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ANATOMICAL ASYMMETRIES IN THE HUMAN HIPPOCAMPAL FORMATION

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ABSTRACT

Unbiased stereological methods were used to evaluate whether in humans there are right/left asymmetries in the morphology of the hippocampal formation. The quantitative parameters analyzed were the volume of the layers, the total number of neurons and the mean neuronal volume. In the dentate gyrus, the estimations were carried out in the granule cell layer and in the polymorphic layer (hilus); in the hippocampus proper they were undertaken in the pyramidal cell layers of the CA1 and CA3-2 fields. The analyses were performed in glycolmethacrylate-embedded sections, sampled using a systematic random procedure, from six right and six left hippocampal formations. The volume of the layers was estimated by applying the Principle of Cavalieri; the total number of neurons in each layer was estimated by means of the optical fractionator; the mean somatic and nuclear volumes of the granule, hilar and pyramidal neurons were estimated by using vertical sections and the nucleator method.

The right hippocampal formation contained 20% more granule cells and 14% more CA3-2 pyramidal neurons than the left. No right/left asymmetries were found in the number of hilar and CA1 pyramidal cells. The volume of the cell-containing layers and of the hilus was similar in the right and left hippocampal formations. Likewise, no right/left differences were detected in the mean somatic and nuclear volumes of the granule, hilar and pyramidal neurons.

Key words: asymmetry, hippocampal formation, human, neuronal numbers, vertical sections, volumes.

INTRODUCTION

It has been recognized since long that the two cerebral hemispheres are not similar as regards the way they process information. Formerly, it was accepted that only subtle anatomical and physiological asymmetries were present in the human brain but, in spite of this, they were regarded as underlying the still controversial problem of hemispheric dominance (Galaburda et al., 1978). However, the studies carried out during the past three decades have shown that many cognitive and behavioral asymmetries can be ascribed to striking anatomical and/or neurochemical asymmetries of the brain. Probably because it is considered the hallmark of human evolution, the neocortex has

been the area most intensively investigated for the presence of morphological differences between the right and the left hemispheres. As a result, there are reports showing asymmetries in parameters such as the length and course of the cerebral sulci, the size and shape of specific cortical areas, the thickness of the gray matter, the number of neurons, the branching density of dendritic trees and the length of dendrites (Hellige, 1993b; Paus et al., 1996; Pakkenberg and Gundersen, 1997).

Conversely, the human hippocampal formation has not been much investigated despite its recognized involvement in functions of language, learning, memory and emotion (Nolte, 1993), which are known to display right/left asymmetry in humans (for review see Hellige, 1993a). Since the pioneering work carried out in the human hippocampal formation by Mouritzen Dam (1979), which showed that there are no right/left differences in the numerical density of neurons in the hippocampus and dentate gyrus, no further morphological studies have explored such possibility. However, in a recent study in which the volume of the hippocampal formation was estimated using magnetic resonance imaging techniques it was shown that the right hippocampal formation is larger than the left (Giedd et al., 1996).

Based on these reports and on animal studies which suggest that the organization of the hippocampal formation might not be identical in both hemispheres (Valdes et al., 1981; Diamond et al., 1982, 1983; Slomianka and West, 1987) we have searched for the presence of right/left asymmetries in the morphology of the human hippocampal formation. For this purpose, we have used unbiased stereological methods to estimate absolute quantities, e.g. volumes, total number of neurons and mean somatic and nuclear volumes, in the cellular layers of the dentate gyrus and hippocampus proper.

Because evidence has been accumulating for the presence of gender-related differences in cerebral lateralization and cognitive functioning (Falk, 1986; Shaywitz et al., 1995; Kimura, 1996), and animal studies have also shown that the morphology of the hippocampal formation is sexually dimorphic (Madeira et al., 1991a, b, 1992), only males were incorporated in this study.

MATERIAL AND METHODS

Material

Brains from autopsy cases were collected between 1992 and 1994 at the Medical Legal Institute of Porto from individuals who died suddenly after traumatic accidents not involving the skull. The material used in this study comprised brains from twelve right-handed males, average age 30 years (range 20-54 years), that were collected after a mean postmortem delay of 32.3 h. None of the subjects had shown signs of neurological or psychiatric disorders prior to death neither medical records of alcohol or drug abuse.

