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Catecholaminergic Innervation of Pyramidal Neurons in the Human Temporal Cortex

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In the human neocortex, catecholaminergic connections modulate the excitatory inputs of pyramidal neurons and are involved in higher cognitive functions. Catecholaminergic fibers form a dense network in which it is difficult to distinguish whether or not target specificity exists. In order to shed some light on this issue, we set out to quantify the catecholaminergic innervation of pyramidal cells in different layers of the human temporal cortex (II, IIIa, IIIb, V and VI). For this purpose, pyramidal cells were labeled in human cortical tissue by injecting them with Lucifer Yellow, and then performed immunocytochemistry for the rate limiting catecholamine synthesizing enzyme tyrosine hydroxylase (TH) to visualize catecholaminergic fibers in the same sections. Injected cells were reconstructed in three dimensions and appositions were quantified ($n = 1503$) in serial confocal microscopy images of each injected cell ($n = 71$). We found TH-immunoreactive appositions (TH-ir) in all the pyramidal cells analyzed, in both the apical and basal dendritic regions. In general, the density of TH-ir apposition was greater in layers II, V and VI than in layers IIIa and IIIb. Furthermore, TH-ir appositions showed a regular distribution in almost all dendritic compartments of the apical and basal dendritic arbors across all layers. Hence, it appears that all pyramidal neurons in the human neocortex receive catecholaminergic afferents in a rather regular pattern, independent of the layer in which they are located. Since pyramidal cells located in different layers are involved in different intrinsic and extrinsic circuits, these results suggest that catecholaminergic afferents may modify the function of a larger variety of circuits than previously thought. Thus, this aspect of human cortical organization is likely to have important implications in cortical function.

Keywords: catecholamines, connectivity, cortical circuitry, intracellular injections, tyrosine hydroxylase

Introduction

In the human brain, catecholamines are involved both in higher cognitive functions and in different pathologies such as Alzheimer's disease and schizophrenia (Palmer, 1996; Goldman-Rakic, 1998; Lidow *et al.*, 1998; Lewis and Lieberman, 2000; Benes *et al.*, 2000; Akil *et al.*, 2003). Indeed, it has been seen in a variety of species that catecholaminergic transmission in the cerebral cortex primarily modulates excitatory inputs to pyramidal neurons (Cepeda *et al.*, 1992; Williams and Goldman-Rakic, 1995; Jedema and Moghddam, 1996; Gao *et al.*, 2001; Tseng and O'Donnell, 2004), the dendrites of which are the main targets for catecholaminergic axon terminals (Goldman-Rakic *et al.*, 1989; Smiley *et al.*, 1992; Smiley and Goldman-Rakic, 1993; Cowan *et al.*, 1994; Carr and Sesack, 1996; Carr *et al.*, 1999; Erickson *et al.*, 2000; Benavides-Piccione and DeFelipe, 2003). This is consistent with the fact that various dopaminergic receptors are preferentially localized in the dendritic spines and shafts of pyramidal cells (Smiley *et al.*, 1994; Bergson *et al.*, 1995; Khan *et al.*, 2000). However, in these electron microscopy studies relatively few tyrosine hydroxylase (TH)-immunoreactive (TH-ir) axon terminals were examined, which, as previously pointed out by Benavides-Piccione and DeFelipe (2003), is an important constraint for establishing general principles of circuitry. Therefore, the use of double labeling for TH and other markers to visualize potential targets of axons is a valuable complementary method to study cortical circuits. Since a large number of TH-ir axon terminals can be examined in this way, it is more feasible to establish whether specific neurons or particular targets, such as the somata or dendrites, are innervated or not. For example, a quantitative analysis in the monkey prefrontal cortex combining immunostaining for TH and intracellular injections with Lucifer Yellow (LY) revealed the widespread innervation of both pyramidal and non-pyramidal dendritic arbors by TH-ir axons (Krimer *et al.*, 1997). These authors also found that the density of catecholaminergic inputs to the dendrites of pyramidal cells varied from layer to layer, although the distribution of these inputs appeared to remain remarkable uniform (Krimer *et al.*, 1997). This is important when considering that pyramidal cells represent the most common type of cortical neuron, and that the different circuits in which they participate depend on the layer in which they are located (Jones, 1984; White, 1989; Felleman and Van Essen, 1991; Morrison *et al.*, 1998). Nevertheless, there is a lack of information regarding the spatial relationship between catecholaminergic fibers and the dendritic arbors of pyramidal neurons in the human cerebral cortex. Therefore, the aim of this study was to map and quantify catecholaminergic appositions with the apical and basal dendritic arbors of pyramidal cells in different layers (II, IIIa, IIIb, V and VI) of the human temporal cortex.

