both stern.

In training periods, all animals received 10 trials daily in which the forced-trials and choiced-trials were used. In the forced-trial, the subjects were forced to enter one of the arms in which the escape platform was placed. In the choiced-trial, the arm was not bloched, but the escape platform was in the same arm that the forced-trial, «win stay».

The intertrials interval was 1 mins, in which periods the animal was on the platform (Castro, C. A., Paylor, R. and Rudy, 1987; Einon, D. and Morgan, M. J., 1980).

We controled the errors numbers and the latency time used by the animals. Parallely was controled the food intake, was given 30 g. daily, because lesions in the MR would involved hyperphagys effects. In the last days, all trials were unblocked.

Surgery

For surgery, the animals were anesthetized with Ketolar (10 mg./1 kg.) intraperitoneal injection and then, subjects was positioned in stereotaxis apparatus, a stainless-steel electrode, insolated except st the tip was lowe into the brain and electrolytic lesion were produced by anodal current (1 mA and 1,3 mA for 10 secs.). Sham-lesioned rats underwent exactly the same surgical procedure, except that no current was passed.

The coordinates, anterior (0.1 mm.) from the bregma, lateral (0.5 m.) to the midline and (9.2 mm.) depth were selected with Pellegrino and Cushman atlas.

At the end the surgery, the animals received Benzetacil (0.1 cc./100 g.) intramuscular injection.

Following surgery, the animals were allowed one week for recovery. Postoperative testing were carried out in the su-

cessive days and all behavioral patterns, neurological tests and spatial retention task had been verified. In the spatial retention, the subjects received 10 trials in one day, in which, all trials were unblocked arms.

Histology

At the end of the experimental, the animals were sacrificed and perfused with 10% formalin in 0.1 M phosphate buffer (pH 7.3); then the brains were removed and fixed in Formaldehyde for some days and then embedded into paraffin.

These brains were cut serially in microtome and the sections obtained had 5 μ m thick and then, were stained with gallocianin-chromalum because this stained marked only DNA and nucleic acids.

Stereology

Estimates of volume have been carried out stereology methods. The Cavalieri's principle was essential for this methods (Cruz-Orive, L., 1987; Pakkennberg, D. and Gundersen, H. J., 1988).

For this analysis must obtained parallels sections with thick estimate:

$$\text{est}(v): \bar{T}x(a/p) \times M^{-2} \times \Sigma Q$$

- Q: Average number of particles counted per sampling frame of area.
- a: The area associated with each point in the systematic point-set.
- P: Number of points which lie over the nucleus profile.
- M: Microscopic fields.
- t: Thickness of the sections.

RESULTS

In the study that has taken place we have obtained that following conductual results.

The neurologic and equilibrium test (under observation), proved the maintenance of the reflex behavioral that we used at the beginning of the experiment, not noticing any kind of change in these ones.

In the equilibrium test, the animals didn't seem to demonstrate any kind of abnormality, staying and maintaining themselves over the bar of equilibrium during the same time.

In this test a reliability interjudges analyses was made, maintaining a coefficient of reliability from 0.89 to 0.90, which could be considered as the maximum coefficient.

The animals saw to maintain a normal attitude which was specially defined by oral activity, grooming, but other kind of conducts as enrichment did not appear during the tests.

The food intake and corporal weight: at the beginning of the experiment, the patterns of intake between females and males was different. The females saw to consume little quantity of food daily (30 g. every day), the males ate everything, but the females only ate around 17 g. daily.

That is the reason why we can see that loose weight during the learning experiment.

All the patterns were maintained even during the postoperative period, that is how we can leave out any kind of hiperphagic effects produced by lesion.

- Spatial learning task: in this process, the TRs and the errors number were considered. If we have to consider the number of errors in the learning period it came to be 10%, but the female got to 20%, this was the fact used to verify the act of learning.

The number of errors in the retention's period and the learning period were similar, though the number of errors was a bit little (Figure 2) (Table 1).

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	Learning					Posttest				
	right responses				\bar{x}	right responses				\bar{x}
G ₁	10	10	10	10	10	10	10	10	8	9.5
G ₂	10	9	9	10	9.5	10	9	8	10	9.2
G ₃	10	10	10	10	10	10	10	9	10	9.7
G ₄	9	10	10	9	9.5	10	10	8	10	9.5
G ₅	9	10	9	10	9.5	8	9	9	9	8.7
G ₆	10	10	10	10	10	10	10	10	10	10

Table 1.- Right responses and means for all groups in two periods: 10th day of learning and posttest.

