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Spatial memory and *c-fos* expression in supramammillary nucleus, anterior cingulated gyrus and entorhinal cortex

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To investigate brain substrates of spatial memory, the cellular expression of c-Fos protein in rats was studied after training the animals to perform a spatial reference memory task and a working reference memory task in a Morris water maze. The number of c-Fos positive neuronal nuclei was quantified in several brain regions: entorhinal cortex, anterior cingulate gyrus, and supramammillary nucleus. The results showed that spatial training in reference and working memory tasks increased the number of entorhinal cortex activated neurons (c-Fos positive neurons). No clear association was found between *c-fos* activation in the anterior cingulate gyrus and either spatial reference or working memory tasks. The number of c-Fos immunoreactive neuronal nuclei in the supramammillary neurons was greater in the spatial working memory groups than in the spatial reference memory groups suggesting that neurons of the supramammillary nucleus plays an important role in spatial processing.

Memoria espacial y expresión de c-fos *en núcleo supramamilar, giro cingulado anterior y corteza entorrinal.* Este trabajo se aproxima al estudio de los substratos cerebrales de la memoria espacial en ratas, empleando la expresión celular de la proteína c-Fos. Para ello, se analizó la expresión de la proteína c-Fos después de la ejecución de una tarea de memoria de referencia y otra de trabajo espacial. De este modo, se cuantificó el número de núcleos neuronales c-Fos positivos en varias regiones cerebrales: corteza entorrinal, giro cingulado anterior y núcleo supramamilar. Los resultados mostraron que el entrenamiento espacial en tareas de memoria, incrementa el número de neuronas activadas en la corteza entorrinal (neuronas c-Fos positivas). No se halló una relación clara entre la activación *c-fos* en el giro cingulado anterior y las demandas de memoria de las tareas. El número de núcleos neuronales c-Fos positivos en las neuronas del núcleo supramamilar, fue mayor en los grupos de memoria de trabajo que en los grupos de memoria de referencia, sugiriendo que las neuronas de este núcleo desempeñan un papel importante en el procesamiento espacial.

A great deal of research has focused on studying the neurobiologic substrate of learning and spatial memory (Jarrad, Okaichi, Steward & Goldschmidt; 1984; Morris, Schenk, Tweedie & Jarrad, 1990; Neave, Nagle & Aggleton, 1997; O´Keefe & Nadel, 1978; Olton & Papas, 1979; Olton, 1978; García-Moreno, Santín, Rubio, García & Arias, 1993; Santín, Rubio, Begega & Arias, 1999a; Santín, Rubio, Begega & Arias, 1999b). Following the works by Olton and collegues (Olton & Papas, 1979; Olton & Samuelson, 1976; Olton, 1978), in which two kinds of spatial memory are described (reference memory and working memory) in the radial arm maze a number of authors have studied the cerebral substrate of these two kinds of memory. Reference memory (RM) is trial-independent and is used to learn the general rules required for the performance of a task. The information available for solving reference memory tasks was constant throughout the trials (Frick, Baxter, Markowska, Olton & Price, 1995) and was reinforced by repeated training (Young, Stevens, Converse & Mair, 1996). Working memory (WM) is a temporary memory that is trial-dependent (it is only relevant for one trial) (Frick et al., 1995). These two kinds of memory can be assessed by studying the use of spatial information in the rat (Nagahara, Otto & Gallagher, 1995; Santín et al., 1999a). In spite of advances in research in this area in recent years, the precise role of the different regions of the brain in spatial RM and WM is still unclear.

Many studies have demostrated that the prefrontal cortex (PFC) plays a role in short-term spatial memory (Funahashi, Bruce & Goldman-Rakic, 1993; Granon, Vidal, Thinus-Blanc, Changeux, & Poucet, 1994). However, lesions of the medial prefrontal cortex (mPFC) do not appear to produce detrimental effects on the performance of a spatial reference memory task (DeBruin, Sánchez-Santed, Heinsbroek, Donker & Postmes, 1994). This suggests that the role of the mPFC in learning and memory processes could be restricted to working memory tasks (Granon et al., 1994).

The role of the hippocampus in processing information could be mediated by connections with the entorhinal cortex (ENT) which supply it with information from the neocortex (Hardman, Evans, Fellows, Hayes, Rupniak, Barnes & Higgins, 1997; Jones, 1993; Tamamaki & Nojyo, 1993). Several experimental studies have observed this relationship between the ENT and spatial lear-

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ning processes (Quirk, Muller, Kubie & Ranck, 1992; Goodlett, Nichols, Halloran & West, 1989; Hardman et al., 1997; Nagahara et al., 1995). Moreover, other hippocampal projections have been associated with spatial processing. In this way, Kirk (1998) have demostrated that the SUM is involved in the modulation of the hippocampal theta frequency.

In the other hand, recent studies have used techniques based on immediate early genes activation (IEGs) (Dragunow, 1996; Heurteaux, Messier, Destrade & Lazdunski, 1993; Kaczmarek, 1993; Paylor, Johnson, Papaioannou, Spiegelman & Wehner, 1994; Radulovic, Kammermeier & Spiess, 1998; Rose, 1991; Rose, 1996; Zhu, Brown, McCabe & Aggleton, 1995) to study learning and memory processes. IEGs are activated in the neurons by several second messengers which initiate their transcription. One of these IEGs is the proto-oncogene *c-fos*. c-Fos protein possibly acts via a third intracellular messenger regulating the transcription of genes of late expression. This protein forms part of a dimeric DNA-binding protein (activator protein 1 or AP1) which binds to specific sites of the multiple gene promoter region and enhances transcriptional activation of these genes (Kaczmarek, 1993; Morgan & Curran, 1991; Sheng & Greenberg, 1990; Struhl, 1991). An increase in *c-fos* proto-oncogene is one of the earliest transcriptional events to follow neuronal activation. In the last few years, several works have shown an association between *c-fos* activation and learning and memory processes using different animal models. These studies suggest that *c-fos* activation can be used as a marker of neuronal activity that offers information on cerebral regions underlying learning and memory (Kaczmarek, 1993).

