Journal of Neurocytology **31**, 337–346 (2002)

Cortical area and species differences in dendritic spine morphology

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Received December 7, 2002; accepted January 22, 2003

Abstract

Dendritic spines receive most excitatory inputs in the neocortex and are morphologically very diverse. Recent evidence has demonstrated linear relationships between the size and length of dendritic spines and important features of its synaptic junction and time constants for calcium compartmentalisation. Therefore, the morphologies of dendritic spines can be directly interpreted functionally. We sought to explore whether there were potential differences in spine morphologies between areas and species that could reflect potential functional differences. For this purpose, we reconstructed and measured thousands of dendritic spines from basal dendrites of layer III pyramidal neurons from mouse temporal and occipital cortex and from human temporal cortex. We find systematic differences in spine densities, spine head size and spine neck length among areas and species. Human spines are systematically larger and longer and exist at higher densities than those in mouse cortex. Also, mouse temporal spines are larger than mouse occipital spines. We do not encounter any correlations between the size of the spine head and its neck length. Our data suggests that the average synaptic input is modulated according to cortical area and differs among species. We discuss the implications of these findings for common algorithms of cortical processing.

Introduction

Dendritic spines were discovered by Cajal in 1888 (Ramón y Cajal, 1888), who argued that they were essential structural elements in the nervous system and served to connect axons and dendrites (Ramón y Cajal, 1899). After his early studies, there were no outstanding contributions during the following five decades, until the introduction of electron microscopy confirmed that spines indeed were postsynaptic (Gray, 1959a, b). Renewed interest in the study of pyramidal dendritic spines occurred in the early 1970s, principally as a result of observations indicating that dendritic spines abnormalities were the most consistent anatomopathological correlates of mental retardation (Marín-Padilla, 1972; Purpura, 1974). Another renaissance of spine studies has recently followed the introduction of live imaging techniques to Neuroscience and, in particular, two-photon microscopy (Denk *et al.*, 1994). These studies have demonstrated that spines compartmentalise calcium (Yuste & Denk, 1995), are constantly moving and changing shape (Fischer *et al.*, 1998; Bonhoeffer & Yuste, 2002) and that spine formation, plasticity and maintenance depend on synaptic activity and can be modulated by sensory experience (Yuste & Bonhoeffer, 2001). In spite of these recent results, the function of dendritic spines is still somewhat mysterious. Because excitatory inputs can be made on dendritic shafts (Feldman *et al.*, 1984), spines must be serving a specific function, which could range from implementing learning rules to minimising axonal wire (Swindale, 1981; Shepherd, 1996; Yuste & Majewska, 2001).

An important aspect of the dendritic spines is the enormous diversity in their morphologies, something which was already noted by Cajal and which could be important to understand their function (Ramón y Cajal, 1899). Indeed, there appears to be a clear relationship between the morphology and function of the spine, particularly with relation to the size of the spine head and the length of the neck. For example, the volume of the spine-head is directly proportional to the size of the postsynaptic density, the number of postsynaptic receptors, to the presynaptic number of docked synaptic vesicles and the ready releasable pool of neurotransmitter (Harris & Stevens, 1989; Nusser *et al.*, 1998; Schikorski & Stevens, 1999, 2001). Also, spines with longer necks

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show longer time constants of calcium compartmentalisation than spines with shorter necks (Majewska *et al.*, 2000a, b). Therefore, the morphology of dendritic spines has a direct functional relevance since it reveals key characteristics of synaptic inputs and their biochemical compartmentalisation.

Most studies on dendritic spines of pyramidal cells have been focused on their density and distribution in a specific cortical areas and species (Elston, 2002). However, there are not systematic studies regarding possible differences in the morphology of dendritic spines between different species or different cortical areas. This appears important to us, not just to illuminate the potential function of spines, but also to highlight commonalties in the search for general rules of computation of a potentially "canonical" cortical microcircuit (Douglas *et al.*, 1989). In the present study we have compared the morphology of dendritic spines of pyramidal cells from the occipital and temporal cortex of mice and humans. We report the existence of systematic differences in spine head sizes and neck lengths among these two species and among these cortical areas. We also report the lack of systematic correlations among spine neck and head, indicating that these variables are regulated independently.

Methods

Preparation of human and animal material

In order to compare the size of dendritic spines of human and mouse pyramidal cells, we used intracellular injections in fixed cortical tissue (Elston *et al.*, 1997). Human tissue was obtained from the left hemisphere of two male patients of 28 and 41 years of age, which was removed to gain access to the epileptic focus in the mesial temporal lobe structures during surgical treatment of epilepsy (Department of Neurosurgery, Hospital de la Princesa, Madrid, Spain). Informed consent was obtained from each patient prior to surgery. The neocortical tissue was considered to be normal on the basis of electrophysiological and histopathological examination . Surgically resected tissue was inmediately immersed in cold 4% paraformaldehyde for 24 h. Mice ($n = 2$, 2 months old) were overdosed by lethal i.p. injection of sodium pentobarbitone and their brain perfused intracardially with 4% paraformaldehyde, then, their brains were removed and further immersed in 4% paraformaldehyde for 24 h.