Preparation of the tissue

The left and the right temporal lobes were isolated from the cerebral hemispheres and fixed in 4% paraformaldehyde for at least 3 months after the autopsy. The temporal lobes were then dissected in order to free the hippocampal formations. The left and the right hippocampal formations were alternately selected for the stereological analyses. The sampled hippocampal formations were then embedded in 7% agar and sliced in the coronal plane in 3.7 mm-thick parallel slices, using a device specially built for this purpose (Fig. 1). The position of the first cut with respect to the rostral pole of the

hippocampal formation was random within the first 3.7 mm interval. This way, 12-14 slices were obtained for each hippocampal formation.

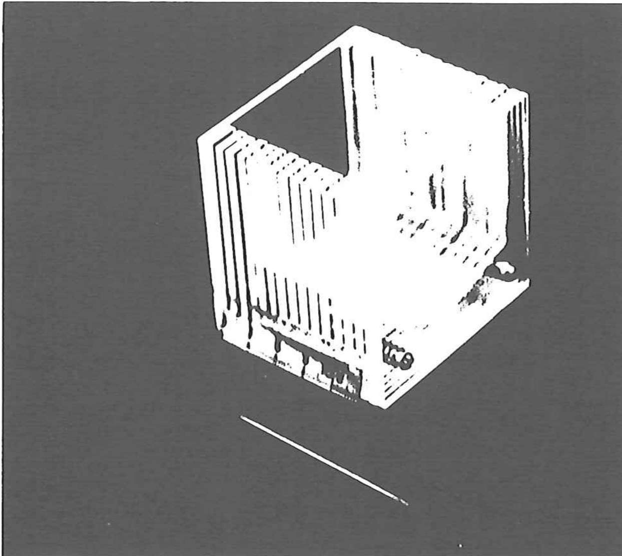

Fig. 1. Apparatus developed for obtaining 3.7-mm-thick slices from the human hippocampal formation. The disposable blade (shown below) is moved through the gaps between the acrylic components of the apparatus. The distance between the gaps can be regulated.

Before starting the histological processing of these slices, a subset of 6-7 slices per each hippocampal formation was temporary formed in order to sample material for generating vertical sections. For this purpose, starting with a random number between the primitive slices 1 and 2 (the two most rostral slices), alternate slices were selected (Fig. 2). From each 3.7 mm-thick selected slices, a 2 mm-thick section was cut in the coronal plane from the caudal face of each slice. These sections were collected and processed separately. Thus, the 12-14 slices obtained from each hippocampal formation for the estimation of total neuron numbers and volumes of the layers had different thickness: half of them were 3.7 mm-thick (the primitive slices), whereas the others, from where the material used to generate vertical sections was removed, were 1.7 mm-thick. Although with different thickness, the 12-14 slices from each hippocampal formation were afterwards ordered the way they were when the whole hippocampal formation was cut at regular intervals of 3.7 mm (Fig. 2).

These slices were dehydrated through a graded series of ethanol solutions, as follows: they were kept overnight in a 70% ethanol solution and thereafter immersed in 90%, 96% and 99% ethanol solutions, for 90 min each; finally, they were dehydrated in absolute ethanol for further 90 min. The slices were infiltrated with resin, without hardener, for 9 days: in the first 3 days the resin was changed daily and thenceforth it was changed every other day; the infiltration solution of the first bath was composed of 50% absolute ethanol and 50% resin. The slices were then embedded in glycolmethacrylate (hydroxyethylmethacrylate, Technovit 7100, Kulzer and Co., Wehrheim, Germany) with

the rostral face down. From each embedded slice, one 50 μm-thick section was cut using a Jung Multicut microtome. The sections were mounted serially and stained with a Giemsa solution modified for use in glycolmethacrylate-embedded sections (West et al., 1991).

