Histol Histopath (1994) 9: 715-721

Histology and Histopathology

Postnatal development of the Ammon's horn (CA1 and CA3 fields). A karyometric and topographic study

M.M. Pérez Delgado, P.G. Serrano Aguilar, A. Castañeyra Perdomo and R. Ferres Torres
Department of Anatomy, Pathology and Histology, Faculty of Medicine, University of La Laguna, Tenerife, Spain

Summary. We have performed a karyometric study of the pyramidal neurons of CA1 and CA3 fields of the Ammon's horn, in male mice aged from the 5th to the 190th postnatal day. Nuclear sizes were measured with the aid of a Magiscan Analysis System, used in an interactive form, in both superficial and deep layers of the stratum pyramidal in those fields. The measurements were made at three different topographic levels: rostral; intermediate; and caudal, to detec any possible difference related to the topography of the neuron in the same field. We have found that both CA1 and CA3 fields are correlated in the postnatal development of their nuclear pyramidal sizes and that all topographic levels of the hippocampus reach their highest karyometric sizes at the 10th-15th postnatal day. Caudal levels show higher karyometric values than the other levels and some differences between neurons of the superficial and deep layers of both fields are also described here and analysed in relation to the different ontogenetic gradients of these cells.

Key words: Ammon's horn, Morphometry, Mouse, Development, Topography

Introduction

We have previously described the postnatal nuclear development of the nuclear cells of the dentate gyrus (Pérez Delgado et al., 1992), in relation to their topographic location and the ontogenetic gradients described by Schlessinger et al. (1975) for those cells. Now, we have centred our interest in the Ammon's horn, specifically CA1 and CA3 fields, in order to analyse their karyometric postnatal development in relation to the topographic location of the cells and/or their ontogenesis (Bayer, 1980).

Ammon's horn presents a relatively simple and homogeneous structure, the pyramidal cells being the predominant ones in it. Bayer (1980) found that the pyramidal stratum of Ammon's horn appears between the 17th and 19th embryonic day, and greatly lengthens between the 21st embryonic day and the first postnatal day; cell migration to the pyramidal stratum of the hippocampus would end during the early neonatal phase (Minkwitz and Holz, 1975). Bayer (1980) described different ontogenetic gradients in the hippocampal formation; the deepest cells would be generated before the superficial ones and cells closer to the rhinal fissure before those lying farther away, and they also described a «sandwich gradient» (i.e., later forming cells are flanked by earlier forming superficial and deep cells).

However, no data exist on the development of the nuclear sizes of the pyramidal cells of CA1 and CA3 in the postnatal phase, and we have asked ouselves if both CA1 and CA3 fields show similar or different postnatal karyometric development and if this postnatal development could be affected by the ontogenetic gradients or by different afferents, depending on the topography of the neuron in the hippocampal formation, as several authors have reported differences in the serotoninergic innervation, depending on the zone of the hippocampus (Gage and Thompson, 1980; Kohler, 1982; Ihara et al., 1988; Oleskevich and Descarries, 1990). The aim of our study is to add new data about these questions that could help to a better morphological and functional understanding of Ammon's horn, serving as a base for future experiments in this hippocampal formation. We have analysed the nuclear size, which is an index of the functional activity of the neuron (Edstrom and Eichner, 1960; Pérez Delgado, 1984; Smialowska et al., 1988) of the pyramidal cells of CA1 and CA3 in Ammon's horn, in a superficial and a deep layer, and at three topographic levels of Ammon's horn: rostral, intermediate and caudal.

Materials and methods

We studied 60 male albino mice, divided into 12 age groups (5 mice per age) between the 5th and the 190th

Offprint requests to: Dr. M.M. Pérez Delgado, Departamento de Anatomía, Anatomía Patológica e Histología, Universidad de La Laguna, 38320 Santa Cruz de Tenerife, Spain

postnatal day. Animals were anaesthetized with Nembutal and perfused intracardically with Bouin's solution. The brain was postfixed for 24 hours in the same solution, dehydrated and embedded in paraffin following standard protocol. Serial coronal sections of 10 µm were performed and thereafter stained by the Klüver Barrera method. Fig. 1 shows the three topographic levels studied of the total rostro-caudal length of the hippocampus in an adult mouse: rostral (1.5/10), intermediate (5/10) and caudal (6/10).

We analysed CA1 and CA3 pyramidal cells in a superficial and a deep stratum separated by a subjective visual plane passing through half of the total thickness of the pyramidal layer (Fig. 2). With the help of a Magiscan Analysis Image System (Joyce-Loebl), and using the Genias program in an interactive form, we determined the nuclear sizes in the different centres and topografic locations. We measured all the whole pyramidal nuclei presenting one or more clearly visible nucleoli. To obtain a security index of 96%, in each location we meassured 30-40 nuclei. The nuclear parameters analysed were: nuclear perimeter; nuclear area; nuclear maximum diameter; and form factor, an index which expresses the approximation of the nuclear shape to a circle. As the three first parameters show a similar evolution of their values, we will only describe the nuclear area results. The form factor did not show significant variations in any of the studied centres, and we will omit to refer to it. Magnification used for the study was x2675. Data were statistically analysed by an ANOVA and Newman-Keuls test, to compare the results between the different ages. Pearson's test was also employed to establish the correlation between the different patterns of development, and Student's t-test was used to compare values at the different topographic values.

