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 patiens 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 Whishaw, 1986; Mair, W. G. P., Warrington, E. K. and Weiskrantz, 1979). The reason of these deficits, would reported in thiamine deficits because a excesive intake of alcohol and a decrease intake of

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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 Whishaw, 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 caractheristic in this pathology, because the patients with this syndrome cannot retain some familiary 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 partipation 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 differents 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 norvergicus*, Wistar strain were used for this experiment with weighing about 290-330 g. at the beginning of the experiment.

All experimentals animals were housed individually with free access to food and water in a constant enviromental temperature (20 ± 2° 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 construted of Plexiglas. It alleys were 10 cms. wide, 50 cms. long and 40 cms. high and was covered of black papper 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 experimentals animals were testing in different tasks:

a) *Neurological test:* For reported the neurological normality of the animals, they were testing in various and differents (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 inmediately withdrawn to the surface of table. And the rats is held by the tail and slowly moved from the edge so that wibrissal estimulation 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, differents activities were quantitatives for example: grooming, oral activity, pasitity, 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 plataforms (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 plataform was placed in both sterm.

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 plataform was placed. In the choiced-trial, the arm was not bloched, but the escape plataform 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 plataform (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 MB would involved hyperphagys effects. In the last days, all trials were unbloked.

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 sucessive 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 unbloked arms.

Histology

At the end of the experimental, the animals were sacrified 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 um 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, B. and Gundersen, H. J., 1988).

For this analisys must obtained paralels 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 behaviorals that we used at the begining of the experiment, not noticing any kind of change in these ones.

In the equilibrium test, the animals didn't seen to demostrate 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 actitude 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 begining 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 durire the postoperatory 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).

	Learning					Postest				
	right responses				x̄	right responses				x̄
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 postest.

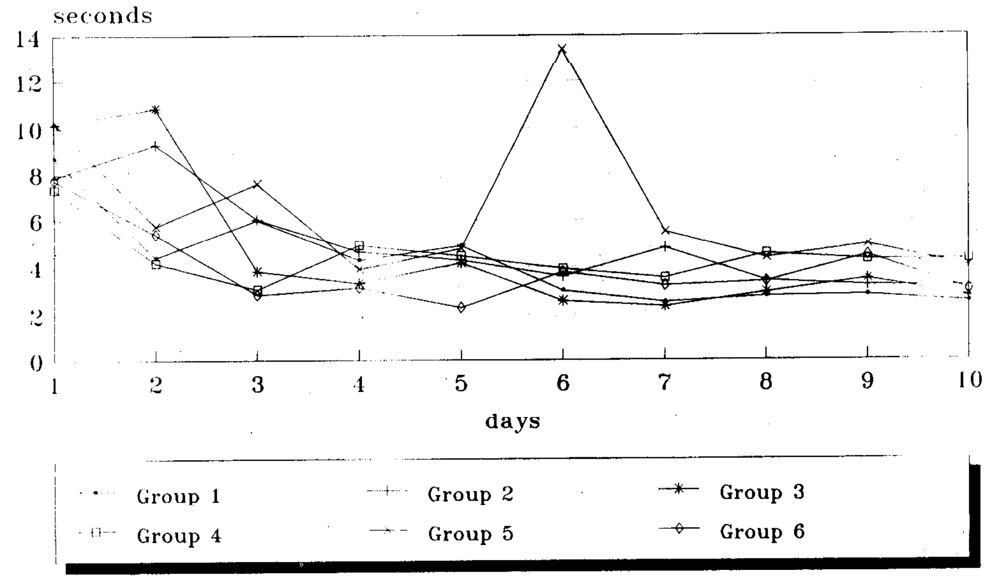

Figure 1.– RT in free trials for learning period.

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 reactive gliosis an excesive 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.