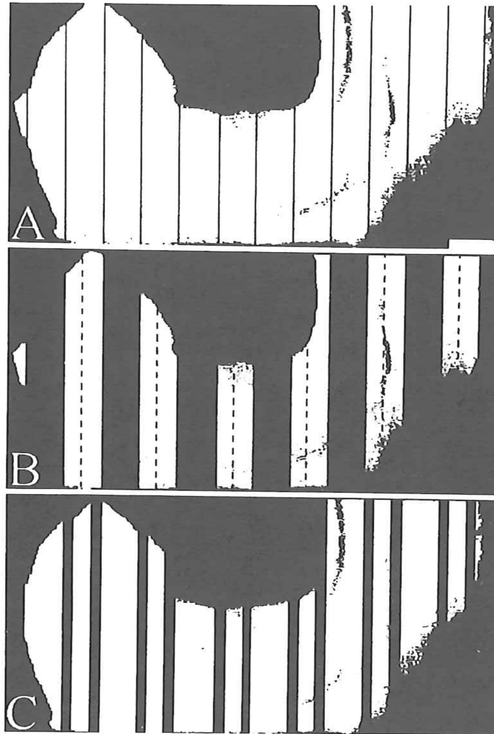

Fig. 2. Schematic illustration of the technique used to obtain sections for estimating neuron numbers, volumes of the layers and mean cell volumes from the same hippocampal formation. A. Lateral view of a left human hippocampal formation. Lines represent the section cuts performed at 3.7 mm intervals. Magnification bar = 5 mm. B. Subset of 7 alternate slices of the hippocampal formation shown in A from which material was obtained for generating vertical sections. Dotted lines represent section cuts between rostral 1.7 mm-thick and caudal 2 mm-thick slices. C. Reconstruction set of 13 slices with alternating thickness, 3.7 and 1.7 mm, respectively. The 50 μm-thick sections used to estimate the volumes of the layers and the total number of neurons were obtained from all the represented slices.

In order to generate vertical sections (Baddeley et al., 1986) each 2 mm-thick slice was cut roughly perpendicular to the alvear surface of the hippocampal formation (the horizontal plane), at random orientations around the vertical direction, into 2 mm-wide tissue bars (Fig. 3). These bars were processed as described above, systematically randomly rotated around their longitudinal axes and embedded side-by-side in glycolmethacrylate. The blocks containing the tissue bars were sectioned exhaustively using a microtome setting at 50 µm. Starting randomly, every 18th section was collected, resulting in a total of 12-14 sections per each hippocampal formation. Sections were then stained as stated above.

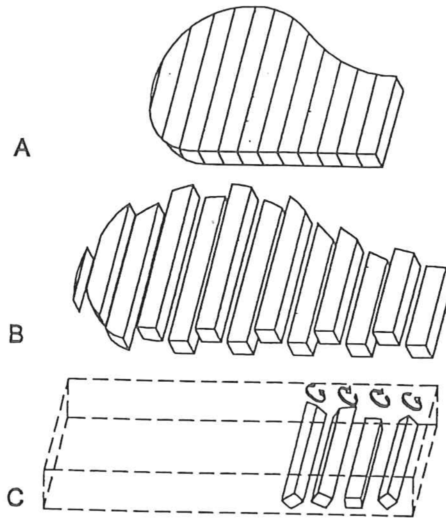

Fig. 3. Schematic illustration of the method followed to generate vertical sections. A. A 2 mm-thick slice of the hippocampal formation was cut into 2 mm-wide tissue bars roughly perpendicular to the alvear surface (horizontal plane). B. 2 mm-wide tissue bars are seen separately prior to random rotation around the vertical axis. C. Randomly rotated tissue bars ready to be embedded side-by-side in glycolmethacrylate.

Volume estimation

The volumes of the granule cell layer and polymorphic layer (hilus) of the dentate gyrus and of the pyramidal cell layers of the CA1 and CA3-2 hippocampal fields were estimated using the Principle of Cavalieri (Gundersen and Jensen, 1987). All 50 µm-thick sections collected from each hippocampal formation were used. The boundaries of the cellular layers and of the hilus, described in detail by West and Gundersen (1990), were drawn using a camera lucida attachment and a 3x objective lens (final magnification of 24x). The area of the sectional profiles of each layer was estimated by point counting using a grid of test points in which the area per point, a(p), was 0.14 mm^2 for the granule cell layer, 0.56 mm^2 for the hilus, 0.27 mm^2 for the CA3-2 pyramidal cell layer and 1.67 mm^2 for the CA1 pyramidal cell layer. The volume of the layers was then calculated

from the total number of points that fell on each layer, $\sum P$, and the distance between the systematically sampled sections - t (3.7 mm) - (Gundersen and Jensen, 1987). Twelve to 14 sections were analyzed per each hippocampal formation. On average, the $\sum P$ counted on the granule cell layer was 200, on the hilus 140, and on the pyramidal cell layers of the hippocampal fields CA3-2 and CA1 170 and 150, respectively.

Estimation of the tissue shrinkage

Two slices were randomly sampled per hippocampal formation before being processed. The rostral face of each slice was photographed and the respective cross-sectional area determined using an image analysis system (Videoplan). The section cut from each of these slices was also photographed after the dehydration, embedding and staining procedures, and the respective cross-sectional area measured. The tissue shrinkage factor (SF_V) was calculated from the ratio of the areas of the sections photographed before and after the histological processing, as described in detail elsewhere (Uylings et al., 1986; Madeira et al., 1991a, 1992). The SF_V value was found to be negligible (0.995).