Results

Ammon's horn: CA1 (Figs. 3, 4)

As both the superficial and deep layers showed a similar development in all the topographic levels, we represented only the graphs of development of the

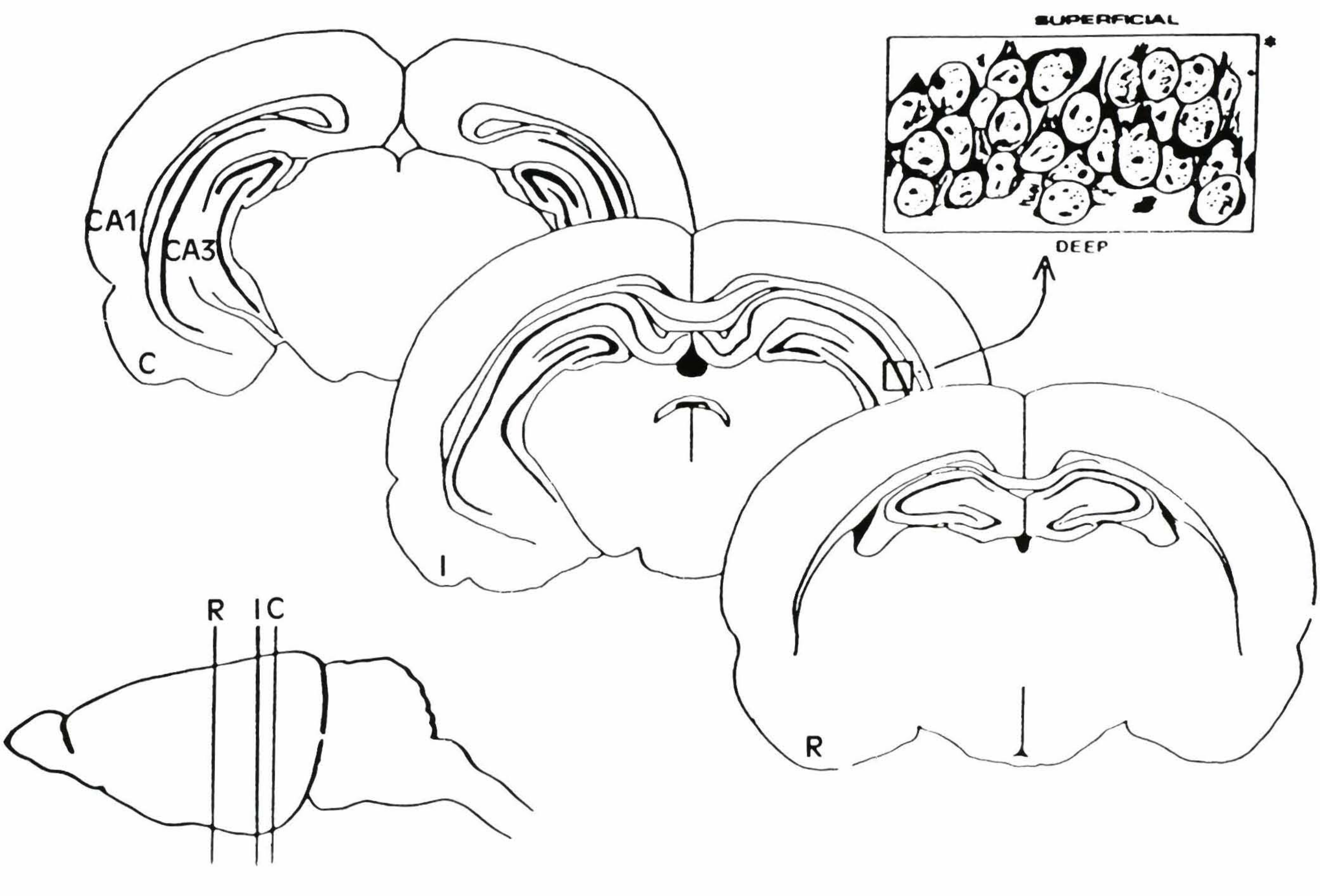


Fig. 1. Representation of the three studied topographic levels in the brain of the mouse. **A)** rostral level; **B)** intermediate level; and **C)** caudal level. With respect to the surface of the brain the superficial and deep layers of CA1 and CA3 are measured separately. The lateral view of the mouse brain shows the location of the three levels that correspond, approximately, to 1.5/10 (A), 5/10 (B) and 6/10 (C) of the total rostrocaudal length of the hippocampus of the mouse.