We were worried that differences in fixation method (perfusion vs. immersion) could account for the morphological differences that we report. To test this we fixed a mouse hemisphere by immersion, following exactly the same protocol used with the human tissue. Spines in the immersion-fixed temporal mouse material were indistinguishable in density and size from those in the perfusion-fixed temporal mouse material (head area $0.35 \pm 0.01\ \mu^2$, mean $\pm$ SEM, $n = 376$, for immersion vs. $x = 0.37 \pm 0.01$, $n = 1306$ for perfusion; Bonferroni $p = 1$; average neck length 0.64 ± 0.02, $n = 231$ for immersion vs. 0.73 ± 0.01, $n = 759$ for perfusion; Bonferroni $p = 0.19$; average spine density 12.5 ± 0.96 spines/10 μm, $n = 10$ for immersion vs. 10.9 ± 0.46, $n = 40$ for perfusion; Bonferroni $p = 0.68$).

Intracellular injections

For both species, the cerebral cortex was cut tangentially to the cortical surface with the aid of a Vibratome. Our cell injection methodology has been described in detail elsewhere (Elston *et al.*, 1997, 2001). Briefly, cells in the flat portion of the occipital and temporal cortex of mice (approximately corresponding to areas V1M/V1B and A1/S2 of Franklin and Paxinos (Franklin & Paxinos, 1997) and third temporal gyrus (Broadman's area 20) of the human cases) were individually injected with Lucifer Yellow by continuous current. Following injections, the sections were processed with an antibody to Lucifer Yellow as described in Elston *et al.* (2001) (Fig. 1).

Reconstruction and analysis

Only neurons whose basal dendritic tree was completely filled were included in this analysis. To preserve a high signal to noise in our analysis we only reconstructed lateral spines, neglecting spines located on the top or bottom surface of the dendrites. To avoid potential differences among neuronal classes and dendritic branches and create a homogeneous sample, we only reconstructed spines from basal dendrites of layer 3 pyramidal neurons. Because spine density (Ruiz-Marcos & Valverde, 1969; Elston & DeFelipe, 2002), and possible also spine size (Konur & Yuste, unpublished observations), changes as a function of distance from the soma, we sought to compare similar segments of dendrites between different cells, by selecting segments of basal dendrites which were located at the same proportional distance from the soma. More specifically, we selected the basal dendrites segments which, according to our previous work (Elston & DeFelipe, 2002) has the highest density of spines. To perform the morphometric analysis of dendritic spines, we studied the same proportional segment of 20 randomly-selected horizontally projecting pyramidal cell basal dendrites of different cells in each area and case. Only one dendrite per cell was analysed. The proportional segments initiated at 45 μm from the soma in mice and 75 μm in humans. These dendritic segments (30 μm long in mice and 50 μm in humans) correspond to the highest density of spines in these two species (Elston *et al.*, 2001; Dierssen *et al.*, 2003 and unpublished obsevations). Images of each portion of dendrite were captured at different focal planes using a BX51 Olympus microscope (100x objective) attached to a Nikon 995 camera at a final magnification of 3100x. Thereafter, images were used to make composite projection drawings of the dendritic spines. Only spines arising form the lateral surfaces of the dendrites were included in the study.

The analysis was carried out blindly by a different investigator. Spine density was measured by counting the number of spines located in the lateral portion of each dendrite segment. Therefore, the results obtained were presumably an underestimate of the total number of spines present on the mentioned portion of dendrite. The area, major and minor axis of the head of spines and the length of the necks in each portion of the dendrites analysed were determined with the aid of a digitizing tablet (SummaSketch III) and NIH image software (NIH Research Services, Bethesda, MD). Spine necks were measured from the point of attachment of the dendrite to the beginning of the spine head, as estimated by the investigator.