Estimation of the total number of neurons

The total number of neurons in the granule cell layer and hilus of the dentate gyrus and in the pyramidal cell layers of the CA3-2 and CA1 hippocampal fields was estimated using the optical fractionator (West et al., 1991). The optical disector equipment used consists of an BH-2 Olympus microscope with a high numerical aperture (NA = 1.4) x100 oil-immersion objective lens and an object rotator (BICO, Denmark), a digital length gauge (Heidenhain, Germany) and a microcator (Heidenhain type VRZ 401, Germany). The microscope is connected to a video monitor where the computer-generated counting frames (GRID stereological software for Commodore Amiga computers, Version 2.01, Olympus, Denmark) are superimposed on the video images of the sections.

All sections containing the subdivisions of the hippocampal formation analyzed, i.e., 12-14 sections per each hippocampal formation, were used for the estimation of neuron numbers. For every subdivision, fields of vision were systematically sampled and in each sampled field neurons were counted, at a final magnification of 2250x, using optical disectors (West and Gundersen, 1990). Starting 5 μm below the upper face of the section, the disectors were performed using a fixed height of 20 μm for all subdivisions analyzed. The area of the counting frames, a(frame), of the disectors and the interframe distances used for the systematic sampling of the fields of vision are shown in Table 1.

Table 1. Information about the a(frame) of the disectors and the distances between consecutive frame centers used along the x-axis and y-axis to estimate neuron numbers in the different subdivisions of the hippocampal formation

Subdivision	a(frame)	x-axis	y-axis
Granule cell layer	391 μm^2	468 μm	480 μm
Hilus	9130 μm^2	700 μm	616 μm
CA3-2 pyramidal cell layer	3588 μm^2	472 μm	490 μm
CA1 pyramidal cell layer	3588 μm^2	1464 μm	1376 μm

The average numbers of disectors performed and neurons counted were, respectively: 122 and 165 in the granule cell layer, 142 and 184 in the hilus, 166 and 186 in the CA3-2 pyramidal cell layer, and 138 and 152 in the CA1 pyramidal cell layer.

Estimation of the mean somatic and nuclear volumes

The number weighted mean volumes (V_N) of neuronal cell bodies and neuronal nuclei were estimated in all cell types analyzed except the granule neurons, in which only the mean nuclear volume was estimated due to the scarcity of their cytoplasm. In the vertical sections used for this purpose, the identification of the neurons as belonging to a specific subdivision of the hippocampal formation was based on their cytological features and staining properties. The mean volume of the neuronal somata or neuronal nuclei were measured according to the nucleator principle (Gundersen, 1988) applied to vertical sections. Neurons were sampled with optical disectors, as described above. At the optical level where the nucleolus was in focus, the unique reference point in the cell was marked. Lines were then provided by a computer program (GRID stereological software for Commodore Amiga computers, Version 2.01, Olympus, Denmark) and the intersections between these lines and the cell or nuclear boundaries were marked. In each cell, the distance between the unique reference point and the cell or nuclear boundaries were measured in four directions. Whenever cells had more than one nucleolus, the measurements were made from the largest. For the granule neurons, which do not display a well-defined nucleolus, the center of the nucleus was considered as the unique reference point. An average of 70 granule neurons, 65 hilar neurons, 60 CA3-2 pyramidal neurons and 60 CA1 pyramidal neurons was measured per each hippocampal formation.

Statistical analysis

The precision of the individual estimates of neuron numbers and volume of the layers, evaluated as the coefficient of error (CE), was estimated as a function of two independent factors: the "Nugget effect" (Gundersen and Jensen, 1987; H.J.G. Gundersen, E.B. Jensen and A. Baddeley, personal communication) and the variance due to sampling between systematically random sampled sections, as shown by West et al. (1996). The precision of the individual estimates of mean somatic and nuclear volumes was calculated according to Matheron (1971) and Gundersen and Jensen (1987), using the following equation: $CE^2 = CV^2/n$. The mean CE was calculated from the estimates for an individual using the relationship: Mean CE = $\sqrt{\text{mean}CE^2}$ (West et al., 1991).

The observed variance among individuals was estimated using the coefficient of variation (CV = S.D./mean) and the biological CV (CV_{biol}) was calculated using the relationship: $CV^2 = CV^2_{biol} + CE^2$ (Kroustrup and Gundersen, 1983).

To test for the effect of (right/left) side, a one-way ANOVA was performed. Differences were considered to be significant if $P < 0.05$.