The aim of the present research was to study the effect of training in spatial RM and WM tasks on neuronal activation in three brain regions: ACG, ENT and SUM. To achieve this purpose, we studied the effect of training in spatial reference and working memory tasks on *c-fos* expression by immunohistochemical detection of the c-Fos protein. Two control groups were included in the study (spatial reference memory control and spatial working memory control) that permitted *c-fos* activation not specific to the spatial working memory tasks, such as motor activity and sensorial stimulation associated with the training process, to be ruled out.

Method

Animals

Twenty-two male Wistar rats weighing on average 312 ± 21g from the central vivarium of the University of Oviedo, were used. All rats were given free access to food and water. Rats were housed individually in a temperature-controlled colony (20 ± 2 ºC) on a constant light-dark cycle (lights on 08:00-20:00). Animals were divided into four treatment groups: RM group (n=5), RM control group (n=5), WM group (n=6) and WM control group (n=6). The care and use of animals were in accordance with the Spanish regulation for the use of animals in research .

Apparatus

The apparatus consisted of a circular pool with the following dimensions: diameter: 150 cm, walls: 43 cm high. The pool was filled with water (21 ± 2 ºC) that was made opaque with non-toxic white paint. The goal platform (11cm diameter) could be placed anywhere in the pool at a distance of 30 cm from the pool edge. The platform was submerged to a depth of 2 cm beneath the surface of the water. The pool was placed in an experimental room furnished with several extra-maze cues. The pool remained immobile in the room throughout the experimental period. An automatic video system (Ethovision. Noldus) was used to record the animals' movements in the pool.

Behavioral tasks

The day before starting the behavioral experiments all the animals were submitted to two 60s sessions of free exploration.

1. Spatial reference memory task: The place learning consisted of training the rats to escape from the water using the submerged platform. The pool was divided into four quadrants (A, B, C, D). The platform was placed in the center of quadrant B where it remained throughout the experiment. The rats were introduced into the pool from one of the four release positions (quadrant A, B, C or D). Each animal was submitted to 6 trials.

The trial finished when the animal found the platform. When a rat did not find the platform within 60 s, the experimenter placed the animal on the platform where it remained for 15 s. After this period the rat was returned to its cage for 30 s after which it was introduced in the pool again. As a control, in order to rule out *c-fos* activation not specific to place learning a control group was submitted to a period of free exploration in the circular pool without the escape platform.

2. Spatial working memory task: The animals were submitted to two trials, one acquisition and one retention trial, per day. In the acquisition trial the animal had to find a submerged platform in order to escape from the water. If the animal did not find the platform in 60 seconds the experimenter placed the animal on the platform where it remained for 15 seconds before being placed in its cage for 30 seconds. After this interval the animal was again introduced into the circular pool for the retention trial. The same exit and escape quadrants were used for the acquisition and the retention trial on the same day but this varied pseudorandomly over 8 days. In this task, the control group was submitted to a daily 30 second trial in the circular pool in the absence of the escape platform.

Immunohistochemical analysis

Ninety minutes after the end of the behavioral task, the animals were deeply anaesthetized with equithesin (3ml/kg) and perfused via the ascending aorta with cold physiological saline solution followed by a cold formaline buffer (4% paraformaldehyde in 0.16 M phosphate buffer, pH 6.9). The perfusion was continued for 5 min and the brain were postfixed in the same fixative for 2 h. The brains were then transferred successively into phosphate buffered saline (PBS, pH 7.2) containing 10%, 20% and 30% sucrose until they sank for cryoprotection. Coronal sections (16 mm) of the brain were cut at –20 ºC in a cryostat. The slices were mounted on gelatinezed slides. c-Fos antiserum (Santa Cruz Biotechnology Inc., CL, USA) was used to detect c-Fos protein. The avidin-biotin complex (ABC, Vector Laboratories) immunoperoxidase method was used to visualize c-Fos immunoreactivity (c-Fos IR). Briefly, the slides containing section were washed in PBS followed by a wash in a solution of 0.1 M PBS containing 0.3% Triton X-100 and 1% normal goat serum. The sections were then incubated at 4ºC in c-Fos primary antiserum (diluted 1:10.000 in the sa-

me solution) overnight. The antiserum was a rabbit polyclonal antibody directed against the aminoacids 3-16 of the N-terminal region of the human c-Fos p62. It is not cross-reactive with c-Fos B, Fra-1 or Fra-2. Sections were washed in PBS and then incubated in biotinylated donkey anti-rabbit secondary antibody (Pierce, Illinois) (diluted 1:200 in incubating solution) for 2 h. They were further washed in PBS and incubated in an avidin-biotinylated horseradish peroxidase complex (Vector Laboratories Standard Kit: 1:100 in incubating solution). After two washes in PBS, the reaction was visualized treating the sections for about 5 min in an immuno-pure-metal-enhanced diaminobenzidine tetrahydrochloride (0.025%) substrate solution (Pierce, Illinois). The reaction was terminated by washing sections in cold PBS. Finally, the slides were dehidrated through a graded series of alcohols and coverslipped for microscopic observation.