Materials and Methods

Tissue Preparation

A total of eight human cases were used in this investigation, each patient providing informed consent prior to participating in the study. Tissue was obtained from the anterolateral inferior temporal gyri (Brodmann's area 20; see Garey, 1994) of patients suffering pharmaco-resistant temporal lobe epilepsy (Department of Neurosurgery, 'Hospital de la Princesa', Madrid, Spain): cases 1–3 were females, aged 24, 29 and 48 years, respectively; cases 4–8 were males, aged 25, 27, 30, 41 and 48 years, respectively. In each case, video-EEG recording from bilateral foramen ovale electrodes was used to localize the epileptic focus in mesial temporal structures. Furthermore, subdural recordings with a 20-electrode-grid (lateral neocortex) and with a 4-electrode-strip (uncus and parahippocampal gyrus) were used at the time of surgery to further identify epileptogenic regions. In this study, only neocortical tissue that

showed no abnormal spiking, as characterized by normal ECoG activity, and without histopathological abnormalities was used. Although we cannot rule out the possibility that the tissue used may have been indirectly influenced by epileptogenic activity, we are reasonably confident that the results are representative of normal conditions.

Surgically resected tissue was immediately immersed in cold 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4 (PB). After 2–3 h, the tissue was cut into small blocks (~15 × 8 × 8 mm) and post-fixed in the same fixative for 24 h at 4°C.

Intracellular Injections

Coronal sections (250 μm) from cases 2, 3 and 7 were obtained with the aid of a Vibratome and labeled with 4,6 diamino-2-phenylindole (DAPI; Sigma, St Louis, MO) to identify cell bodies. Then, pyramidal cells were individually injected with LY (8% in 0.1 M Tris buffer, pH 7.4), in cytoarchitectonically identified layers II, IIIa, IIIb, V and VI of the inferior temporal cortex. LY was applied to each injected cell by continuous current until the distal tips of each cell fluoresced brightly, indicating that the dendrites were completely filled and ensuring that the fluorescence did not diminish at a distance from the soma (for a detailed methodology of the cell injections, see Buhl and Schlote, 1987; Elston and Rosa, 1997; Elston *et al.*, 2001). Since intracellular injections of pyramidal cells were made in coronal sections, the part of the dendritic arbor nearer to the surface of the slice from which the cell somata were injected (typically at ~30 μm from the surface) was lost. Furthermore, with this method the apical dendrites that run for further than ~900 μm from the somata were not filled with dye, and therefore apical tufts were not included in the analysis. Using a similar method of intracellular injection, Krimer *et al.* (1997) estimated that reconstruction of neurons represented approximately two-thirds of the total dendritic arbor of pyramidal cells.

Immunocytochemistry

Single Labeling Procedure

Sections (100 μm) from cases 1, 4, 5, 6 and 8 were preincubated in 3% normal serum (horse or goat) in PB with Triton X-100 (0.25%) for 2 h at room temperature. These sections were then incubated for 24 h at 4°C in the same solution containing mouse anti-tyrosine hydroxylase (1:1000; Diasorin, Stillwater, MN). They were then washed in PB and incubated for 1 h at room temperature in biotinylated goat anti-rabbit IgG or horse anti-mouse diluted 1:200 in PB (Vector, Burlingame, CA). Thereafter, the sections were processed using the Vectastain ABC immunoperoxidase kit (Vector) and the antibody distribution was detected histochemically with 0.05% 3,3′-diaminobenzidine tetrahydrochloride (DAB; Sigma) and 0.01% hydrogen peroxide. Finally, they were mounted, dehydrated, cleared with xylene and coverslipped.