RESULTS

Characteristics of the sample

The average age, body weight, height and brain weight of the subjects from whom the six right and the six left hippocampal formations were collected was as follows. Right hippocampal formation: age, 29 years (range 21-49 years); body weight,

74 Kg (range 58-78 Kg); height, 168 cm (range 158-180 cm); brain weight, 1475 mg (range 1300-1675 mg). Left hippocampal formations: age, 31 years (range 23-54 years); body weight, 69 Kg (range 51-78 Kg); height, 172 cm (167-174 cm); brain weight, 1434 (range 1370-1485 mg).

No statistical significant differences were detected in the body weight ($F_{(1,10)}$ = 0.74; P = 0.407), height ($F_{(1,10)}$ = 2.20; P = 0.169) and brain weight ($F_{(1,10)}$ = 0.01; P = 0.916) between the two groups analyzed.

Granule cell layer

The quantitative data estimated on the granule cell layer are shown in Fig. 4. No right/left difference ($F_{(1,10)}$ = 0.01; P = 0.909) was found in the volume of the layer (Fig. 4A). However, there was a right/left asymmetry in the total number of granule cells because the total number was 20% higher ($F_{(1,10)}$ = 11.19; P = 0.007) in the right than in the left hippocampal formation (Fig. 4B). Although no significant right/left difference ($F_{(1,10)}$ = 1.39; P = 0.267) was detected in the mean nuclear volume of the granule cells (Fig. 4C), the volume of the nuclei was 5% greater in the left than in the right granule neurons.

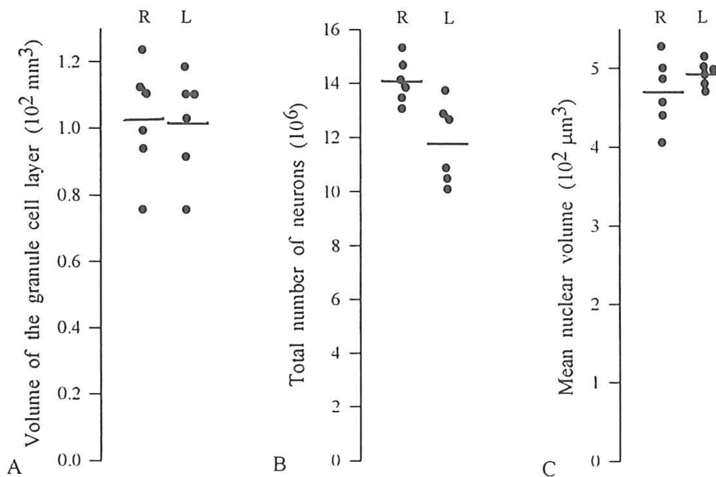

Fig. 4. Graphic representation of the data obtained in the granule cell layer of the right (R) and left (L) dentate gyrus. A. Volume of the layer. B. Total number of granule neurons. One-way ANOVA: right vs left, P = 0.007. C. Mean nuclear volumes of granule neurons. Horizontal bars represent mean values.

The average interindividual variability in the volume, total number of neurons and mean nuclear volume was 16% (right, CV = 0.16; left, CV= 0.15), 9% (right, CV = 0.06; left, CV = 0.12) and 6% (right, CV = 0.09; left, CV= 0.03), respectively. The mean CE of the estimates was 0.079, 0.059 and 0.033, respectively.

Hilus

The quantitative data from the hilus of the dentate gyrus are shown in Fig. 5. No right/left differences were found in the volume of the hilus ($F_{(1,10)} = 1.86$; $P = 0.202$), in the total number of hilar neurons ($F_{(1,10)} = 0.63$; $P = 0.446$), nor in the mean volumes of the neuronal somata ($F_{(1,10)} = 0.29$; $P = 0.603$) and neuronal nuclei ($F_{(1,10)} = 0.08$; $P = 0.777$).

The mean CV of the volume of the hilus was 16% (right, CV = 0.17; left, CV = 0.15) whereas the mean CV of the neuron numbers was 10% (right, CV = 0.15; left, CV= 0.04); the average CVs of the mean somatic and nuclear volumes were, respectively, 6% (right, CV = 0.07; left, CV = 0.05) and 15% (right, CV = 0.17; left, CV = 0.13). The mean CE of the estimates was 0.090, 0.044, 0.039 and 0.043, respectively.

