

## Functional differences between dorsal and ventral hippocampus revealed with AgNOR staining

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Recientemente se han encontrado diferencias en cuanto a la actividad neural de la formación hipocámpica en sus zonas dorsal y ventral. Estas diferencias podrían jugar un importante papel en cuanto a la implicación diferencial de estas zonas en el aprendizaje y la memoria. Aplicamos aquí la técnica histológica de impregnación argéntica de las regiones organizadoras del nucleolo (AgNORs) con el fin de analizar la actividad biosintética de las áreas hipocámpicas CA1 y CA3 en las zonas dorsal y ventral de ratas Wistar. Se cuantificaron el número y área media de los AgNORs presentes en sus neuronas, que son indicadores indirectos de la actividad de síntesis proteica. Los resultados muestran una actividad biosintética significativamente mayor en la región dorsal de ambas áreas estudiadas, que concuerda con estudios anteriores y se discuten sus implicaciones funcionales.

*Diferencias funcionales entre el hipocampo dorsal y ventral reveladas con la tinción AgNOR.* Recently differences were reported concerning the dorsal and ventral zones of hippocampal formation. These differences may play an important role regarding the differential involvement of both zones in learning and memory. We use here the silver staining of nucleolar organizer regions (AgNORs) histological technique in order to analyze the biosynthetic activity of CA1 and CA3 hippocampal areas in dorsal and ventral zones from Wistar rats. We quantified the mean number and area of AgNORs in their neurons, which are considered as indirect indexes of protein synthesis activity. The results show a significantly higher biosynthetic activity in the dorsal region from the areas studied, which agrees with previous research. Functional implications derived from our results are discussed in this paper.

Since the first descriptions of the famous case of patient H.M. by Scoville and Milner (1957), the important role of the hippocampus in the formation of memory has been extensively documented. Until now, a vast majority of actual experimental work with animals and humans has been done considering the hippocampus as a homogeneous structure with a unified functional role (Eichenbaum et al., 1999; O'Keefe et al., 1998). However, numerous studies report the morphological, neurochemical and functional differences between dorsal and ventral zones from hippocampus (Moser et al., 1993; Jung et al., 1994; Bannerman et al., 1999). According to these findings, the experimental research made on the hippocampus, mainly by lesion techniques, shows that the effect of such disruption is different depending on the affected site (Moser et al., 1993; Lorenzini et al., 1997; Levin et al., 1999). Additionally, the behavioral effects are supported by other neurochemical and physiological heterogeneity (Bruinink and Bischoff, 1993; Jung et al., 1994).

Although a number of techniques offer a good approach to the functional study of central nervous system structures, silver staining of nucleolar organizer regions (AgNORs) is considered a ra-

pid and easy way to obtain an estimation of protein synthesis rate (Crocker, 1996). This technique makes it possible to label the fibrillar centers, a nucleolar component involved in the synthesis of ribosomal RNA. The fibrillar centers are related morphologically with the NORs, sites composed of RNA and proteins, many of them argyrophilic. By using this technique these NOR-associated proteins are selectively stained; the result of this reaction is known as AgNOR dots, and the number and area of AgNORs are an accurate index of activity and cell proliferation in terms of protein synthesis (Derenzini et al., 1992; Ploton et al., 1992). The AgNOR technique alone or combinations with other histological stains have been used in various studies of the CNS with promising results in animals and humans (Fushiki et al., 1995; González-González et al., 1996; García-Moreno et al., 1997).

By taking into consideration these studies, the present work aimed to evaluate the biosynthetic activity in CA1 and CA3 hippocampal areas from both the dorsal and ventral zones. The above-mentioned AgNOR parameters were selected as adequate indexes of neural transcriptional activity.

### Materials and method

Eight male adult Wistar rats (120 days of age) from the University of Oviedo vivarium were used. They were housed at a constant temperature of  $21 \pm 1$  °C and a 12-hour light/dark period (lights on from 8:00 to 20:00 hours) with water and food available ad libitum. All animals were handled following strictly the Euro-

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pean directives for care and use of laboratory animals (EC 86/609).

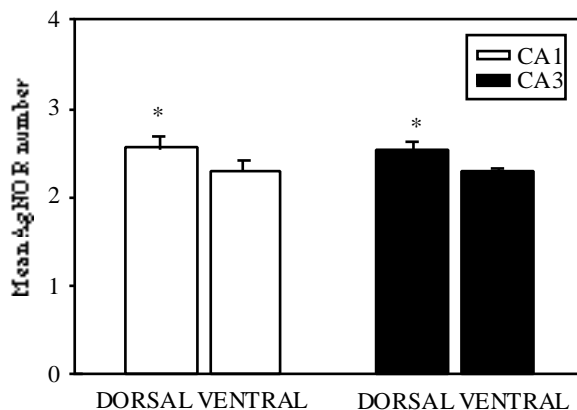
Rats were anesthetized with ether and transcardially perfused with 10% formaldehyde in phosphate buffer (0.1M, pH 7.4). Their brains were carefully removed and maintained in the same fixative for several days. Later, a tissue block comprising the region between the optic chiasm and posterior hypothalamus was embedded in paraffin. The blocks were sectioned in 15  $\mu\text{m}$ -thick coronal sections, selecting 2 out of 12 sections across the hippocampus. From each pair, only one was selected for AgNOR stain according to Ploton et al. (1992) method, with a total of 11-15 equidistant sections obtained from the entire hippocampus.