Quantification of c-Fos IR

The number of c-Fos IR neuronal nuclei was quantified in three brain regions: ACG, SUM and ENT (Figure 1). In the ACG and ENT regions, quantifications were done unilaterally in the right hemisphere. Brain regions were located using the stereotaxic atlas of Paxinos and Watson (Paxinos & Watson, 1997) (Figure 1). Three sections of the ACG and ENT and two sections of the SUM were sampled. c-Fos IR nuclei were counted with a computerised system (Leica QWIN) and the results expressed as number/μm^3 (Nv). The quantification was done by systematically sampling each of the regions selected in each section using frames superimposed over the preparations. In order to obtain a comparable metric unit the following formula was used: Nv = N/V(ref) or Nv = $\sum(Q^-) / \sum(h \times a(fra))$. Where Q = total number of c-Fos IR nuclei counted in all the frames used; a(fra) = area of the frames used, h = thickness of the section (West, 1999). The thickness of the sections was determined using a microcator (Heidenhain. Germany).

Statistics

Behavioral data of the reference memory task were analyzed with the Friedman ANOVA by ranks and data of the working memory task using the Wilcoxon matched pairs test to compare the acquisition trial and the retention trial. The data obtained by c-Fos IR quantification were analyzed with one-way ANOVA for each brain region. Post hoc comparisons were done with the Games-Howell test, to study the differences between the four groups studied in each brain region.

Results

Behavioral results

1. Spatial reference memory task: results of the statistical analysis show that trained animals successfully performed a place learning in the pool reflected by the shorter escape latencies (χ^2 =

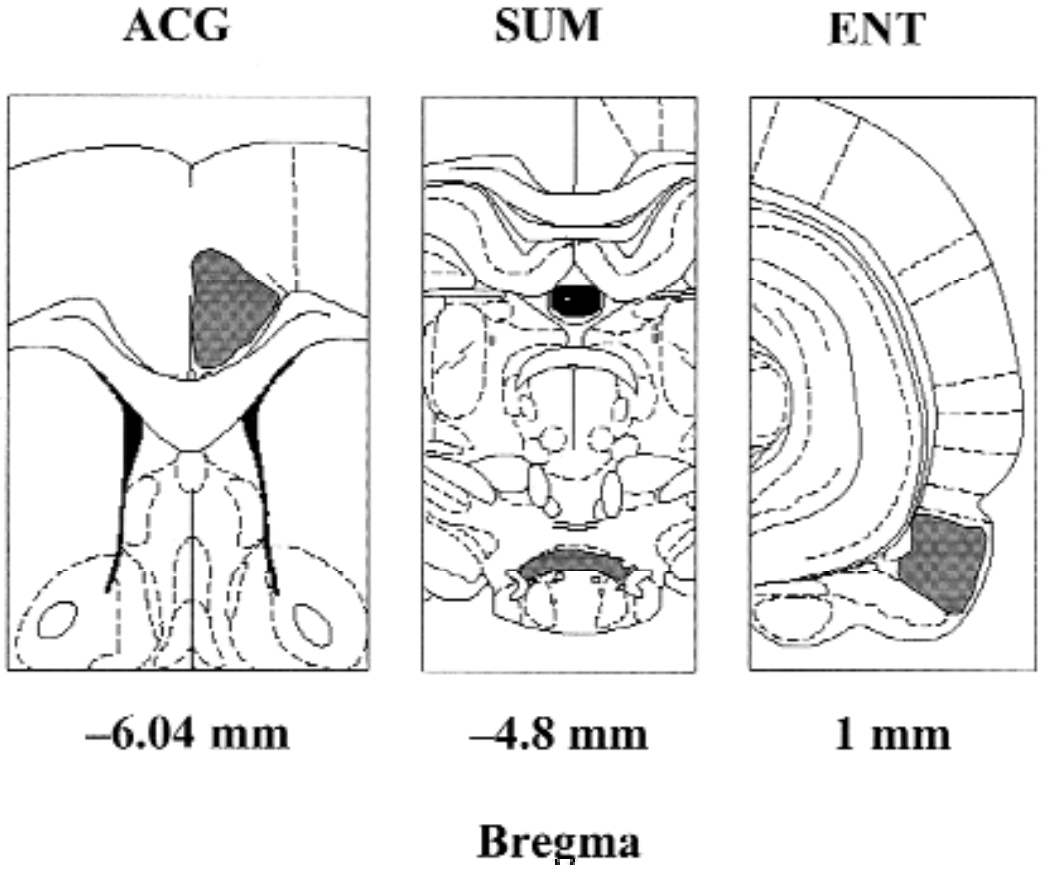

Figure 1. *Diagrams of coronal sections of the rat brain showing the sampled regions. ACG = anterior cingulate cortex; ENT = entorhinal cortex; SUM = supramammillary nucleus. (Adapted from Paxinos & Watson (1997)*

13.78698, $p \leq 0.017$) and distances swam by the animals in the maze ($\chi^2 = 13.91$, $p \leq 0.01618$) during development of the reference memory task (Figure 2).