Confocal Laser Microscopy for LY and Tyrosine Hydroxylase (TH)

Following the intracellular injection of pyramidal neurons, the sections were double-stained for LY and TH using rabbit antisera against LY (1:400,000; generated at the Cajal Institute) and TH (mouse, 1:1000; Diasorin) diluted in stock solution (2% bovine serum albumin, 1% Triton X-100, 5% sucrose in PB). The sections were then incubated in biotinylated donkey anti-rabbit IgG (1:100; Amersham, Buckinghamshire, UK) and in a mixture of Alexa fluor 594-conjugated goat anti-mouse (1:1000; Molecular Probes, Eugene, OR) and Alexa fluor 488 streptavidin-conjugated (1:1000; Molecular Probes). Finally, sections were mounted in 50% glycerol in PB. Control sections were processed as above but the primary antibody was omitted, or alternatively, the secondary antibody was replaced with an irrelevant secondary antibody. No specific labeling was observed under these control conditions. Double-labeled sections were studied with the aid of a Leica TCS 4D argon/krypton mixed gas confocal scanning laser attached to a Leitz DMIRB fluorescence microscope. Fluorescent labeling profiles were imaged through separate channels, using excitation peaks of 585 and 491 nm to visualize Alexa fluor 594 and 488, respectively.

Quantitative Analyses

The relative density of TH-ir fibers in each cortical layer was quantified in sections where the labeled fibers were visualized after TH immunostaining using the immunoperoxidase method and DAB. We counted the number of intersections of TH-ir fibers using a superimposed grid (62 × 47 μm) of 10 μm spaced lines (Verney *et al.*, 1993), and a ×100 objective lens at a final magnification of ×3000. A total of 500 images were sampled from layers II, III, V and VI (20 images per cortical layer). TH-ir fibers were examined with the aid of a microscope equipped with a Hitachi CCD color video camera. Images were captured at a single focal depth in a random *Z*-plane of the section where the primary antibody had penetrated.

In double labeled sections, three dimensional reconstruction of pyramidal cells was performed (n = 71). Putative appositions of TH-ir fibers were marked from of serial stacks confocal microscopy images (30–55 images per stack; 2 μm intervals in the *Z*-axis) of each injected cell (*Z*-axis width, mean ± SD, 71,8 ± 17,3; range, 28–116 μm). In order to confirm that differences in antibody penetration did not alter the immunolabeling of TH at different depths of the scanned stacks, TH-ir fiber density was measured at the most superficial and the deepest region of the stacks in layer III of all tissue slices (n = 6). Sets of six serial images were sampled from the top and bottom of the stacks (two stacks per slice). No significant differences were observed in the density of the TH-ir fibers between the superficial region (mean ± SEM, 0.93 ± 0.09 μm/100 μm^2) and the deep region of the stacks (0.89 ± 0.08 μm/100 μm^2; paired sample two-tailed t-test, P = 0.45). Therefore, we assumed that penetration of the antibody was homogeneous throughout the region in which the labeled pyramidal cells were analyzed. Appositions between TH-ir axon terminals and dendritic spines and/or shafts of labeled pyramidal cells were determined by studying each single serial confocal image of the stack from every injected pyramidal cell at a final magnification of ×100. Only confocal images that contained immunostained processes for both antigens were analyzed. TH-ir axonal boutons were considered to contact dendrites when both labeled elements appeared to be in direct apposition, in the same confocal microscope image (TH-ir appositions). The Neurolucida package (MicroBrightField Europe, Magdeburg, Germany) was used to trace the dendritic arbor of pyramidal cells three-dimensionally and map the TH-ir appositions. The density of TH-ir appositions to pyramidal cells as a function of the distance from the soma was determined using concentric spheres of increasing 75 μm radii for each cell (centered on the cell body).