superficial layer of CA1 in the three topographic levels (Fig. 3). Figure 4 shows the superficial and deep layers of CA1 at the rostral level for comparison of their developmental patterns. We observed a significant increase in the values from the 5th to the 10th day ($p< 0.01$), and a posterior stabilization in the rostral level, while in the intermediate and caudal levels, values increased up to the 15th day. In the intermediate level, nuclear area decreased at the 20th day ($p< 0.05$), while in the caudal level, it stabilized after the 15th day. Pearson's test between both layers was highly significant ($p< 0.001$), indicating a high positive correlation in their development in the three studied topographic levels. Student's t-test was used to compare the values of the superficial and deep layers, and was significative at the 5th day, at the three levels, but with a lower value in the deep layer ($p< 0.05$) (Fig. 4).

Rostrocaudal analysis (Table 1):

The correlation between the different topographic levels (rostral, intermediate and caudal) of the deep layer was high ($p< 0.001$), while in the superficial layer the rostral and intermediate levels were the most related ($p< 0.001$).

The rostral level, in the superficial and deep layers, tended to show lower values than the intermediate and the caudal levels, with significant differences at different ages.

Ammon's horn: CA3 (Fig. 5)

As the correlation between the layers was high ($p< 0.001$) in all levels, we only represent in Figure 5 the superficial layer, pointing out in the text the statistical differences found in some cases with respect to the deep layer. Figure 4 shows graphically the comparison between the superficial and deep layers of CA3 in the rostral level. In the rostral level, although values of nuclear area increased significantly in both layers from the 5th to the 10th day ($p< 0.01$), the posterior evolution was different. In the superficial layer, values decreased from the 10th to the 85th day ($p< 0.01$), showing significantly higher values at the 190th day than those obtained at the first studied age ($p< 0.01$). In the deep layer values decresased at the 20th day ($p< 0.01$) and stabilized and no differences were detected between the 5th and the 190th day (Fig. 4).