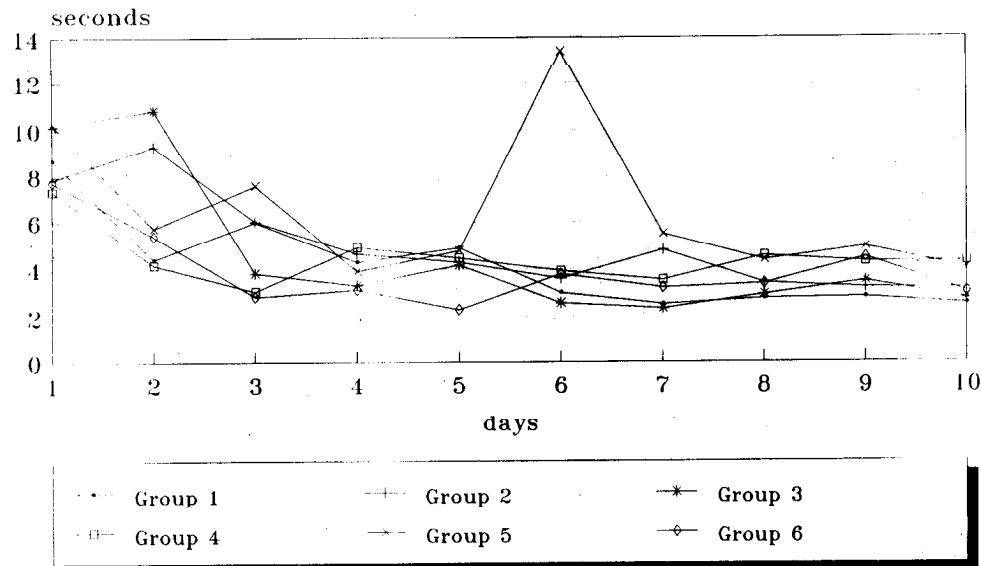


Figure 1.- RT in free trials for learning period.

The TRs seen to have an important decrease this fact was more obvious during the first learning day (Figure 1). During the retention period, the TRs were stable if we compare it with the last learning day.

The results of the histologic study (using an optic microscope), we can confirm the lesion in the LM and also that the damage has been extended to the anteriorpart of this subnucleus (Figure 3).

This lesion is distinguished by a reac-

tive gliosis an excessive presence of astrocytic cells and also of blood capillaries near to the lesioned area, facts that are present in every regeneration neuronal process (Kimelberg, H. and Norenberg, M., 1989).

If we have to set a high value the volumen that we have lesioned to determine this volume we used stereologicals methods obtaining 4.4% of the lesioned zone with and intensity of 1 mA and 9.5% if we use 1.3 mA intensity.

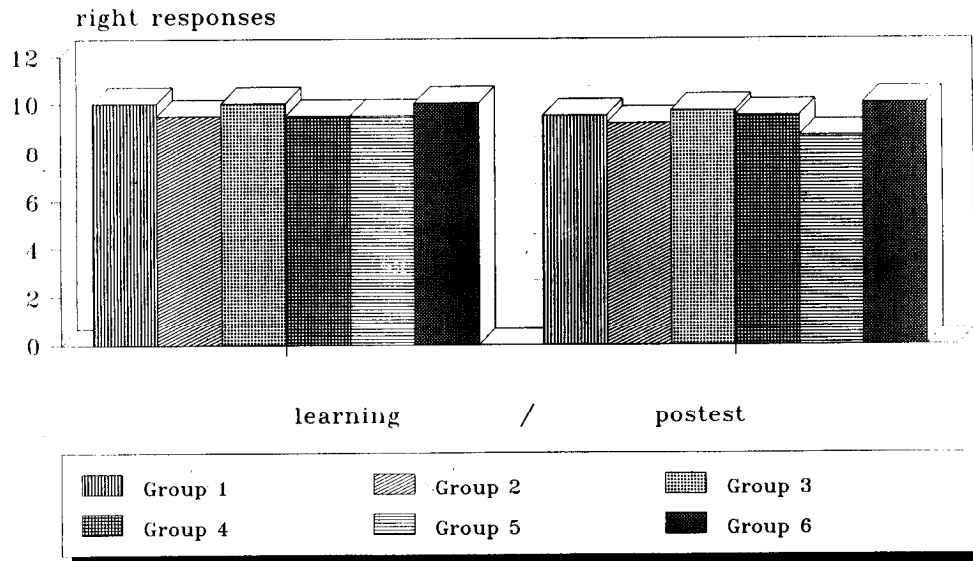


Figure 2.- Means distribution for right responses.

In all this study, the analysis is revealed that groups did not differ significantly, Student's *t* was used for dependent samples:

$$t(7) = 0.84, \alpha = 0.05.$$

$$t_{teoric}(7) = 1.89.$$

DISCUSSION

When we are talking about memory, we must understand that a whole system is implicated: the hippocampal system (Becher, J. et al., 1980) and that as a complex of nuclei those are going to take part in memoristic processes and, to be more specific, in spatial tasks (Morris, R., 1984; Eison, D. and Morgan, M. J., 1980).

There are also some other authors that defend the existence of a general memoristic system as Lashley and Penfield (we must remember that this way of thinking is a cognitivist one). They affirmed that a nonspecific functional system of en-

cephalon exists and that it is the reason why the coordination of the activity at this general system takes place.