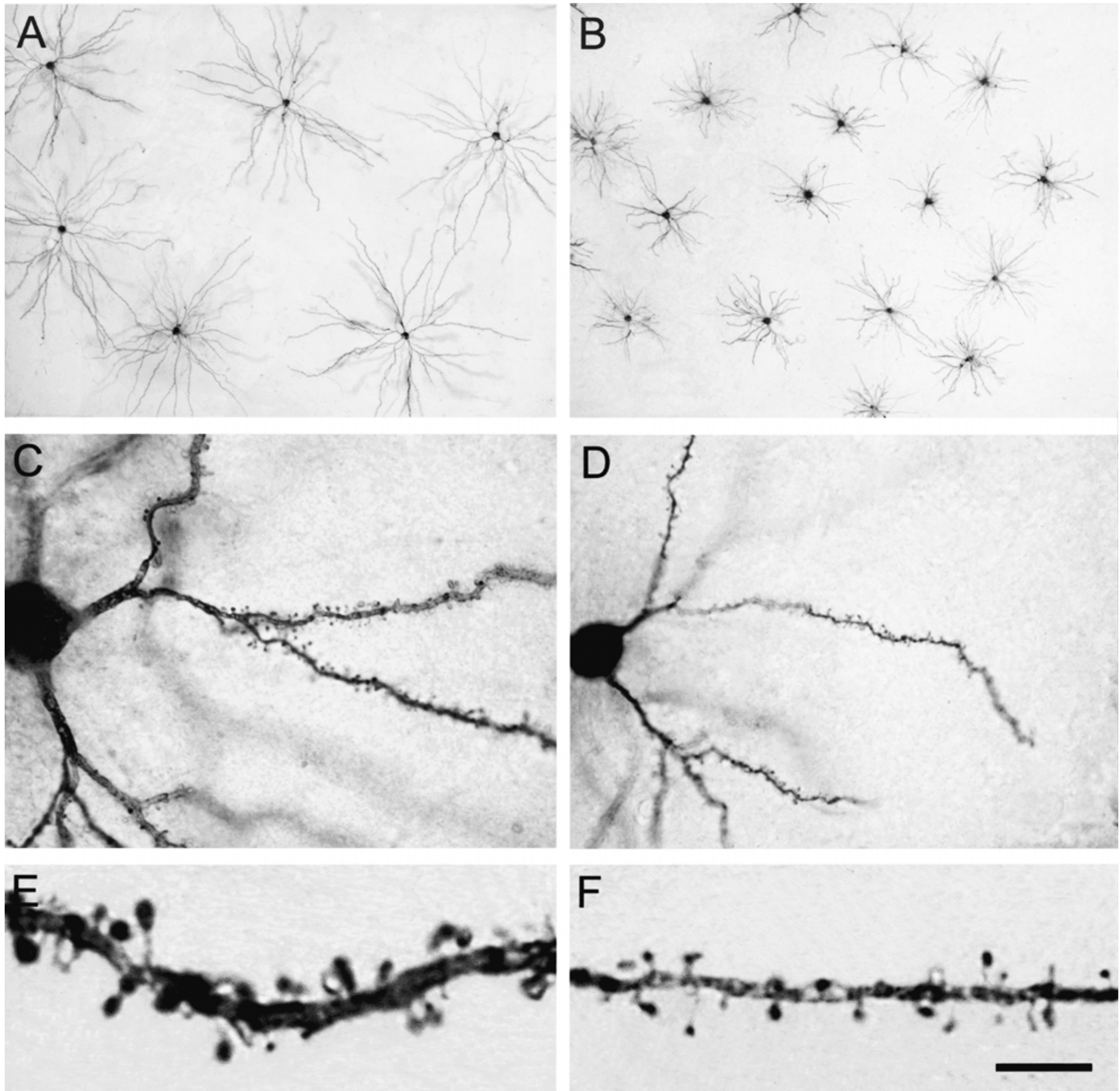

Fig. 1. Photomicrographs of pyramidal cells in the human and mouse neocortex. A, B. Low-power photomicrographs of layer III pyramidal cells injected with Lucifer Yellow and processed with DAB in human (A) and mouse (B) temporal cortex. Note the smaller size of mouse cells. Section is parallel to the cortical surface. C, D: Photomicrograph of horizontally projecting dendrites of a human (C) and mouse (D) pyramidal cell. E, F: High-power photomicrographs of the basal dendrite segments of human (E) and mouse (F) pyramidal cells illustrating individual dendritic spines. Note the smaller size of the mouse spines. Scale bar: 425 μm in A, B; 45 μm in C, D; 10 μm in E, F.

Results

SPECIES DIFFERENCES IN SPINE DENSITY

In order to explore whether systemic differences in spine morphologies or densities exist among species or among cortical areas, we reconstructed and measured spines from human temporal cortex ($n = 2768$ spines, 40 cells, 2 patients) and mouse temporal ($n = 1306$ spines, 40 cells, 2 animals) and occipital ($n = 1226$ spines, 40 cells, 2 animals) cortex. Spines were labelled using intracellular Lucifer Yellow injections in fixed material and immunocytochemistry (Elston *et al.*, 1997, 2001) (Fig. 1) and they were reconstructed by tracing high magnification digital microphotographs.