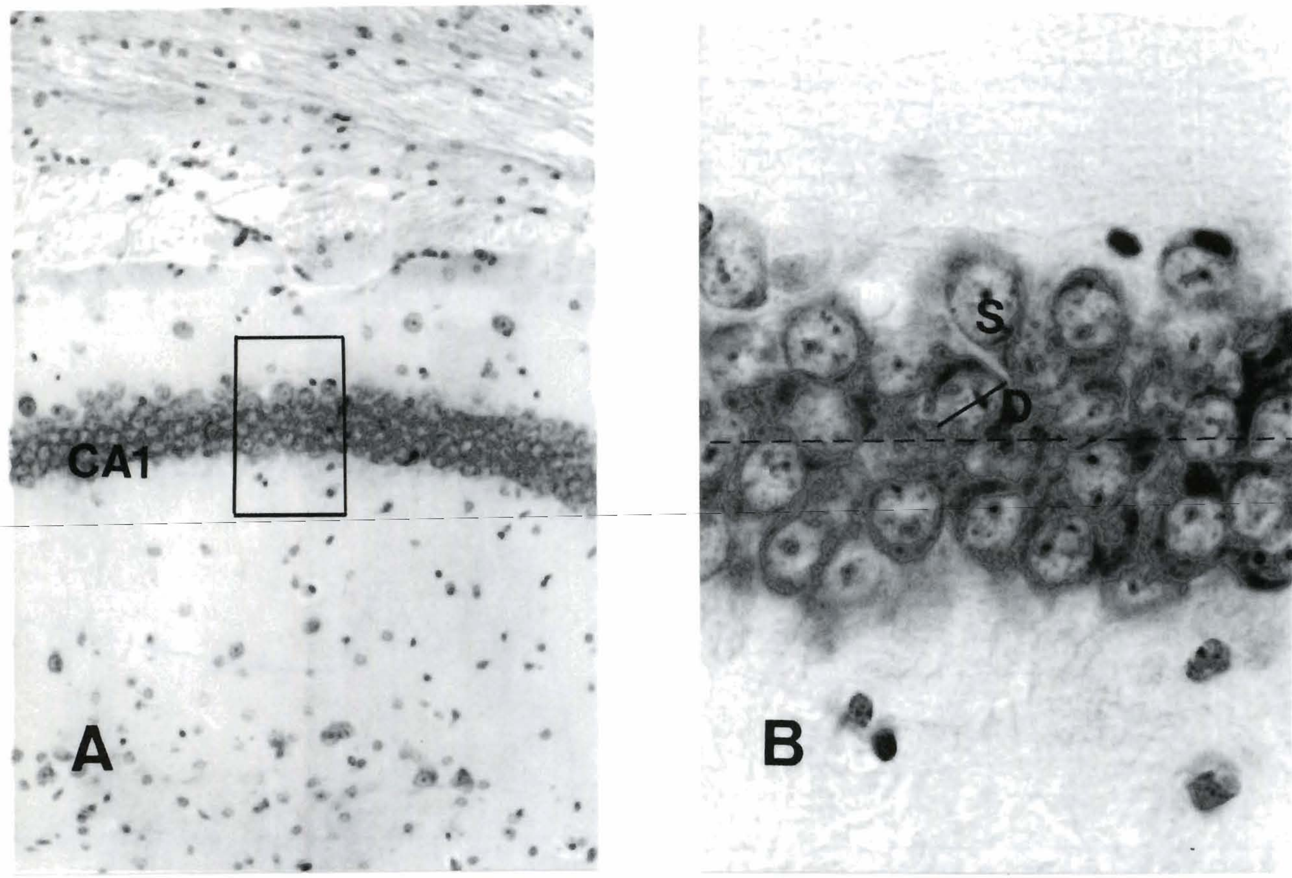


Fig. 2. Pyramidal layer of the CA1 field in the Ammon's horn of the adult male mouse **(A)**. A visual plane passing through the half of the total thickness of the layer **(B)** constitutes the separation between the superficial and deep layers; only the nuclei presenting visible nucleoli and clearly located in the external or the internal stratum were measured. S: nuclear area; D: maximum diameter. A: x 125; B: x 1,250

Table 1. Signification of the Pearson's test of correlation between the different topographical levels of the CA1 field of the Ammon's horn in both superficial and deep layers..

LEVELS	LAYER	SIGNIFICATION (P value)
Rostral-intermediate	Superficial	0.001
	Deep	0.001
Rostral-caudal	Superficial	N.S.
	Deep	0.001
Intermediate-caudal	Superficial	0.05
	Deep	0.001

N.S.: not significant.

Table 2. Signification of the Pearson's test of correlation between the different levels and layers of CA3.

LEVELS	LAYER	SIGNIFICATION (P value)
Rostral-intermediate	Superficial	0.001
	Deep	0.01
Rostral-caudal	Superficial	0.01
	Deep	0.05
Intermediate-caudal	Superficial	0.001
	Deep	0.001

Table 3. Significations of the Pearson's test of correlation between the different levels and layers of CA1 respecting to CA3.

LEVELS	LAYER	SIGNIFICATION (P value)
Rostral	Superficial	0.001
	Deep	0.01
Intermediate	Superficial	0.01
	Deep	0.001
Caudal	Superficial	0.01
	Deep	0.001

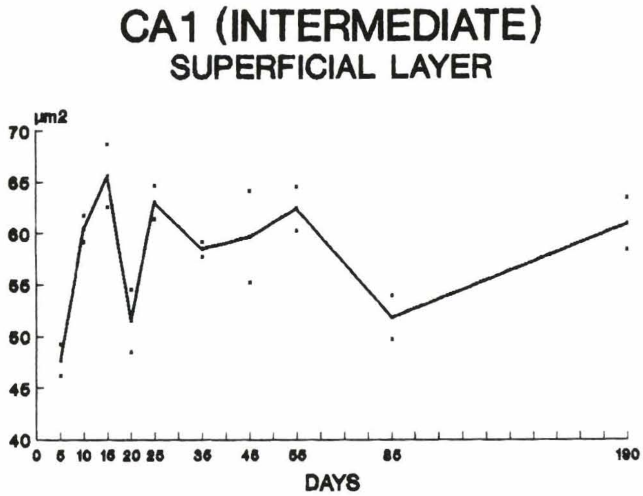
In the intermediate level, values increased from the 5th to the 10th day ($p< 0.01$) and, in the superficial layer, decreased from the 15th to the 190th day ($p< 0.05$), while in the deep layer no more significant differences were detected after the 10th day.

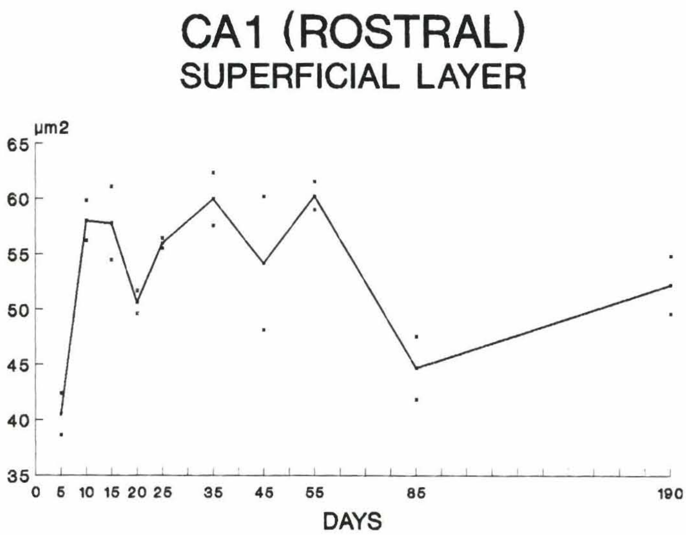
In the caudal level, values increased in both layers, from the 5th to the 15th day ($p< 0.01$) and stabilized, with significantly higher values at the 190th day than those at the 5th day ($p< 0.05$).