Results

The presence of TH-ir fibers in all layers of the human temporal cortex has been previously documented showing a bilaminar pattern of distribution (Gaspar *et al.*, 1987; Hornung *et al.*, 1989; Kuljis *et al.*, 1989; Lewis, 1992; Benavides-Piccione and DeFelipe, 2003). In the present study, we estimated the relative density of TH-ir fibers in layer I and in those cortical layers where pyramidal cells were injected. We found that layer I had the highest density of fibers, followed by layer VI, layer II, V and finally layer III (arbitrary units: layer I, 8.25; layer II, 5; layer III, 1.63; layer V, 3.42; layer VI, 6.79). Hence, we detected significant differences in the density of TH-ir fibers in all layers [one-way analysis of variance (ANOVA), $F(4,499) = 81.7$, $P < 0.001$; post-hoc Bonferroni analysis, $P < 0.001$).

Seventy-one injected pyramidal cells (Fig. 1) were reconstructed in three dimensions and the putative contacts with TH-ir fibers (appositions; n = 1503) were quantified in the reconstructed serial confocal microscopy images of each injected cell. TH-ir axons were considered to be in apposition with a dendrite when both labeled elements were directly juxtaposed in the same confocal image (Fig. 1*h,i*). We found that all pyramidal neurons examined received catecholaminergic inputs to their dendritic spines or shafts in both the apical and basal dendritic regions (Fig. 1*h,i*). Furthermore, while a given catecholaminergic fiber was usually in apposition with a labeled dendrite only once, in some cases a single fiber