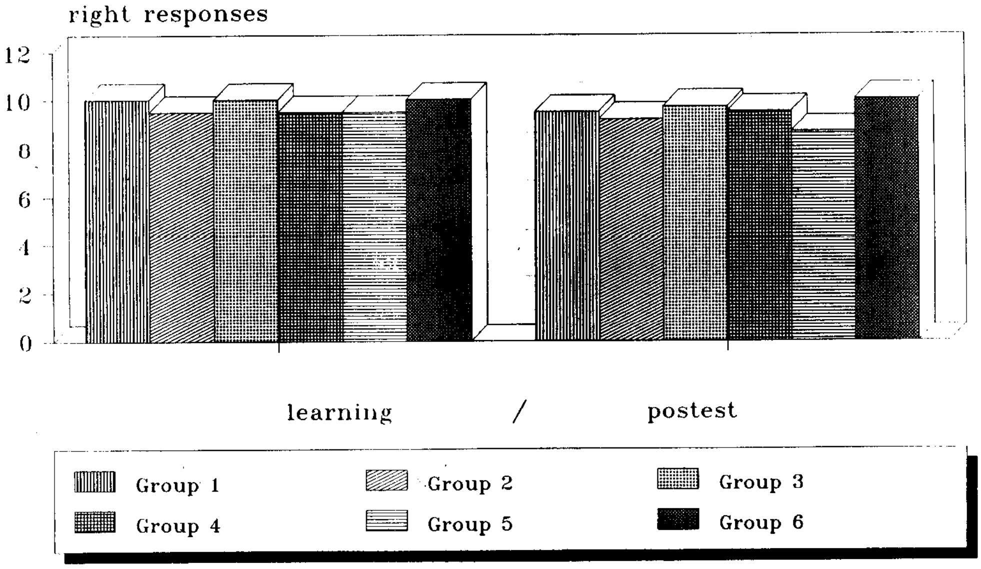

Figure 2.– Means distribution for right responses.

In all this study, the analysis is reveleaad that groups did no differ significantly, Sthudent't was used for dependent sames:

$$t(7) = 0.84, \text{alpha} = 0.05.$$
$$t \text{ teoric}(7) = 1.89.$$

DISCUSSION

When we are talking about memory, we must understand that a whole system is implicated: the hippocampal system (Becher, J. et als., 1980) and that as a complex of nucleuses those are going to take part in memoristics processes and, to be more specific, in spatial tasks (Morris, R., 1984; Einon, 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 inespecific funtional system of encephalon 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 differents areas of these funtional 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 bilaterals 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 invalvable when we are talking about bilateral lesions, because in this case the effects are in deed more serious and permanent (Irle, E., 1987).

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

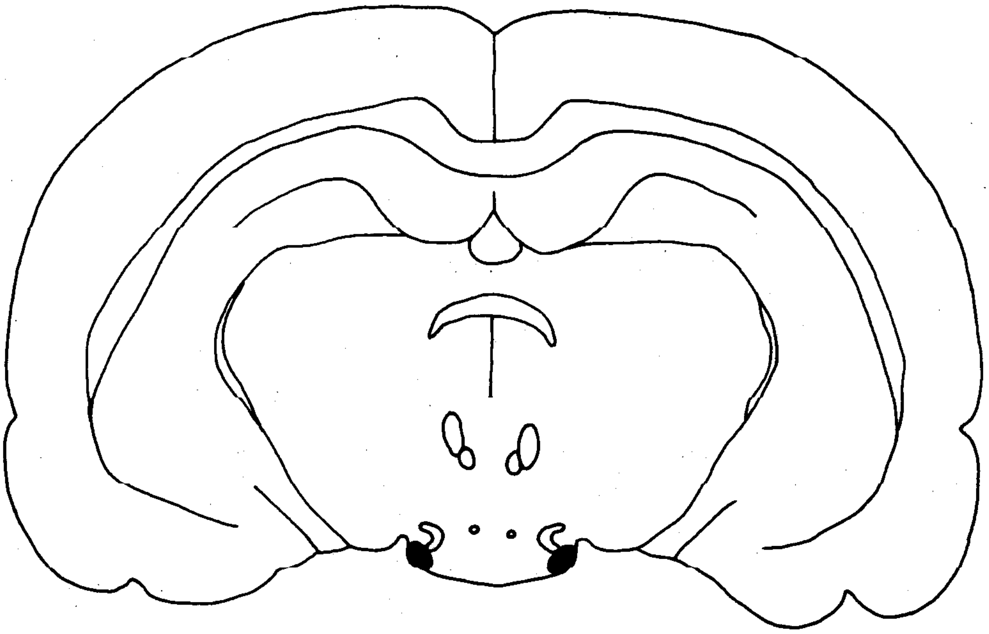

Figure 3.– Lesioned area, corresponding to the LM of mammillary complex.

some changes in behavior. That is the reason why a lesion unespecific and constitutive area of this system, does not produce significative 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 eliminited when it is made in enrichment enviromental (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 citologic, 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 citologic and topographic 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 medialdorsal thalamic nucleus regulates the intake functions and the short-term memory, while the anteroventral thalamic nucleus is directly relationed 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 relationed with the long-term memory and this area is also relationed with the MM.

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

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