We first wondered if there were differences in spine densities between species or areas. Quantification of

Table 1. Morphometric values (mean ± sem) of dendritic spines from layer III pyramidal cells of the occipital and temporal cortex of mice and human.

	Mouse occipital cortex	*Mouse temporal cortex*	*Human temporal cortex*
Spine density (per 10 μm)	10.21 ± 0.49 (n = 40 cells)	10.88 ± 0.46 (n = 40 cells)	14.19 ± 0.43 (n = 40 cells)
Area of the head (μm^2)	0.31 ± 0.01 (n = 1226)	0.37 ± 0.01 (n = 1306)	0.59 ± 0.01 (n = 2768)
Major axis (μm)	0.77 ± 0.01 (n = 1226)	0.85 ± 0.01 (n = 1306)	1.08 ± 0.01 (n = 2768)
Minor axis (μm)	0.48 ± 0.01 (n = 1226)	0.53 ± 0.01 (n = 1306)	0.66 ± 0.01 (n = 2768)
Length of the neck (μm)	0.67 ± 0.01 (n = 1226)	0.73 ± 0.01 (n = 1306)	0.94 ± 0.01 (n = 2768)

the spine density of the corresponding segment of basal dendrites in mouse and human revealed that the mean number (mean ± SEM; for all measurements) of spines per 10 μm segment was 10.2 ± 0.5 and 10.9 ± 0.5 for cells in occipital and temporal cortex of mice, respectively and 14.2 ± 0.4 for the temporal cortex of humans (Table 1). Statistical analysis (one-way ANOVA) revealed differences to be significant between mice and humans, but not between the two areas of the mouse analysed (Table 2; see also Fig. 1E, F). To explore whether there were different classes of neurons with systematic differences in spine density we plotted the individual data for our three samples, finding that different cells covered a continuum of spine densities, although some outliers were evident in the mouse and human temporal data (Fig. 2A). We concluded that human temporal cortical neurons have on average a higher (∼30%) spine density (in the basal dendritic segment with the highest density of spines) than mouse temporal or occipital neurons (see also Elston & DeFelipe, 2002).

SPECIES AND AREA DIFFERENCES IN SPINE HEAD AREA

We then analyzed whether there were any systematic differences in the size of the spine head. As explained, the volume of the spine is linearly correlated with a variety of pre- and postsynaptic physiological parameters (Harris & Stevens, 1989; Nusser *et al.*, 1998; Schikorski & Stevens, 1999, 2001). As an approximation to the estimation of the spine volume, we measured the maximal cross-sectional area of the spine head, as determined from reconstructions of several images taken at different focal points. The study of the size of spine heads revealed that the mean area in the temporal cortex of mice was smaller than that in humans (mean ± sem: 0.37 ± 0.01 μm^2 and 0.59 ± 0.01 μm^2, respectively; Table 1). Statistical analysis showed the difference to be significant (Table 2; Fig. 1 E, F). Moreover, the area of the head of spines in the occipital cortex of mice was significantly smaller (0.31 ± 0.01 μm^2) than that of mouse temporal cortex.

To explore whether these differences were due to differences in spine heterogeneity, we plotted histograms of the relative frequency of spines in the three samples, as a function of head area (Fig. 2B). For each of the three populations of spines, these histograms showed a unimodal α-type function, without any clear indications of a separate classes of spines with different head sizes. The peak of this function was clearly shifted in the three samples, with the mouse occipital spines distribution peaking around 0.2 μm^2, the mouse temporal around 0.3 μm^2 and the human temporal around 0.4 μm^2. We concluded that the distribution of heterogeneous spine head areas is systematically different between human and mouse temporal cortex and even between mouse temporal and occipital cortices.

SPECIES DIFFERENCES IN SPINE NECK LENGTH

We then searched for potential differences in the length of the spine neck. While the diameter of the spine neck appears relatively constant among spines (Harris &

Table 2. Statistical comparisons among dendritic spines from layer III pyramidal cells of occipital and temporal cortex of human and mouse.

	Mouse occipital–Mouse temporal	*Mouse occipital–Human temporal*	*Mouse temporal–Human temporal*
Spine density (per 10 μm) one-way ANOVA, $F_{(3.129)} = 14.0$, $p < 0.001$		*	*
Area of the head (μm^2) one-way ANOVA, $F_{(3.5675)} = 343.7$, $p < 0.001$	*	*	*
Length of the neck (μm) one-way ANOVA, $F_{(3.3489)} = 58.9$, $p < 0.001$		*	*

*Post-hoc Bonferroni analysis, $p < 0.001$.