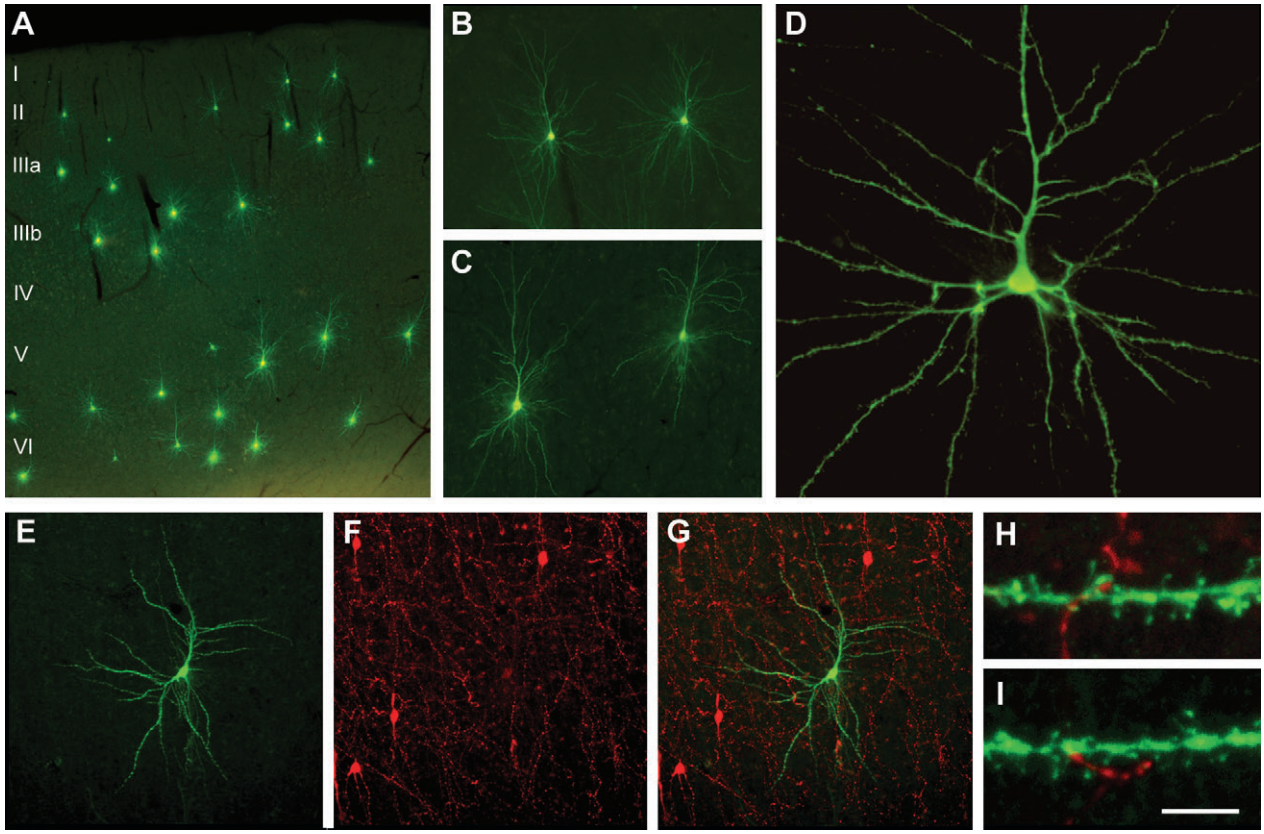

Figure 1. (*A–D*) Confocal microscopy images of neurons injected with LY. (*A*) Labeled pyramidal cells in layers II, IIIa, IIIb, V and VI of the human temporal cortex. (*B*, *C*) Pyramidal cells from layer II (*B*) and V (*C*) at higher magnification. (*D*) A high-power confocal image of a labeled pyramidal cell from layer III illustrating the extent of the labeling. (*E*, *F*) A pair of pseudo-colored confocal images, each from the same microscopic field in layer VI, showing an injected pyramidal neuron (*E*) as well as TH-ir neurons and fibers (*F*). (*G*) By combining these images (*E* and *F*), the relative location of TH-ir axons with respect to the pyramidal cells could be established. (*H*, *I*) High-magnification images showing examples of TH-ir axonal appositions with the dendritic spines (*H*) and shafts (*I*) of a labeled pyramidal cell. Scale bar = 450 μm in *A*, 170 μm in *B* and *C*, 25 μm in *D*, 130 μm in *E–G* and 7.5 μm in *H* and *I*.

established 2–4 appositions with the same dendritic segment. In order to quantify this observation, we counted the number of TH-ir appositions that were at a distance ⩽ 5 μm from each other in the same dendritic segment in all neurons. We found that 16% (n = 242) of the TH-ir appositions were seen in clusters of two and 11% (n = 177) were seen in clusters of three or more. These clusters of two and of three or more appositions were found with a frequency of 1.7 and 0.83 per pyramidal neuron, respectively. Only 0.4% (n = 6) of appositions were observed on the labeled somata.

The apical and basal dendritic arbors in each layer showed a remarkable similar density of TH-ir appositions, in spite of the individual cell variability (Fig. 2*a*,*b* and Table 1). Indeed, the only significant difference we found was between the apical and basal dendrites of layer IIIb [one-way ANOVA, $F(1,21) = 11.4$, $P < 0.005$]. Moreover, the average number of TH-ir appositions per pyramidal neuron in the different layers was also similar (18.8, 18.6, 21.0, 23.5 and 26.3 in layers II, IIIa, IIIb, V and VI; Table 1). The total density of TH-ir appositions in pyramidal cells from layers II, V and VI was similar, and significantly higher than that of layers IIIa and IIIb (see Table 2 for statistical analysis). Since the three human cases that we examined were of different ages (29, 41, and 48 years old), we investigated possible effect that age might have on the density of TH-ir appositions on pyramidal cells. We found no evidence that age influenced the densities of these appositions (Fig. 2*c*).

The analysis of the distribution of catecholaminergic appositions as a function of the distance from the soma of pyramidal cells was calculated in increasing radii of 75 μm (Fig. 3). This value was chosen since the highest density of dendritic spines in the basal dendritic arbors of pyramidal neurons in the human temporal cortex corresponds to 75–150 μm from the soma (Elston *et al.*, 2001; Elston and DeFelipe, 2002). We found that TH-ir appositions were evenly distributed in both apical and basal dendritic regions in all cortical layers, except for those in layer V (Fig. 2*d–f*), where the distal dendritic compartments showed a significantly lower density of catecholaminergic appositions [one-way ANOVA, apical region: $F(3,45) = 5.03$, $P < 0.005$. Post-hoc Bonferroni analysis, $P < 0.005$ between the 151–225 μm and 226–300 μm intervals; basal region: $F(3,45) = 3.53$, $P < 0.05$. Post-hoc Bonferroni analysis, $P < 0.05$ between the 76–150 μm and 226–300 μm intervals]. The study of the distribution of TH-ir appositions by branch order revealed no significant differences except for the basal region of pyramidal cells of layer VI and IIIa in the dendritic orders 1, 3 and 4 [one-way ANOVA, order 1, $F(4,68) = 3.67$, $P < 0.01$; order 3, $F(4,67) = 3.61$, $P < 0.01$; order 4, $F(4,61) = 2.78$, $P < 0.05$].