At the 5th day, values at the superficial layer were significantly lower than those of the deep layer in the rostral ($p< 0.01$, Fig. 4) and caudal level ($p< 0.05$), contrary to CA1.

Rostrocaudal analysis (Table 2):

Pearson's test showed that the most related levels were the intermediate and the caudal ones.





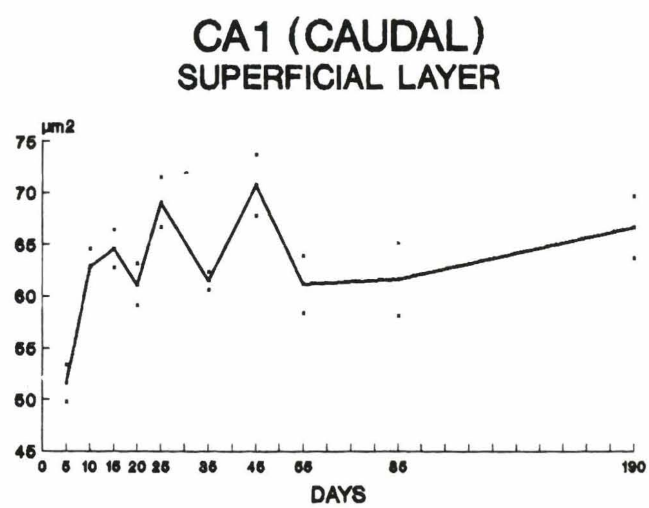


Fig. 3. Graphs showing the development from the 5th to the 190th postnatal day of the nuclear area sizes in the superficial layer of CA1 in the three topographic levels analysed: rostral, intermediate and caudal. The points above and below the line indicates the SEMs.

Similarly to CA1, CA3 also tended to show lower values at both layers of the rostral level.

Correlation CA1/CA3 (Table 3)

Both fields were significantly correlated at all levels through postnatal development of their nuclear sizes, as shown in Table 3 and in figure 4.

Discussion

Nuclear size of the neurons of the stratum pyramidal of CA1, increased from the 5th to the 10th day in the rostral level, while in the intermediate and caudal level they increased up to the 15th day, with caudal levels showing higher karyometric values than the other levels. Bayer (1980) described a deep-superficial gradient for the neurogenesis of the pyramidal neurons of the hippocampus. We have found that deep cells, although originating first, developed their nuclear sizes more slowly than the superficial cells, as at the 5th day they showed lower values than the superficial ones, without significant differences in the other studied ages.

Our results agree with those of Smollich and Matzke (1986), who also described an increase in the nuclear volume of pyramidal CA1 neurons between the 5th and the 10th postnatal day, coinciding with the period of general growth of the CNS. This increase of nuclear volume of pyramidal cells has also been described by Rami et al. (1986). Dendritic spines of CA1 pyramidal neurons also show a similar pattern of growth, although more protracted in time (Meyer and Ferrers Torres, 1978; Meyer et al., 1978), and Rabie et al. (1979) described an increase of mitotic activity and cellularity