2. Spatial working memory task: the results show that the animals can successfully perform place learnings in a task with a daily acquisition and retention trial in the pool, reflected by the shorter escape latencies in the retention trial compared to the acquisition trial ($z = 1.991$, $p \leq 0.046$). Nevertheless, although graphically the animals can be observed to swim further in the retention compared to the acquisition trial, these differences are not

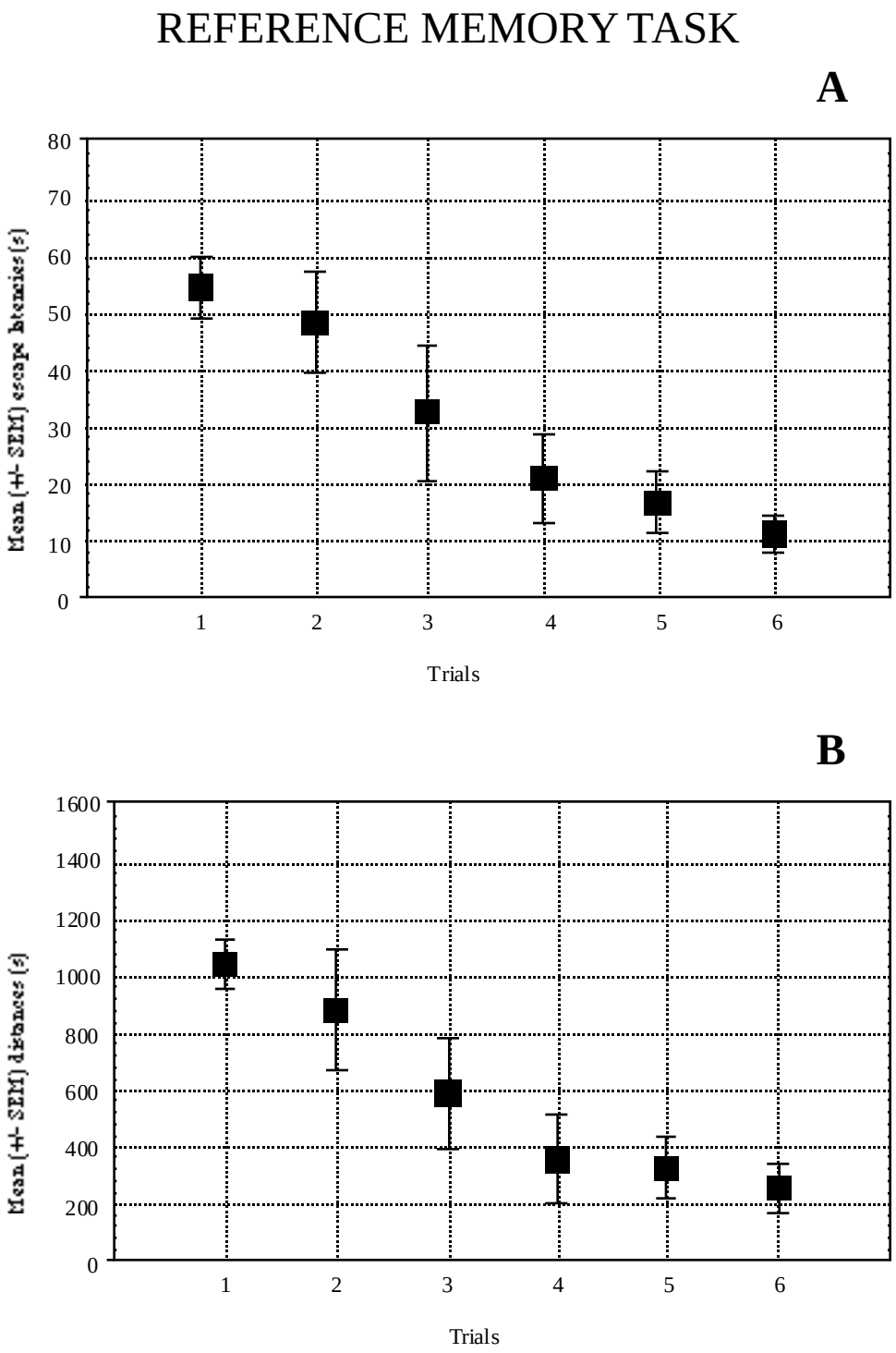

Figure 2. *Graphical representation of spatial reference memory task that shows an improved ability of the rats to locate the submerged platform in the Mo - rris water maze after training. The place learning is reflected in the reduced escape latencies (p≤0.017) (A) and the decr ease in the distances swam in the pool (p≤0.017) (B)*

statistically significant indicating that the animals swim a similar distance in the circular pool during the acquisition trial and the retention trial ($z = 1.15$, $p \geq 0.248$) (Figure 3).

Quantification results

Entorhinal cortex: The statistical results reflect the existence of differences between the groups ($F(3, 18) = 6.256$, $p \leq 0.004$). Post hoc comparisons showed differences between the following groups: RM control and RM, WM, WM control ($p \leq 0.05$). Anterior cingulate gyrus: The one-way ANOVA did not show differences between the four groups ($F(3, 18) = 2.475$, $p \geq 0.095$). Supramamillary nucleus: There were differences between the four groups ($F(3, 18) = 7.801$, $p \leq 0.002$) and post hoc comparisons showed differences between the groups: WM and RM, WM and RM control, WM control and RM control ($p \leq 0.05$). (Figures 4 and 5).