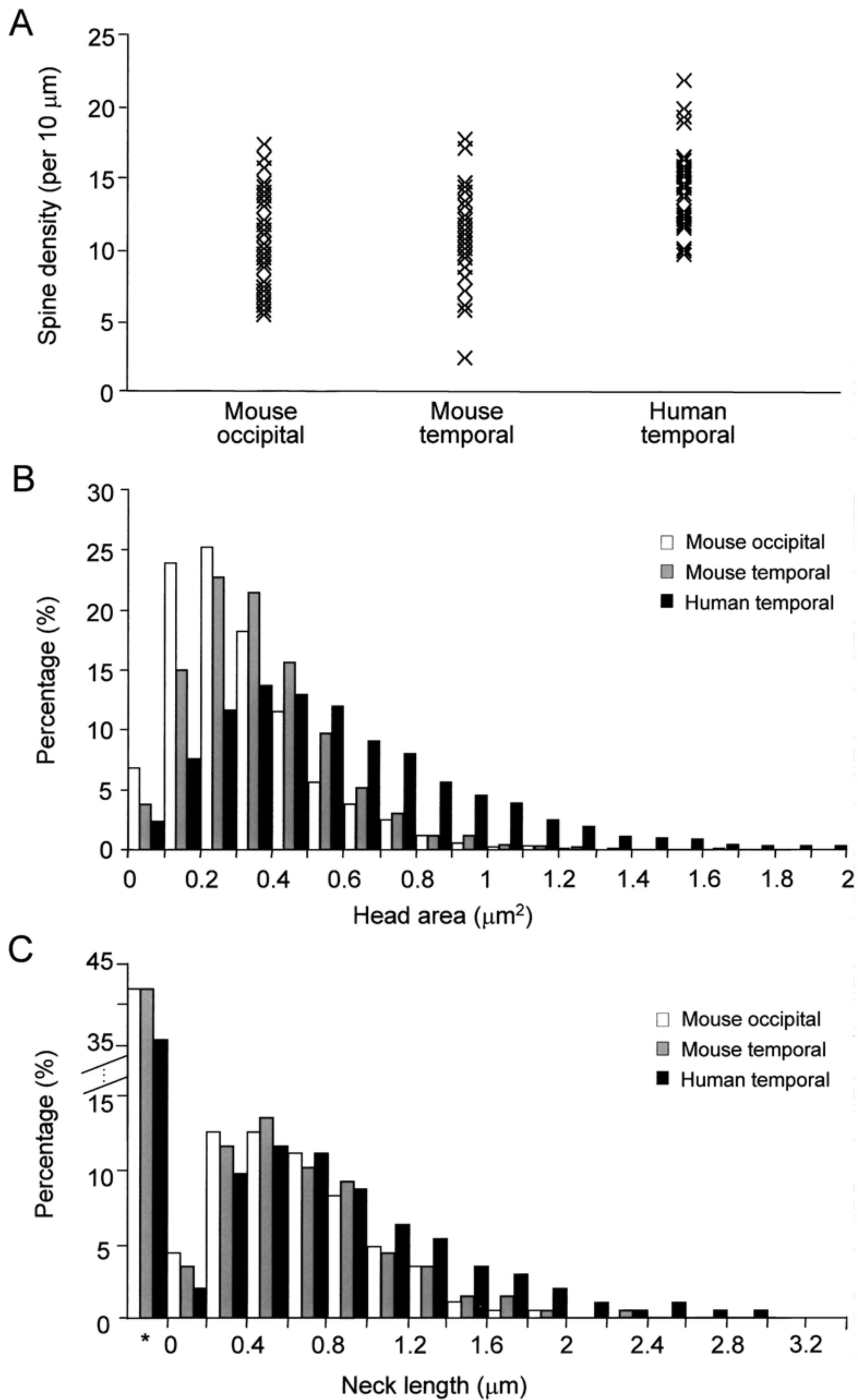

Fig. 2. Species and area differences in spine densities and morphologies. Distribution of spine density (A), area of spine heads (B) and length of necks (C) for the occipital and temporal cortex of the mouse and the human. *includes stubby spines and spines whose neck were not distinguishable from their head.

Stevens, 1989), there is a large variability in its length (Ramón y Cajal, 1899). Moreover, it is likely that the length of the spine neck controls the time constant of calcium compartmentalization, which could be the major function of the spine (Majewska *et al.*, 2000a, b). Specifically, the diffusion of calcium out of the spine can be substantial in stubby spines with short necks but is prolonged or perhaps even non-existent in spines with long necks (Majewska *et al.*, 2000a; Sabatini *et al.*, 2002).