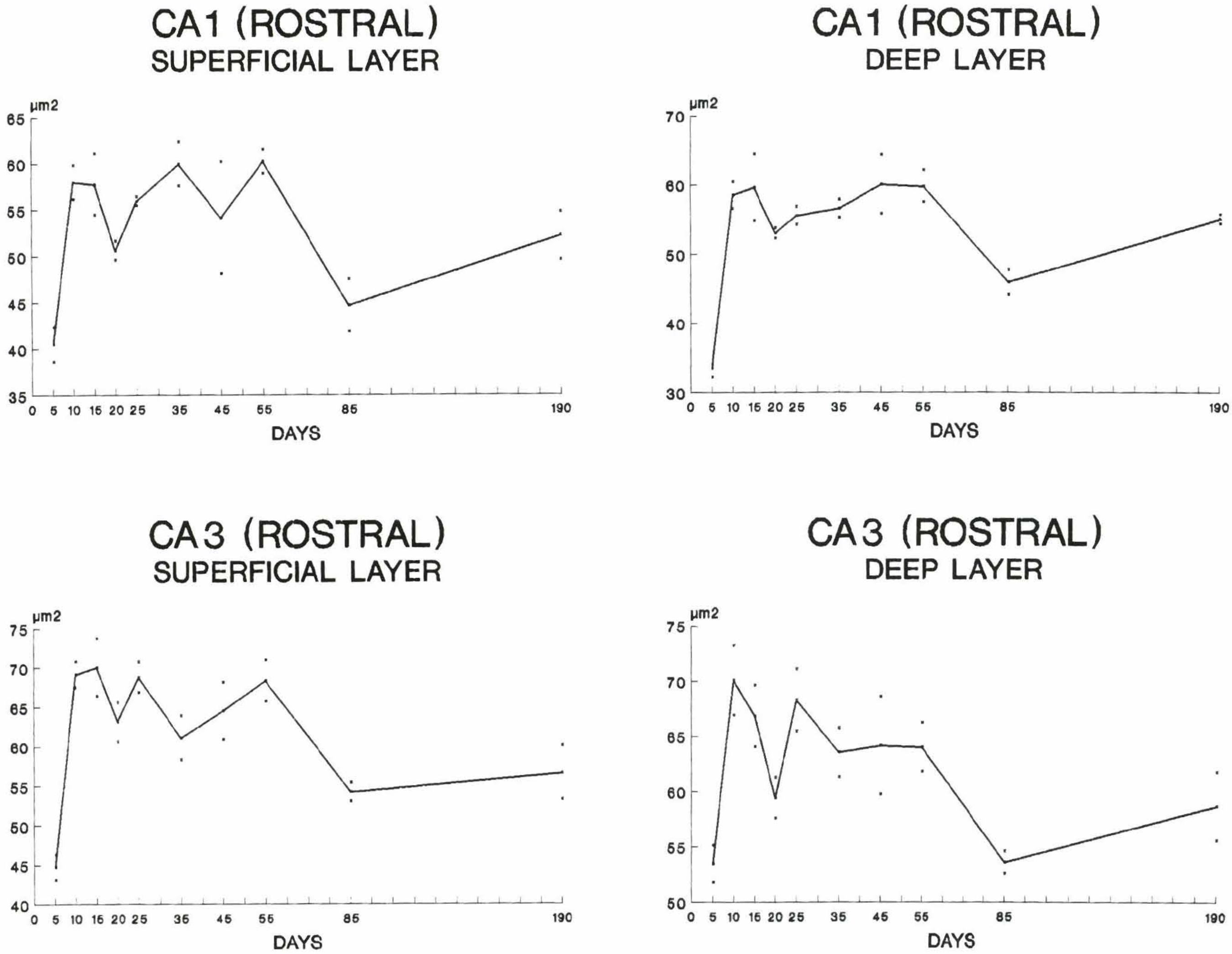


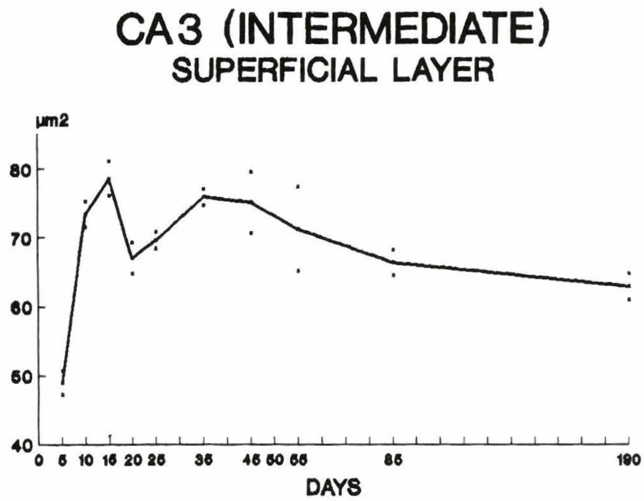
Fig. 4. Graphs showing the development of the nuclear area sizes in the postnatal period analysed (5-190 days) of the fields CA1 and CA3 of the Ammon's horn, in the rostral topographical level and in both superficial and deep layers of both fields. The points above and below the line indicates the SEMs.

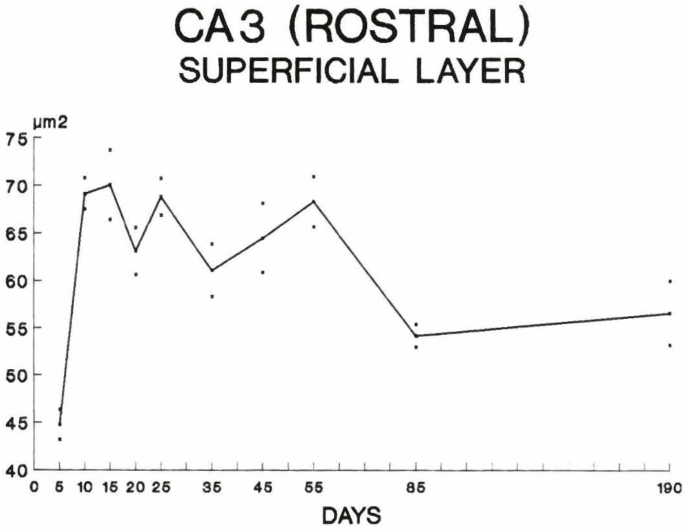
in the hippocampus during the first 10 postnatal days. We relate the increase of nuclear sizes with an increase in the functionality of the neuron, in accordance with other authors (Edstrom and Eichner, 1960; Pérez Delgado, 1984; Smialowska et al., 1988).