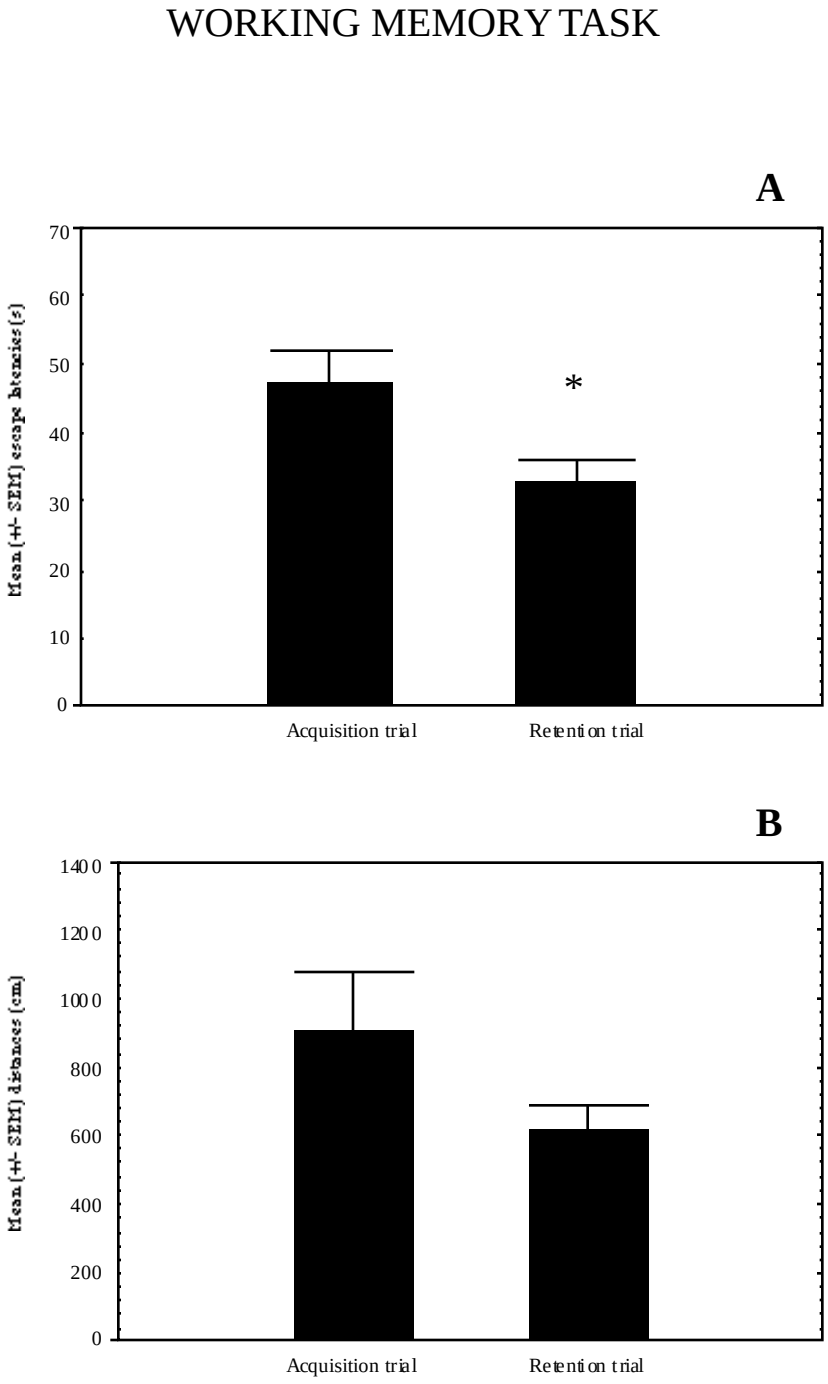

Figure 3. *Graphical representation of spatial working memory task. The rats found the submerged platform in the shortest time when they had been previously shown its position in a stimular context (retention trial) compared to when they did not know its location (acquisition trial) ($p \leq 0.046$) (A). Nevertheless, significant differences were not found between the two trials in the distances swam in the pool ($p \geq 0.248$) (B)*

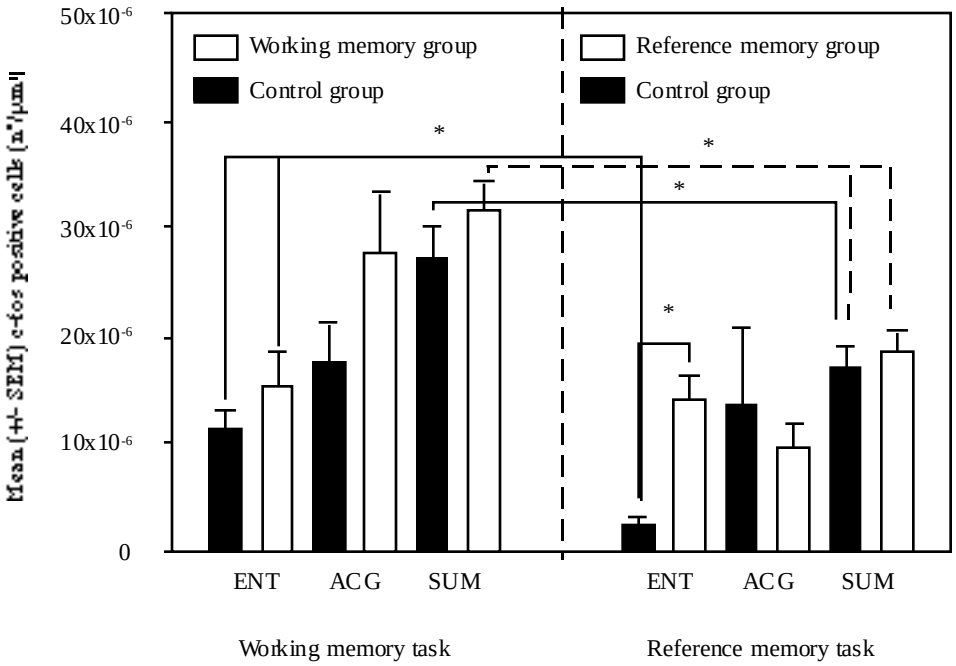

Figure 4. *Graphical representation of the quantification of the number of c-Fos IR neurons in different brain regions. As can be observed in the figure the ENT has the greatest number of c-Fos IR neurons when the animals are processing spatial information. Neurons of the SUM appear to be more closely associated with the general processing of spatial information than with reference and working memory processes. Neurons of the ACG show no clear association with either spatial RM or WM (* p≤0.05). ENT = entorhinal cortex; SUM = supramammillary nucleus; ACG = anterior cingulate gyrus*