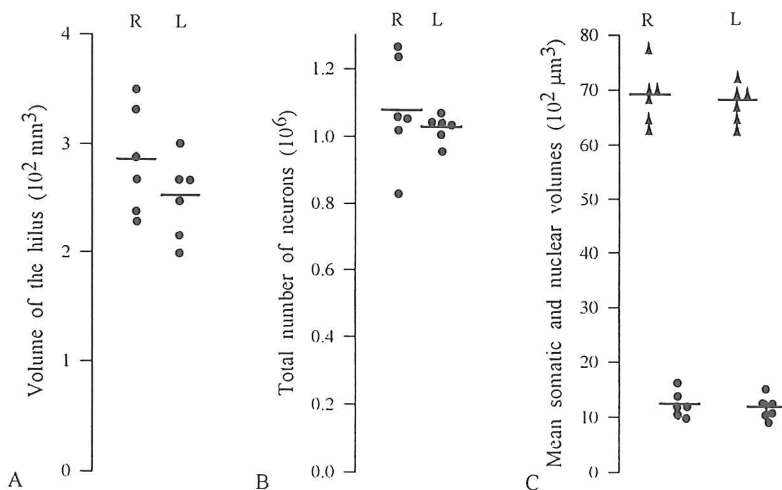

Fig. 5. Graphic representation of the data obtained in the hilus of the right (R) and left (L) dentate gyrus. A. Volume of the hilus. B. Total number of hilar neurons. C. Mean somatic (triangles) and mean nuclear (circles) volumes of hilar neurons. Horizontal bars represent mean values.

CA3-2 pyramidal cell layer

The volume of the CA3-2 pyramidal cell layer (Fig. 6A) was similar in the right and left hippocampus ($F_{(1,10)} = 0.21$; $P = 0.659$). However, the right hippocampal CA3-2 subdivision contained 14% more neurons than the left (Fig. 6B), a difference which proved to be significant ($F_{(1,10)} = 6.44$; $P = 0.029$). No significant right/left differences were detected either in the mean somatic volume ($F_{(1,10)} = 1.30$; $P = 0.279$) or in the mean nuclear volume ($F_{(1,10)} = 3.35$; $P = 0.097$) of the CA3-2 pyramids (Fig. 6C); however, the mean somatic volume was 11% higher and the mean nuclear volume 6% greater in the left than in the right hippocampal formations.

The average CV of the volume was 15% (right, CV = 0.21; left, CV = 0.08) whereas that of the total neuron numbers was 9% (right, CV = 0.09; left, CV = 0.09); the average CVs of the mean somatic and nuclear volumes were, respectively, 9% (right, CV = 0.07; left, CV = 0.10) and 10% (right, CV = 0.12; left, CV = 0.07). The mean CE of the estimates was 0.129, 0.046, 0.049 and 0.040, respectively.

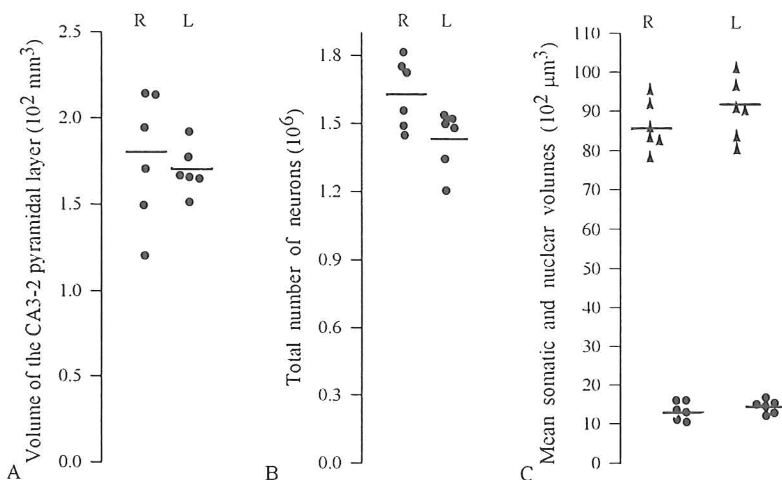

Fig. 6. Graphic representation of the data obtained in the CA3-2 pyramidal cell layer of the right (R) and left (L) hippocampus. A. Volume of the layer. B. Total number of CA3-2 pyramidal neurons. One-way ANOVA: right vs left, P = 0.029. C. Mean somatic (triangles) and mean nuclear (circles) volumes of CA3-2 pyramids. Horizontal bars represent mean values.

CA1 pyramidal cell layer

Data obtained in the CA1 pyramidal cell layer are graphically represented in Fig. 7. No right/left differences were found either in the volume of the layer ($F_{(1,10)}$ = 2.08; P = 0.179) or in the total number of its neurons ($F_{(1,10)}$ = 1.07; P = 0.324). Likewise, no differences were detected between the right and the left pyramids as regards the mean somatic ($F_{(1,10)}$ = 0.087; P = 0.774) and the mean nuclear ($F_{(1,10)}$ = 0.12; P = 0.742) volumes.