We found that temporal cortex of mice showed shorter neck lengths (0.73 ± 0.01 μm) than that of humans (0.94 ± 0.01 μm). A one-way ANOVA demonstrated that these differences were significant between the two groups (see Tables 1 and 2). However, this was not the case when comparing the values from the occipital cortex of mice (0.67 ± 0.01 μm) and those from the temporal region.

There was a wide distribution of lengths of necks in both mice and human (Fig. 2C). These distributions were clearly bimodal for our three samples, with a peak at zero and a second peak around 0.5 μm. Indeed, almost half of the spines analyzed are represented as "no neck". This bin included stubby spines and also those spines whose head was not distinguishable from the neck. This bimodal distribution suggest that there are two populations of spines: a population with no neck, and a population with substantial necks. The differences between human and mouse spines that we encountered appeared due both to a reduction of the "no neck" spines in humans, as well as the systematic shift in the rest of the human spines towards longer neck lengths.

CORRELATIONS AMONG SPINE DENSITY, HEAD AREA AND NECK LENGTH

We finally studied the possible correlation of spine density, head area and neck length (Fig. 3). This appeared important to us in order to uncover potential causal links between the regulation of these three variables. For every pair of variables in the three samples, we plotted the average for each cell and fitted linear regression equations to the data. We found that none of the correlations (head area vs. neck length, length vs. density and area vs. density in the three samples) were statistically significant from the null hypothesis of zero slope in the linear fits. Moreover, the correlation coefficients were low, all below 0.34 and most hovering around zero. Within each pair of variables, no systematic trends was observed, with the exception of the negative correlation found between head area and spine density in the three samples of cells. Albeit not significant ($p = 0.15$), we cannot rule out that a larger sample could uncover a potential relation between the size of the spine head and spine density, whereby larger spines are spaced farther away from each other than smaller spines.

Discussion

METHODOLOGICAL CONSIDERATIONS

The morphological diversity of dendritic spines has baffled neuroscientist since their first description by Cajal (Ramón y Cajal, 1888). Spines must be playing a fundamental role in the nervous system, and it is likely that their morphological diversity reflects the diversity of this elusive function of the spine. In this work we are applying a new technique to this old question and seek to explore whether there are differences in the morphology and density of in spines from different species or different cortical areas. We use Lucifer Yellow injections of layer III neurons from human and mouse specimens and reconstruct and measure spines from proportionally identical segments of their basal dendrites. The advantage of this novel technique (*e.g.* Elston *et al.*, 1997; reviewed in Elston & DeFelipe, 2002; Elston, 2002) with respect to more established staining methods is its superb signal to noise, that make light microscopic measurements of spine morphologies feasible. In addition, we find that the staining of the dendritic branches is complete in most of our samples, which makes the potential errors due to incomplete staining of spines less likely.

Nevertheless, our results should be interpreted with care and there are several caveats that limit our conclusions. First, our sample of spines only includes those located in a particular region of the basal dendrites from layer III cells. Although the strength of our approach is its tight focus, at the same time this restricts its universality and it is possible that a different sample could result in different conclusions. Secondly, we only reconstructed spines that protruded laterally from the dendritic tree, neglecting to reconstruct or analyze all spines that were located directly above or below the dendrite. This obviously results in a large underestimate of the number of spines and of the spine densities. In fact, our numbers of human spine densities are roughly half from those that we have previously reported from human tissue (Elston *et al.*, 2001). At the same time, our study was essentially a comparative one, so we feel entitled to make conclusions about relative differences in spine densities across our samples. Also, the spine head area and neck length should not be affected by our choice to reconstruct only lateral spines. Finally, we want to emphasize that the spines morphologies that we have analyzed are the result of conventional aldehyde fixation. Because spines are full of actin networks (Matus *et al.*, 1982), which are very susceptible to fixation, it is possible that the dimensions of the spine head area or neck length that we report are different from those from spines in living tissue.