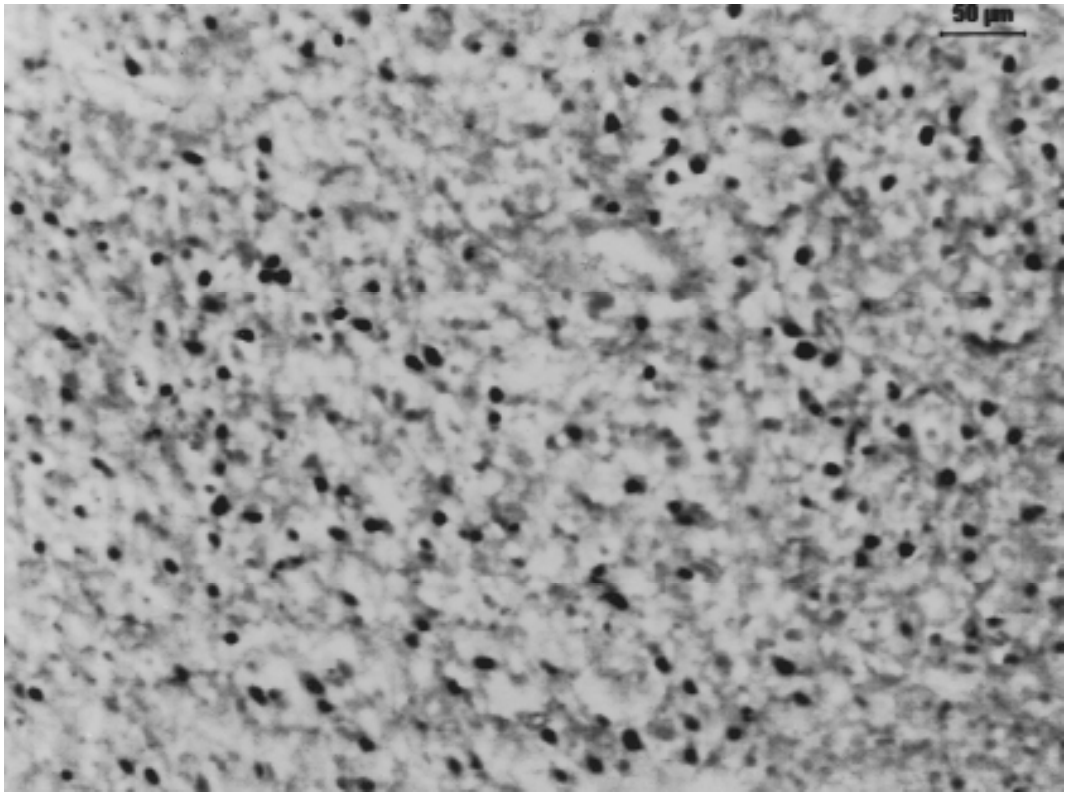

Figure 5. *The microphotograph shown coronal section of the rat brain showing the c-Fos IR on AG*

Discussion

Some studies have demonstrated an increase in c-Fos protein in the central nervous system (CNS) in animals submitted to behavioral experiments. Hence, a rise in *c-fos* mRNA was found in an aversive conditioning task in rodents (Maleeva, Ivolgina, Anokhin & Limborskaja, 1989; Nikolaev, Kaminska, Tischmeyer, Matthies & Kaczmarek, 1992). In an active avoidance task performed in rats, increased levels of *c-fos* mRNA were observed during the first training session (Nikolaev et al., 1992). Moreover, copulatory behavior in rats provokes an accumulation of *c-fos* mRNA in the sensorial cortex and a rise in c-Fos protein in the olfactory bulb of female rats exposed to mating (Brennan, Hancock, & Keverne, 1992). Zhu et al. (1995) determined *c-fos* expression in different brain regions associated with recognition memory and observed a rise in the expression of c-Fos protein in ACG (among other structures) with new objects and a milder expression of this protein with more familiar objects. Moreover, the IEGs such as *c-fos* could play an important role in the establishment of spatial memory processes. Paylor et al. (1994) observed an impairment of *c-fos*-deficient animals in the spatial Morris water task, but no impairments in a simple left/right discrimination task.

Our results (and other researchs (Nagahara et al., 1995; Hardman et al., 1997) suggest that ENT is involved in processing spatial information. The group that explore the pool for 30 s (RM control group) shows a few c-Fos IR neurons compared with the other groups, that have a greater spatial knowledge. These differences suggest that ENT is important during repeated training in spatial tasks, when the animals have formed a relational representation of the enviroment, but it is less relevant when the animals have even not formed those representations. Moreover, the number of ENT c-Fos IR neurons is independent of the memory process required (spatial WM, WM control and RM groups present a greater number of c-Fos IR cells than the spatial RM control group). (Figure 4).

Studies on the PFC in rats (Granon & Poucet, 1995; Granon et al., 1994) have shown to play a crucial role in WM. Nevertheless, the results of the two tasks performed here do not clearly reflect this participation of the mPFC. As can be seen in Figure 4, a greater increase in the number of c-fos positive neurons is observed in the group submitted to the spatial WM task compared with the control group and with the animals submitted to the spatial reference memory task. Nevertheless, these differences are not statistically significant and we can not clearly conclude that ACG participate in the spatial WM processes. On the other hand, some works have shown that the mPFC is mainly subdivided into two, possibly functionally diverse, regions (ventromedial mPFC and dorsomedial PFC) (DeBruin et al., 1994; Delatour & Guisquet-Verrier, 1996; Kolb, 1984; Van Eden, Lamme & Uylings, 1992). ACG forms part of the dorsomedial PFC and receives important afferents from the anteromedial nucleus of the thalamus (Shibata, 1993), whereas regions of the ventromedial mPFC (infralimbic cortex and prelimbic cortex) receive important hippocampal afferents (Swanson, 1981). The relationship between the ventromedial PFC and the hippocampus suggests that this ventral region of the PFC plays some part in processing spatial information (Fantie & Kolb, 1990). Moreover, one of the ventral regions of the mPFC, the infralimbic cortex appears to be especially important in memory processes (Brito, Thomas, Davis & Gingold, 1982; Brito & Brito, 1990). These data could explain the absence of significant differences between the groups in our study, suggesting that the system of connections between the hippocampus and the ventral mPFC are more relevant in the processing of spatial information than the system which involves ACG and AT .