The AgNOR parameters chosen were the mean AgNOR number and area per neuron, since it was successfully used in other clinical and experimental studies (García-Moreno et al., 1993; Crocker, 1996; González-González et al., 1996); it seems that the higher the mean AgNOR number and area, the higher the transcriptional activity is. By using an optical microscope (Leica series, Germany) and a computer image analysis system (Leica Q-Win) a total of 100 pyramidal neurons were sampled from each animal in order to measure their AgNOR dots. The lateral geniculate nucleus was used as a reference to mark the boundary between the dorsal and ventral zones of hippocampus in its most caudal part.

### Results

In order to analyze the differences between the dorsal and ventral hippocampus and the regions selected, a two-way (zone  $\times$  region) repeated measures ANOVA was applied regarding the AgNOR parameters evaluated: mean AgNOR area and mean AgNOR number per neuron.

The results for the mean AgNOR number show an statistically significant difference between dorsal and ventral hippocampal zones ( $F_{1,7}=17.5$ ;  $P<0.01$ ), as shown in figure 1. No significant differences were observed for region and its interaction with zone factor. In the case of the mean AgNOR area, no differences between dorsal and ventral zones in CA1 and CA3 were found (Fig. 3). However, significant differences were found for the region factor ( $F_{1,7}=18.6$ ;  $P<0.01$ ), since mean values for CA3 were significantly higher than those from CA1 region for the latter AgNOR parameter.



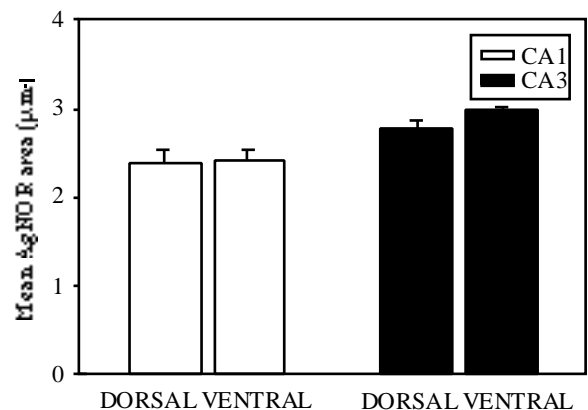
**Figure 1.** Mean AgNOR number per neuronal cell measured in the different hippocampal regions (CA1 and CA3) and zones (dorsal and ventral). Mean  $\pm$  S.E.M. \*( $P<0.01$ )

### Discussion

Our results show that there is a significant difference in the biosynthetic activity from dorsal and ventral regions from both CA1 and CA3 hippocampal areas. Moreover, the activity is higher in the dorsal region in basal condition. The parcellation of hippocampus into dorsal and ventral zones has been considered by other authors, which found morphological and functional differences that could explain the reported results (Moser et al., 1993; Jung et al., 1994). Thus, from a behavioral point of view, whereas dorsal hippocampal lesions facilitate active avoidance response in the shuttle-box, this is not the case for the ventral zone (Lorenzini et al., 1997). Conversely, after dorsal hippocampal lesions an impairment of spatial task performance is observed, not affected by ventral damage (Moser et al., 1993). Recent evidence supports a functional double dissociation between dorsal and ventral hippocampus related with different behaviors involving spatial learning and motor activity or contextually conditioned freezing (Bannerman et al., 1999; Richmond et al. 1999).

By using the silver staining of NORs, many differences were reported in other CNS structures according to their activity levels (Lafarga et al., 1995; Gonzalez-Gonzalez et al., 1996). Changes in neuronal transcriptional activity may be related in adult CNS with protein synthesis, and the latter would reveal the modification of several functional parameters such as long-term potentiation (LTP), since blocking biosynthetic activity ceases (Stanton and Sarvey, 1984; Otani et al., 1992). Additionally, the relationship between transcriptional activity and cell firing rate is well known, a fact that would explain the reported differences in the frequency and duration of electrical responses between dorsal and ventral hippocampus (Jung et al., 1994). We should take into consideration that the dorsal hippocampus receives the majority of sensorial input from the cortex and parahippocampal structures (Room and Groenewegen, 1986; Witter, Van Hoesen and Amaral, 1989); this would explain a higher synaptic activity of the dorsal subdivision, reflected in a higher biosynthetic activity.

The results concerning the higher activity of CA3 region, manifested by a high AgNOR area should be considered in synaptic terms. Particularly, the CA3 region receives a number of inputs by the mossy fibers from the dentate gyrus, and the latter from the entorhinal cortex via the perforant path; this means that all the information that the hippocampal formation receives establishes synap-



**Figure 2.** Mean AgNOR area per neuron in the studied hippocampal subdivisions. Significant differences were observed only between CA3 and CA1 regions ( $P<0.01$ ).

tic contacts mainly with CA3 and not CA1, so that the postsynaptic activity of CA3 pyramidal cell would be logically high and therefore its biosynthetic demands.

In conclusion, although in numerous investigations the hippocampus is considered as a whole, our data question this assumption clearly. Thus, it may be inappropriate to formulate hypotheses about a unitary function of hippocampal function. In this way, this work demonstrates that significant differences in activity are found between the dorsal and ventral hippocampus. By taking this results

into consideration, future research is necessary in order to explain more precisely the behavioral and physiological significance of this heterogeneity in the hippocampal formation.

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