Wimer et al. (1988) found a decrease in the number of granular cells of the dentate gyrus between the 20th and the 27th day of life. We have observed a decrease of nuclear sizes at the 20th day at the intermediate level of CA1, and at the deep layer of CA3 intermediate level. This decrease has also been observed in the paraventricular (Pérez Delgado, 1984), and ventromedial hypothalamic nucleus (Pérez Delgado et al., 1985), and could be related to an overgrowth and posterior stabilization on optimal levels, according to the functionality of the nucleus (Zilles et al., 1976).

Although increases of functionality in the hippocampus between the 35th and 45th day have been reported by several authors (Meyer and Ferres Torres, 1978; Meyer et al., 1978; Smollich and Matzke, 1986) and related to the pubertal increase of testosterone (Jean Faucher et al., 1978), in the rat (Smollich and Matzke, 1986, karyovolumetrically) or in the male mouse (Meyer and Ferres Torres, 1978; Meyer et al., 1978; counting dendritic spines), we have not found a pubertal increase in nuclear sizes in the hippocampus, which we did detect in other centres, such as the paraventricular (Pérez Delgado, 1984) and ventromedial hypothalamic nuclei (Pérez Delgado et al., 1985), and area postrema and subfornical organ (Castañeyra Perdomo et al., 1988), that could be interpreted as a close relationship of these centres to endocrine activity.

Neurons at CA3 level showed similar developmental patterns to those of CA1 in the different topographic divisions. This fact was corroborated by a significant correlation between both CA1 and CA3 fields with Pearson's test. Bayer (1980) found that the CA3 field of Ammon's horn appears before the CA1. This different chronology of formation is not reflected in the karyometric sizes, as both fields show similar patterns of development and reach their maximum values at the same ages. Our results in CA3 also coincided with those described by Rami et al. (1986) for the development of the volume of neuronal soma of pyramidal cells in CA3, and with those reported by Meyer and Ferres Torres (1978) for the dendritic spines in this field. We found that the superficial cells grew more slowly than the deep cells, the difference being significant at the 5th day. This fact could be related to their different ontogenesis (Bayer, 1980). The contradiction between the results here and those described in CA1, could be due to the characteristic folds of the adult mammalian hippocampus, conditioning a different relation of both fields,





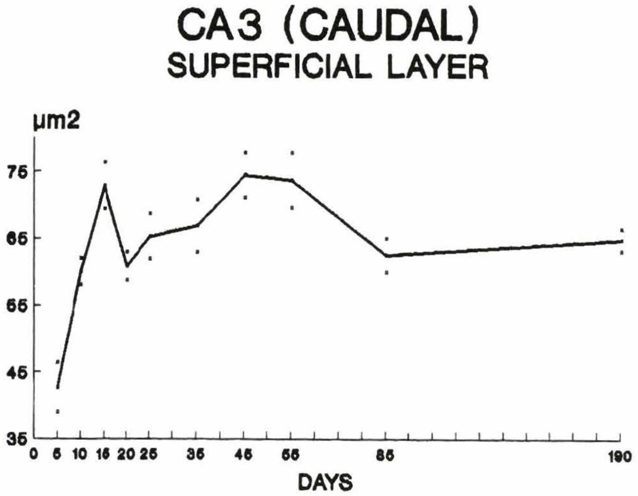


Fig. 5. Graphs showing the development of the nuclear area sizes of the superficial layer of the CA3 field of the Ammon's horn in the three topographical levels analysed (rostral, intermediate and caudal). The points above and below the line indicate the SEMs.

CA1 and CA3, with respect to the brain surface. In the dentate gyrus, we have described different gradients of postnatal karyometric development, which seem to be closely related to the ontogenetic gradients of the granular cells (Pérez Delgado et al., 1992). On the other hand, in Ammon's horn, we have not detected a clear relation between postnatal nuclear size development and neuronal ontogenesis or neuronal input, although our morphological study does not exclude that these factors could influence more indirectly on karyometric development.

Karyometric values of the pyramidal cells of CA3 are larger than those of CA1, except at the oldest ages of this study, and this agrees with the known fact that CA3 and CA2 present the largest pyramidal cells of the hippocampus.

In conclusion, we have found: 1) that CA1 and CA3 are related in their karyometric development; 2) that rostral levels reach their higher karyometric values earlier; 3) generally, the caudal level of the hippocampus shows the highest nuclear sizes in both CA1 and CA3 fields of Ammon's horn; and 4) in the first days of life, differences are found between superficial and deep layers, although these differences are not clearly related to the ontogenetic gradients described by Bayer (1980) in CA1.