In the other hand, Kirk (1998) have shows that the SUM primarily determines the frequency of hippocampal theta rhythm, suggesting that it plays a role in processing spatial information. In our work, the number of SUM c-Fos IR neurons is very similar in the experimental groups and their controls in either of the two tasks (RM and WM tasks). Nevertheless, the expression of c-Fos protein in the SUM, was greater in spatial WM groups compared to spatial RM groups. These differences suggest an involvement of SUM neurons in spatial processing because WM and WM control animals have explored the environment for several days but RM groups have only explored it for one day (Figure 4). Perhaps, SUM neurons are greater involved in the spatial recall than in the acquisition of the spatial learning.

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Referencias

Brennan, P.A., Hancock, D. & Keverne, E.B. (1992). The expression of the immediate-early genes c-fos, egr-1 and c-jun in the accessory olfatory bulb during the formation of an olfatory memory in mice. *Neuroscience*, 49, 277-284.

Brito, G., Thomas, G., Davis, B., & Gingold, S. (1982). Prelimbic cortex, mediodorsal thalamus, septum and delayed alternation in rats. *Experimental Brain Research*, 46, 52-58.

Brito, G.N.O. & Brito, L.S.O. (1990). Septohippocampal system and the prelimbic sector of frontal cortex: a neuropsychological battery analysis in the rat. *Behavioural Brain Research*, 36, 127-146.

DeBruin, J.P.C., Sánchez-Santed, F., Heinsbroek, R.P.W., Donker, A. & Postmes, P. (1994). A behavioral analysis of rats with damage to the medial prefrontal cortex using the morris water maze: evidence for behavioral flexibility, but not for impaired spatial navigation. *Brain Research*, 652, 323-333.

Delatour, B. & Guisquet-Verrier, P. (1996). Prelimbic cortex specific lesions disrupt delayed-variable response tasks in the rat. *Behavioral Neuroscience*, 110 (6) 1282-1298.

Dragunow, M. (1996). A role for immediate-early transcription factors in learning and memory. *Behavioral Genetics*, 26 (3) 293-299.

Frick, K.M., Baxter, M.G., Markowska, A.L., Olton, D.S. & Price, D.L. (1995) Age-related spatial reference and working memory deficits assessed in the water maze. *Neurobiology of Aging*, 16 (2) 149-160.

Fantie, B.D. and Kolb, B. (1990). An examination of prefrontal lesion size and the effects of cortical grafts on performance of the Morris water task by rats. *Psychobiology*, 18,, 74-80.

Funahashi, S., Bruce, C.J. & Goldman-Rakic, P.S. (1993). Dorsolateral prefrontal lesions and oculomotor delayed-response performance: evidence for mnemonic “scotomas”. *Journal of Neuroscience*, 13,1479-1497.

García-Moreno, L.M., Santín, L.J., Rubio, S., García, H. & Arias, J.L. (1993). Effects of etanol and diazepam on Ag-NOR neuronal activity in the medial mammillary nucleus. *Psicothema*, 5(1), 125-134.

Goodlett,C.R., Nichols, J.M., Halloran, R.W. & West, J.R. (1989). Long-term deficits in water maze spatial conditional alternation performance following retrohippocampal lesions in rats. *Behavioural Brain Research*, 32, 63-67.

Granon, S. & Poucet, B. (1995). Medial prefrontal lesions in the rat and spatial navigation: evidence for impaired planning. *Behavioral Neuroscience* 109 (3) 474-484.

Granon, S., Vidal, C., Thinus-Blanc, C., Changeux, J.P. & Poucet, B. (1994). Working memory, response selection and processing in rats with medial prefrontal lesions. *Behavioral Neuroscience*, 108, 883-891.

Hardman, R., Evans, D.J., Fellows, L., Hayes, B., Rupniak, H.T., Barnes, J.C. & Higgins, G.A. (1997). Evidence for recovery of spatial learning following entorhinal cortex lesions in mice. *Brain Research*, 758, 187-200.

Heurteaux, C., Messier, C., Destrade, C. & Lazdunski, M. (1993). Memory processing and apamin induce immediate early gene expression in mouse brain. *Molecular Brain Research*, 18 (1-2) 17-22.

Jarrad, L.E., Okaichi, H., Steward, O. & Goldschmidt, R. (1984). On the role of hippocampal conections in the performance of the place and cue tasks: Comparisons with damage to hippocampus. *Behavioral Neuroscience*, 98, 946-954.

Jones, R.S.G. (1993). Entorhinal-hippocampal connections: a speculative view of their function. *Trends Neuroscience*, 16, 58-64.

Kaczmarek, L. (1993). Molecular biology of vertebrate learning: is c-fos a new beginning?. *Journal of Neuroscience Research*, 34 (4) 377-381.

Kirk. I. (1998). Frecuency modulation of hippocampal theta by the supramammillary nucleus, and other hypothalamo-hippocampal interactions: mechanisms and functional implications. *Neuroscience & Biobehavioral Reviews*, 22 (2) 291-302.