We have taken the map of projections that interconnect the different areas of these functional system, we could obtain a correlation or a clear physiological afferents and efferents projections (Allen, G. V. and Hopkins, H., 1989; Shibata, K., 1989; Yakawa, T. and Zyo, K., 1989).

The electrolytic lesion of bilateral structures, made us lesion both of them. This is totally necessary because if we did not do so functions that have been made in the lesioned part would be assumed by the contralateral.

We must, otherwise remember that unilateral lesions can produce invaluable deficits that are not so invaluable when we are talking about bilateral lesions, because in this case the effects are indeed more serious and permanent (Irle, E., 1987).

This is the usual way to get to interfere with the brain function and to look for

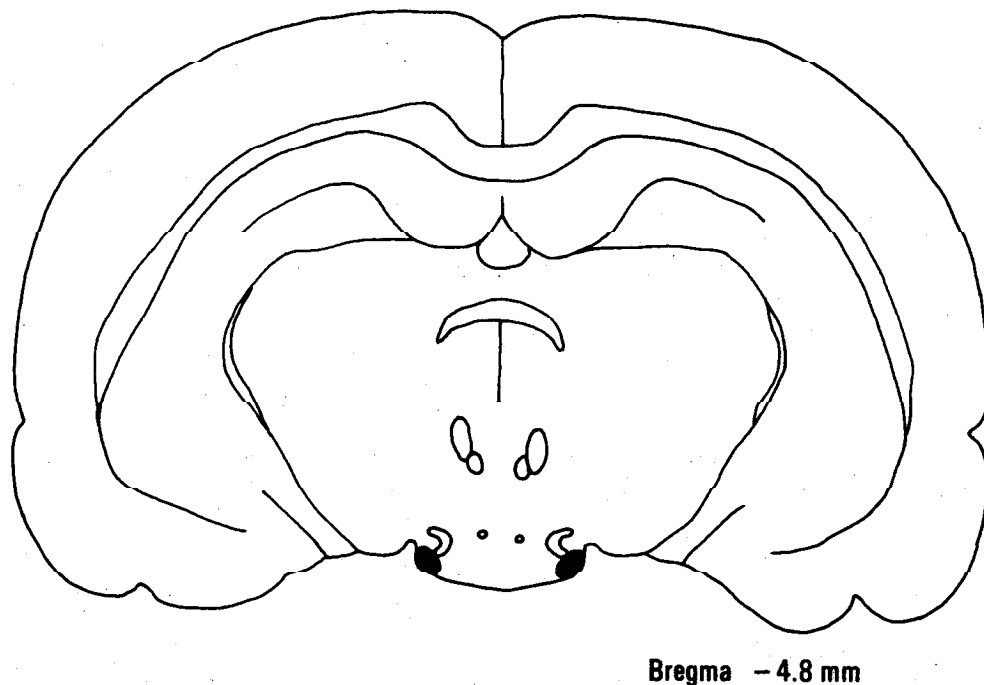


Figure 3.— Lesioned area, corresponding to the LM of mammillary complex.

some changes in behavior. That is the reason why a lesion unespecific and constitutive area of this system, does not produce significant changes, this would not be that same if we lesioned two or more areas. Some other authors think that the changes, that can be produced by the lesion can get to be reduced but not eliminated when it is made in enrichment environmental (Tako, A. et als., 1988).

The MB are situated in the hippocampal system received and connecting areas as the thalamus, tegmental and subiculum.

The complex mammillary can be identified with the group of nucleuses, which can get to be distinguished by cytologic, topografic and even funtional analysis (Bleier, R., Cohnp, P. and Siggelcow, I. R., 1979).

In this complex mammillary two nucleuses have been differend: LM and

MM, by Cajal as the Tuberomammillary these are citologically different because of their magnocells and parvicells (Allen, G. V. and Hopkins, H., 1988).

On the other hand, and trying to make an specific division of the different areas that we can in these nucleuses, we have used the topografic discernment that is how we have subdivided the MM in: lateral, median, medial, basal and posterior.

The citologic study would only provide us information about the predominance of magnoneurons in the LM cytologic and topografic differences remain in the projections that these nucleus realized. The LM connects to the medial ventral and the dorsal then, we will be able to relation the nucleuses with funtions.

The mediodorsal thalamic nucleus regulates the intake functions and the short-term memory, while the anteroven-

tral thalamic nucleus is directly related with the long-term memory.

Our results notify the scart relation of magnoneurons of the MB in long-term funtions, but makes us go a head to possible relations that can exist between the MM and the anteroventral thalamic nucleus, that we can see in patients with the Korsakoff's disease, anterograde amnesic (Mair, W. G. P. et als., 1979).

The anteroventral thalamus is directly related with the long-term memory and this area is also related with the MM.

New hypothessis and future investigations must be in deed maken to go a head in this fascinating study.

ACKNOWLEDGMENTS

This research was supported by a Univ. Oviedo grant Ayudas de Difícil Financiación Exterior to J. L. A.; Marcelino Cuesta is thanked for his assistance with the project.

The authors wishes to thank Susana Suárez for her review of the English translation.

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