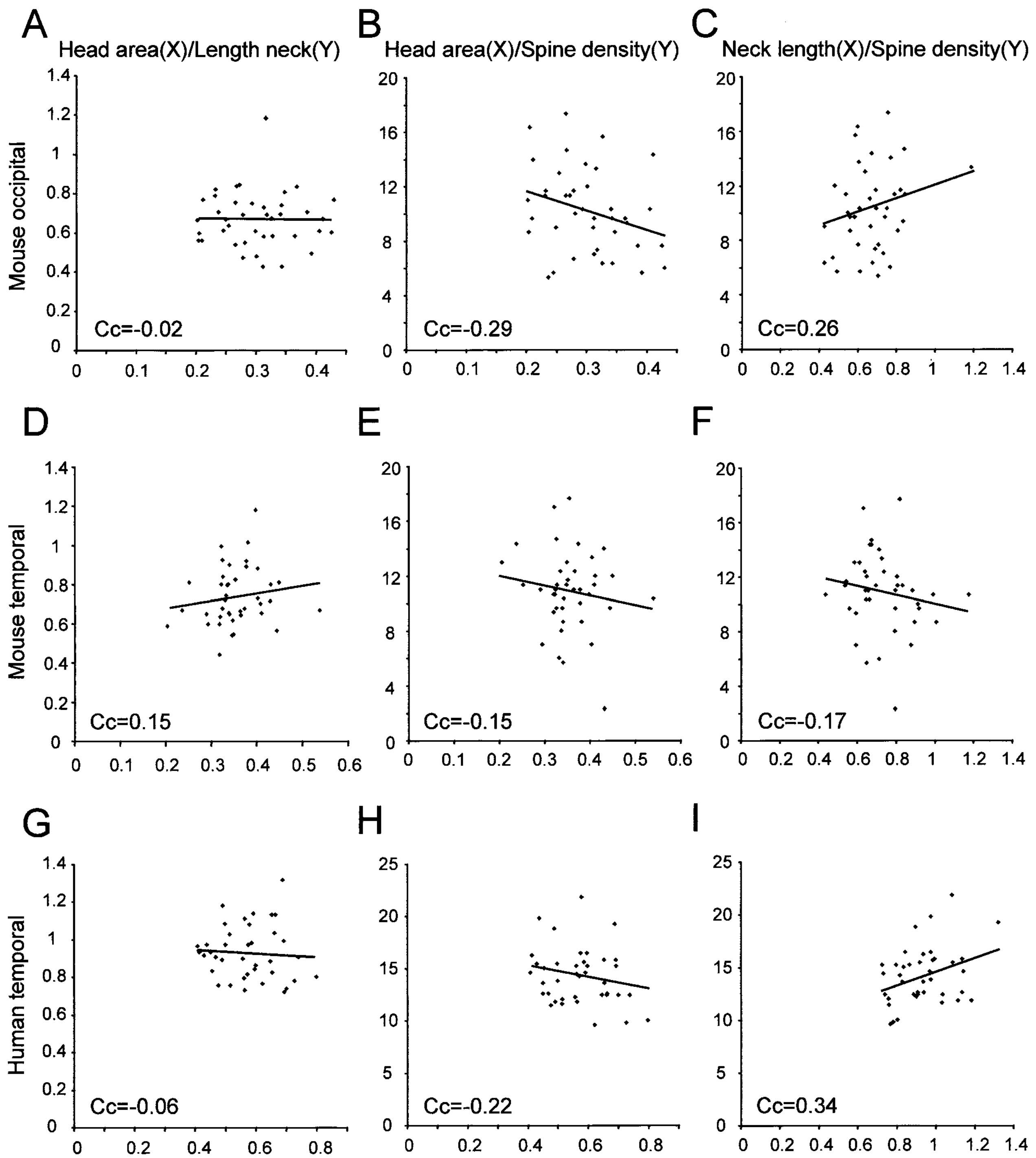

Fig. 3. Lack of correlations between spine head areas, neck lengths and spine densities. A, D, G: Correlations between the average areas of spine heads and the average lengths of necks for the occipital (A) and temporal (D) cortex of the mouse and the human (G). Each point represents one neuron. B, E, H: Correlation coefficient of the average area of spine heads and average spine density for the occipital (B) and temporal (E) cortex of the mouse and the human (H). C, F, I: Correlation coefficient of the average length of necks and average spine density for the occipital (C) and temporal (F) cortex of the mouse and the human (I). Cc = correlation coefficient.

DIFFERENCES AMONG AREAS AND SPECIES

Our first finding is the existence of large differences in spine densities between human and mouse cortex. Differences in spine densities across species have been reported before (Elston *et al.*, 2001; Elston & DeFelipe, 2002; Elston, 2002) and our results confirm and extend these findings. It is commonly assumed that every spine has an excitatory synapse (Gray 1959a, b; Colonnier, 1968), although to our knowledge, this has not been demonstrated unambiguously. Nevertheless, the

large differences in spine densities that we report between mouse and human cells, together with the larger dendritic length of human cells (~70% larger average dendritic length in human vs. mouse basal dendrites, according to our unpublished measurements) implies that human pyramidal neurons can integrate a substantially higher number of inputs that their mouse counterparts. A rough estimation of 30% higher spine densities times 70% larger basal dendrites would produce an approximately ~210% larger number of inputs for human neurons.