Kolb, B. (1984). Functions of the frontal cortex of the rat: a comparative review. *Brain Research Review*, 8, 65-98.

Maleeva, N.E., Ivolgina, G.L., Anokhin, K.V. & Limborskaja, S.A. (1989). Analysis of the expression of the c-fos protooncogene in the rat cerebral cortex during learning. *Genetica*, 25, 1119-1121.

Morgan, J.I. & Curran, T. (1991). Protooncogene transcription factors and epilepsy. *Trends Pharmacology Science*, 12, 343-349.

Morris, R.G.M., Schenk, F., Tweedie, F. & Jarrad, L.E. (1990). Ibotenate lesions of hippocampus and/or subiculum: dissociating components of allocentric spatial learning, *European Journal of Neuroscience*, 2, 1016-1028.

Nagahara, A.H., Otto, T. & Gallagher, M. (1995). Entorhinal-perirhinal lesions impair performance of rats on two versions of place learning in the Morris water maze. *Behavioral Neuroscience*, 109(1) 3-9.

Neave, N., Nagle, S. & Aggleton, J.P. (1997). Evidence for the involvement of the mammillary bodies and cingulum bundle in allocentric spatial processing by rats. *European Journal of Neuroscience*, 9, 941-955.

Nikolaev, E., Kaminska, B., Tischmeyer, W., Matthies, H. & Kaczmarek, L. (1992). Induction of expression of genes encoding transcription factors in rat brain elicited by behavioral training. *Brain Research Bulletin*, 128, 479-484.

O´keefe, J. & Nadel, L. (1978). *The hippocampus as a cognitive map.* Clarendon Press, Oxford.

Olton, D.S. & Papas, B.S. (1979). Spatial memory and hippocampal function. *Neuropsychologia*, 17, 669-682.

Olton, D.S. & Samuelson, R.J. (1976). Remembrance of places passed: spatial memory in rats. *Journal of Experimental Psychology*, 2, 97-116.

Olton, D.S. (1978). *The function of septo-hippocampal connections in spatially organized behaviour*. In Elliot, K. and Whelan, J. (eds), *Funtions of the septo-hippocampal system.* Elsevier, Amsterdam, 327-349 pp.

Paxinos, G. & Watson, C. (1997). *The rat brain in stereotaxic coordinates* (Compact 2th ed.). Academic Press, London.

Paylor, R., Johnson, R.S., Papaioannou, V., Spiegelman, B.M. & Wehner, J.M. (1994). Behavioral assessment of c-fos mutant mice. *Brain Research*, 651 (1-2) 275-282.

Quirk, G.J., Muller, R.U., Kubie, J.L. & Ranck, J.B.(1992). The positional firing properties of medial entorhinal neurons: description and comparison with hippocampal place cells. *Journal of Neuroscience*, 12, 1945-1963.

Radulovic, J., Kammermeier, J. & Spiess, J. (1998). Relationship between fos production and classical fear conditioning: effects of novelty, latent inhibition and unconditioned stimulus preexposure. *Journal of Neuroscience*, 18 (18) 7452-7461.

Rose, S.P. (1991). How chicks make memories: the cellular cascade from c-fos to dendritic remodelling. *Trends in Neuroscience*, 14 (9) 390-397.

Rose, S.P. (1996). Cell adhesion molecules and the transition from short to long-term memory. *Journal Physiology Paris*, 90 (5-6) 387-391.

Santín, L.J., Rubio, S., Begega, A. & Arias, J.L. (1999a). Effects of mammillary body lesions on spatial reference and working memory tasks. *Behavioural Brain Research*,*102*, 137-150.

Santín, L.J., Rubio, S., Begega, A. & Arias, J.L. (1999b). Non-effects of mamillary body lesions on spontaneous alternation: pre and postoperative study. *Behavioural Processes*, *44*, 323-329.

Sheng, M. & Greenberg, M.E. (1990). The regulation and function of c-fos and other immediately early genes in the nervous system. *Neuron*, 4, 477-485.

Shibata, H. (1993). Efferent projections from the anterior thalamic nuclei to the cingulate cortex in the rat. *Journal of Comparative Neurology*, 330, 333-342.

Struhl, K. (1991). Mechanisms for diversity in gene expression patterns. *Neuron*, 7, 177-181.

Swanson, L.W. (1981). A direct projection from Ammons´s horn to prefrontal cortex in the rat. *Brain Res*., 217, 150-154.

Tamamaki, N. & Nojyo, Y. (1993). Projection of the entorhinal layer II neurons in the rat as revealed by intracellular pressure-injection of neurobiotin. *Hippocampus*, 3, 471-480.

Van Eden, C.G., Lamme, V.A.F. & Uylings, H.B.M. (1992). Heterotopic cortical afferents to the medial prefrontal cortex in the rat. A combined retrograde an anterograde tracer study. *European Journal of Neuroscience*, 4, 77-97.

West, M.J. (1999). Stereological methods for estimating the total number of neurons and synapses: issues of precision and bias. *Trends in Neuroscience*, 22, 51-61.

Young, H.L., Stevens, A.A., Converse, E. & Mair, R.G. (1996). A comparison of temporal decay in place memory tasks in rats (rattus norvegicus) with lesions affecting thalamus, frontal cortex or the hippocampal system. *Behavioral Neuroscience*, 110 (6) 1244-1260.

Zhu, X.O., Brown, M.W., McCabe, B.J. & Aggleton, J.P. (1995). Effects of the novelty or familiarity of visual stimuli on the expression of the immediate early gene c-fos in rat brain. *Neuroscience*, 69 (3) 821-829.

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