Acknowledgements. This study was supported by a grant from the Gobierno de la Comunidad Autónoma de Canarias (Spain).

References

Bayer S.A. (1980). Development of the hippocampal region in the rat. I. Neurogenesis examined with 3H-thymidine autoradiography. J. Comp. Neurol. 190, 87-114.

Castañeyra Perdomo A., Pérez Delgado M.M., González Hernández T. and Ferres Torres R. (1988). Development of the subfornical organ and area postrema of the male albino mouse. Karyometric effect of neonatal and prepubertal castration. Acta Anat. 131, 13-25.

Edstrom J.E. and Eichner D. (1960). Qualitative und Quantitative Ribonukleinsaureuntersuchungen an den Ganglienzellen der NN. supraopticus und paraventricularis der Ratte unter normalen und experimentellen Bedingungen (Kochsalzbelastung). Anat. Anz. 108, 312-319.

Gage F.H and Thompson R.G. (1980). Differential distribution of norepinephrine and serotonin along the dorsal ventral areas of the hippocampal formation. Brain Res. Bull. 5, 771-773.

Ihara N., Ueda S., Kawata M. and Sano Y. (1988). Immunohistochemical demonstration of serotonin-containing nerve fibers in the mammalian hippocampal formation. Acta Anat. 132, 335-246.

Jean Faucher Ch., Berger M., de Turckheim M., Veyssiere G. and Jean C.L. (1978). Developmental patterns of plasma and testicular testosterone in mice from birth to adulthood. Acta Endocrinol. 89, 760-788,

Kohler C. (1982). On the serotoninergic innervation of the hippocampal region: an analysis employing immunohistochemistry and retrograde fluorescent tracing in the rat brain. In: Cytochemical methods in neuroanatomy. Chan Palay V. and Palay S.L. (eds). Alan R. Liss. New York. pp 387-405.

Meyer G. and Ferres Torres R. (1978). Quantitative altersabhangige Variationen der Dendritenspines im Hippocampus (CA1, CA3 und Fascia Dentata) der Albinomaus. J. Hirnforsch. 19, 371-378.

Meyer G., Ferres Torres R. and Mas M. (1978). The effects of puberty and castration on hippocampal dendritic spines of mice. A golgi study. Brain Res. 155, 108-112.

Minkwitz H. and Holz L. (1975). Die Ontogenetische Entwicklung von Pyramideneuronen aus dem Hippocampus (CA1) der Ratte. J. Hirnsforsch. 16, 37-54.

Osleskevich S. and Descarries L. (1990). Quantified distribution of the serotonin innervation in adult rat hippocampus. Neuroscience 34, 19-33.

Pérez Delgado M.M. (1984). Karyometric development of four topographic subdivisions of the paraventricular nucleus in normal and castrated mice. Brain Res. 310, 363-366.

Pérez Delgado M.M., Serrano Aguilar P.G. and Ferres Torres R. (1985). Effects of gonadectomy on the karyometric development of the ventromedial nucleus of the hypothalamus in the mouse. Brain Res. 333, 355-358.

Pérez Delgado M.M., Serrano Aguilar P.G., Castañeyra Perdomo A. and Ferres Torres R. (1992). Postnatal development of the dentate gyrus. A karyometric and topographic study. Acta Anat. 144, 160-166.

Rabie A., Patel A.J., Clavel M.C. and Legrand J. (1979). Effect of thyroid deficiency on the growth of the hippocampus in the rat. A combined biochemical and morphological study. Develop. Neurosci. 2, 183-194.

Rami A., Patel J. and Rabie A. (1986). Thyroid hormone and development of the rat hippocampus: morphological alterations in granule and pyramidal cells. Neuroscience 19, 1217-1226.

Schlessinger A.R., Cowan W.M. and Gottlieb D.I. (1975). An autoradiographic study of the time of origin and the pattern of granule cell migration in the dentate gyrus of the rat. J. Comp. Neurol. 159, 149-176.

Smialowska M., Bal-Klara A. and Smialowski A. (1988). Chronic imipramine diminishes the nuclear size of neurons in the locus coeruleus and cingular cortex but not in the hippocampus of the rat brain. Neuroscience 26, 803-807.

Smollich A. and Matzke M.C. (1986). Aktivitätsschwankungen und Sexualdimorphismus hippocampaler Pyramidenneurone im Verlauf der postnatalen Entwicklung der Ratte. J. Hirnforsch. 27, 5-17.

Wimer R.E., Wimer C.C. and Alameddine L. (1988). On the development of strain and sex differences in granule cell number in the area dentata of house mice. Dev. Brain Res. 42, 191-197.

Zilles K., Scheider A. and Wingert F. (1976). Quantitative analyses des Wachstums der Frischvolumina limbischer kerngebiete im Diencephalon und Mesencephalon einer ontogenetischen Reihe von Albinomausen. J. Hirnforsch. 17, 1-10.

Accepted June 22, 1994