The average CVs of the volume of the layer, total number of neurons and mean somatic and nuclear volumes was 11% (right, CV = 0.09; left, CV = 0.12), 6% (right, CV = 0.06; left, CV = 0.05), 9% (right, CV = 0.10; left, CV = 0.08) and 10% (right, CV = 0.11; left, CV = 0.08), respectively. The mean CE of the estimates was 0.070, 0.047, 0.046 and 0.044, respectively.

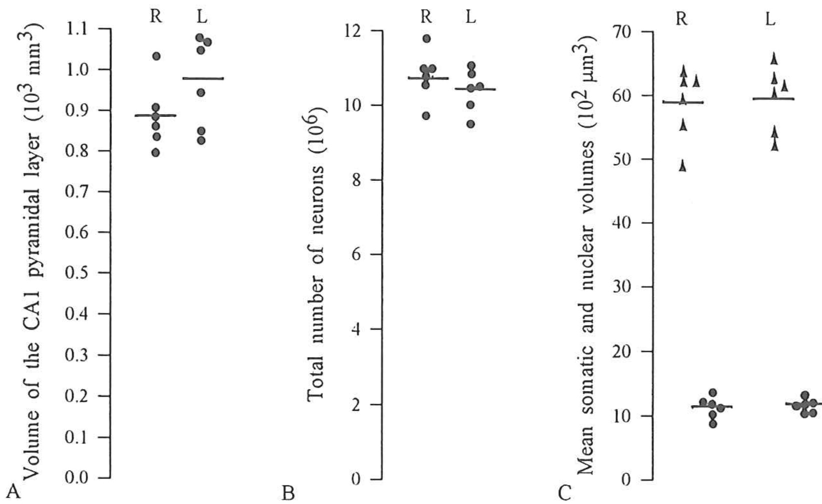

Fig. 7. Graphic representation of the data obtained in the CA1 pyramidal cell layer of the right (R) and left (L) hippocampus. A. Volume of the layer. B. Total number of CA1 pyramidal neurons. C. Mean somatic (triangles) and mean nuclear (circles) volumes of CA1 pyramids. Horizontal bars represent mean values.

DISCUSSION

In this study we have used 3D sampling strategies to obtain unbiased estimates of volumes of the hippocampal layers, and of total numbers and mean volumes of hippocampal neurons. A systematic random sampling procedure was applied for selecting the sections used for volume and neuron number estimates and for sampling the fields of vision where neurons were counted or measured. The sampling variance introduced by the stereological estimations, evaluated as the CE of the estimates, represents a small fraction of the observed interindividual variance, both in the volume and in the total neuron number estimations. In effect, in the volume estimates of the granule cell layer, hilus, CA3-2 and CA1 pyramidal cell layers the biological variance makes up 87%, 83%, 51% and 77% of the observed variance, whereas in neuron number estimates it represents 76%, 90%, 86% and 62%, respectively. These data indicate that the sampling procedures used are satisfactory and suggest that a larger number of individuals would be required to reduce the interindividual variation. An alternative strategy to reduce the interindividual variation would be the use of a paired comparison design, which allows each subject to serve as its own control. Because the hippocampal formations of the subjects used in this study were employed as controls in another investigation (Sá, 1998), we were compelled to apply a two-sample design.

Several previous studies have reported unbiased estimates of total numbers of neurons in the human hippocampal formation (West and Gundersen, 1990; West, 1993;

West et al., 1994; Šimić et al., 1997), but in all cases they were obtained by the multiplication of the volume of the cellular layers by the numerical density of the neurons. To our knowledge, this is the first study in which the optical fractionator was used for the same purpose. The comparison of the results herein presented with those available in the literature shows, as expected, that the estimates are of the same magnitude, but it also reveals that the interindividual variation of the total number of neurons is lower than that shown in the referred studies for all hippocampal subdivisions. However, the mean values of our estimates of hilar and CA3-2 pyramidal neuron numbers are smaller than those previously reported, a finding which is not likely to be ascribed to differences in the criteria used for the definition of the hippocampal regions or delineation of the cell-containing layers because our volume estimates, as well as the respective CVs, are similar to those obtained by the referred authors (West and Gundersen, 1990; Šimić et al., 1997).