In addition, we also encountered major differences in spine head areas in different cortical regions, whereby spines from human temporal cortex are larger than those from mouse temporal cortex, which are themselves larger from those in mouse occipital cortical neurons. These differences in maximal cross sectional area must translate into even larger differences in volume and these volume differences linearly translate into differences in a host of physiological parameters. Therefore, assuming as an approximation that spine heads are spherical, we can estimate that human temporal spines, which are 60% larger in area than mouse temporal spines, should have on average close to 100% larger volume. This could correlate into a doubling of the number of postsynaptic receptors (Nusser *et al.*, 1998), double the number of docked vesicles and of ready releasable pool in the presynaptic terminals (Schikorski & Stevens, 1999, 2001). Therefore the functional impact of human spines, and the current that they inject into the dendrites, must be much larger than those from mouse neurons. A similar estimate, comparing mouse temporal and occipital spines, would suggest that mouse temporal spines, with 20% larger area than occipital spines, could have ~30% larger volumes and similarly larger number of receptors and docked vesicles. It is important that there are significant differences between cortical areas in spine size, because this implies that the average synaptic current is modulated according to cortical region. Finally, we should note that, with respect to their head size, we cannot find evidence for the existence of different types of spines, but find instead a continuum of sizes.

We also observe differences in spine neck length between human and mouse samples, whereby human spines have ~30% longer necks than mouse temporal or occipital ones. Interestingly, in all three populations, the bimodal distribution of spine neck lengths indicates the existence of at least two populations of spines: one with no necks, and another with necks. Given the relation between spine neck length and biochemical compartmentalization (Svoboda *et al.*, 1996; Majewska *et al.*, 2000a), we hypothesize that, rather than a continuum of spine with respect to their neck lengths, there are two distinct populations of spines, ones which are biochemically isolated from the dendrite and another one which are not. In addition, humans spines appear to be on average more biochemically isolated that mouse ones.

LACK OF CORRELATIONS AMONG SPINE SIZE AND NECK

We have searched for co-regulation of spine density, head size and neck length and have failed to encounter significant correlations among these three parameters in our sample. The lack of correlation between spine head area and neck length implies that they are regulated independently. This conclusion is in disagreement with the proposal that larger spines have longer necks (Jones & Powell, 1969), albeit we cannot rule out that with a more complete sample, or a sample from different cell types, such a correlation may exist. Nevertheless, our data implies that the control and potential function of the spine neck is in principle unrelated to the regulation and function of the spine head. It is conceivable that the neck length reflects the consequences of a developmental process, by which the spine grows to different length according to the input it wants to contact. Meanwhile the spine head size could be determined by the nature of that input and the life history and previous use of that synapse.

IMPLICATION FOR DIFFERENCES IN CORTICAL PROCESSING AMONG SPECIES

In summary, we have encountered major differences between mouse and human spines in every morphological variable we have measured. Human spines are more densely packed and are larger and longer than mouse ones. We even encounter significant differences in spine head size between mouse temporal and occipital cortices. We would argue that spines have a specific function, one that is likely to be of central importance in the cortical circuit. Whatever this specific function is, it appears to be carried out more effectively in human cortex than in mouse cortex. If spines are providing the circuit with implementations of local learning rules (Yuste & Majewska, 2001), humans could have a richer and more flexible circuit with more opportunities to regulate inputs. Even a cursory comparison between human and mouse spines underscores this point: human spines are enormous and have large necks and occur in great densities. It is therefore fair to argue that human pyramidal neurons are more "spiny" and is tempting to speculate that mental differences between humans and other mammals could be attributed to the increased number of spines (Elston *et al.*, 2001, 2002). It is pertinent here to recall the old debate between Cajal and Lorente de Nó on the morphological characteristics that could account for human intelligence, where Cajal argued that humans had a richer complement of neuronal classes (Ramón y Cajal, 1899), whereas Lorente argued that mice had a similar complement of neuronal classes than humans, but had fewer total numbers of

neurons (Lorente de Nó, 1922, 1933). These proposals are not mutually exclusive. We would add to this debate the fact that our data provides evidence for there are substantial morphological differences at the spine level, differences that might underlie cognitive differences.

Acknowledgments

We thank Guy Elston for his infinite patience in teaching us the many tricks of performing successful intracellular injections in fixed material and Dr. García-Sola from Hospital de la Princesa for the human samples. R.B.-P. thank the "Comunidad de Madrid" (01/0782/2000) and I.B.-Y. the MEC (AP2001-0671) for support. J.D. is supported by Spanish Ministry of Science and Technology (DGCYT PM99-0105) and the 'Comunidad de Madrid' (08.5/0027/2001.1). RY thanks the NEI (EY13237), NINDS (NS40726) and the John Merck Fund for support and the Cajal Institute for hosting him as a visiting professor. We thank members of both laboratories for help and R. Tapelia for inspiration.

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