As far as we are aware, no previous estimations of neuronal volumes in the human hippocampal formation were performed. To do so, we developed a simple sampling strategy able to provide precise estimates of number-weighted mean neuronal volumes without interfering with the obtainment of absolute neuron numbers in the same hippocampal formation. Neurons were selected for measurements using disectors, thus ensuring that all neurons, irrespective of their size, had equal probability of contributing to the estimates. In addition, the measurements were undertaken in vertical sections which, by allowing to obtain information about the 3D of neurons, overcome any anisotropy of the orientation of the neurons and, thus, increase the precision of the estimates. Because the vertical sections were systematically random sampled along the whole extent of the hippocampal formation the estimates can be considered as representative of the entire neuronal populations. Using this sampling scheme, the relative precision of the estimates of mean nuclear and mean somatic volumes (CE^2/CV^2; calculated according to Eq. 2 of West et al., 1991) ranged between 0.04 and 0.30 and between 0.26 and 0.42, respectively, which indicates that precise estimates, particularly of mean nuclear volumes, could be obtained with fewer measurements. Finally, our estimates are not likely to have been influenced by the tissue shrinkage/swelling induced by the histological processing because we also show that the value of the tissue shrinkage factor, evaluated in the material used for the estimates, is negligible.

The most relevant biological conclusion of this study is that in humans the right and the left hippocampal formations are not similar as regards their morphological organization. Specifically, we found that the right hippocampal formation contains 20% more granule cells and 14% more CA3-2 pyramidal neurons than the left hippocampal formation. However, no significant differences were found in the volumes of the dentate granule and CA3-2 pyramidal cell layers when comparisons between the right and left sides were performed. The lack of parallelism between the right/left variations in neuron numbers and in layer volumes can only be ascribed to right/left differences either in the components of the neuropil of the layers or in the size of their constituent neurons. To investigate this issue we have estimated the volume of the granule and CA3-2 pyramidal neurons and we found that the mean values were 5% higher, for the nuclear volume of granule cells, and 6.1% and 10.5% larger, respectively for the mean nuclear and somatic volumes of the CA3-2 pyramids, in the left than in the right hippocampal formations. Although these differences did not reach statistical significant levels, the larger volume of the neurons in the left hippocampal formations, which contain a smaller number of cells, might well have contributed to abolish the differences in the volume of the layers, that were expected to occur if the volume of the cell-containing layers were exclusively dependent on the total number of neurons.

Unlike these two components of the hippocampal formation, no significant right/left differences were found in the hilus and in the CA1 pyramidal cell layer as regards the volume of the layers and the total number and size of their neurons. This observation associated with the finding of asymmetries in the total number of granule and CA3-2 pyramidal neurons led us to hypothesize that the organization, as well as the activity, of the intrinsic trisynaptic circuit of the hippocampal formation is probably rather different in the right and left hemispheres. In effect, it is known that the granule cells, the CA3 pyramids and the CA1 pyramidal neurons are connected by largely unidirectional excitatory projections, and also that collaterals of the mossy fibers (the axons of granule cells) establish synaptic contacts with hilar neurons, whose axons make inhibitory contacts on the cell bodies and dendrites of granule cells (Amaral and Witter, 1995). We cannot infer, on the grounds of the data estimated in this study, for the existence of right/left differences in the number of synaptic contacts within the hippocampal circuit. Yet, the presence of asymmetries in the number of granule and CA3 pyramidal neurons, but not in the number of hilar and CA1 pyramidal neurons, allows us to infer that the pattern of synaptic organization is more complex in the left than in the right hippocampal formations. Should this be the case, then the balance between excitatory and inhibitory inputs is markedly different in the right and in the left hippocampal circuits. These possibilities are further reinforced by the observation that the entorhinal cortex, which provides a major excitatory input to the dentate gyrus and hippocampus (Amaral and Witter, 1995), contains more neurons in the left than in the right hemisphere (Heinsen et al., 1994). This suggests that the entorhinal-hippocampal input is higher on the left side, an asymmetry that can compensate for the eventual smaller ability of the left hippocampal formation to process information.

Consistent with our observations and lending support to the hypotheses raised above are data from studies which show that the volume of the left, but not of the right, hippocampal formation is positively correlated with specific forms of memory (Lencz et al., 1992; Goldberg et al., 1994) and with the duration of major depression (Sheline et al., 1996). Other investigations have, furthermore, suggested that temporal lobe seizures occur more frequently on the left side (Roberts and Horton, 1992) and that schizophrenic disorders are associated with left hippocampal dysfunction (Trimble, 1991), whereas affective disorders are commonly associated with right hippocampal dysfunction (Post et al., 1992). We can therefore conclude that in human studies the right and the left hippocampal formations should not be indifferently sampled for functional, behavioral or anatomical investigations because their structural organization is likely to be markedly different in the right and in the left hemispheres.

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