Discussion

This study provides overall information on the distribution of putative catecholaminergic inputs to pyramidal cells located in

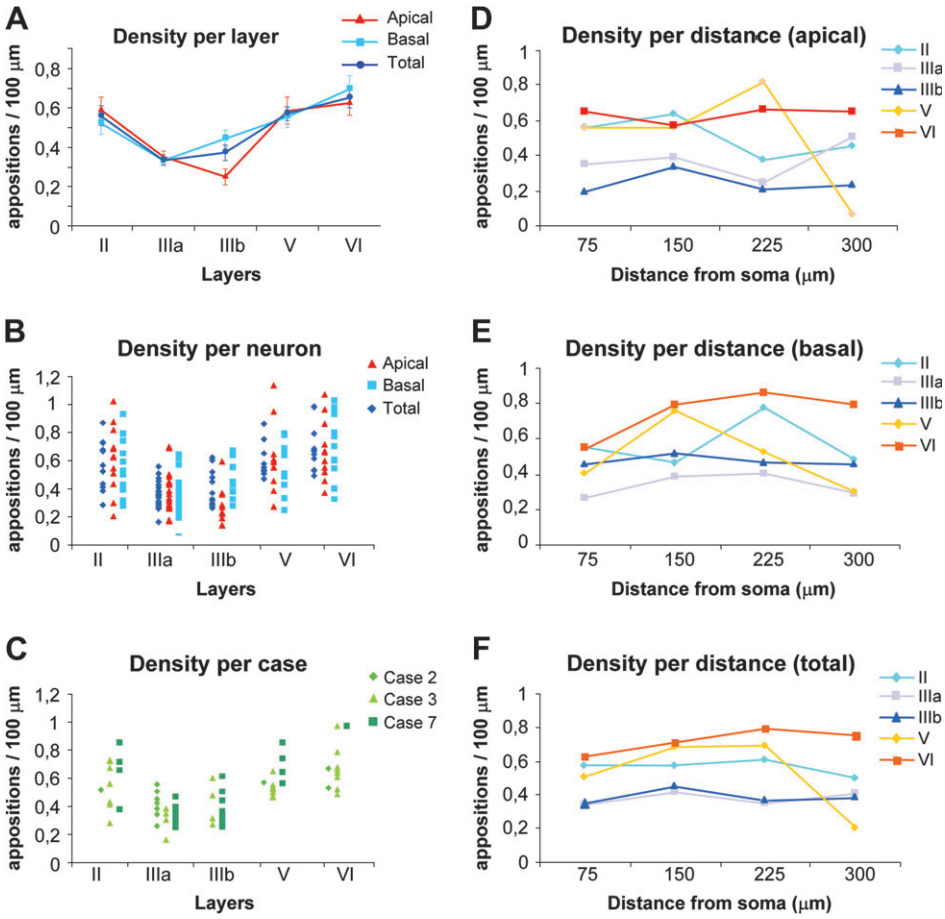

Figure 2. (*A*) Graphical representation of the density of TH-ir appositions on apical, basal and total dendrites of pyramidal cells from layers II, IIIa, IIIb, V and VI of the human temporal cortex. (*B*) Total density of TH-ir appositions per neuron in each cortical layer showing the individual cell variability on apical, basal and total dendrites. (*C*) Cell variability of the density of total TH-ir apposition across layers I–VI in human cases 2, 3 and 7 (aged 29, 48 and 41 years, respectively). (*D–F*) Density of TH-ir appositions along the apical (*D*), basal (*E*) and total dendritic region (*F*), as a function of the distance from the pyramidal cell somata (75 µm segments) in layers II–VI.

layers II–VI of the human temporal neocortex. To our knowledge, this is the first detailed study performed in the neocortex to examine the distribution of catecholaminergic inputs in three-dimensionally reconstructed pyramidal cells. While the methods applied do not enable us to determine the nature of these appositions, they do reveal significant features of catecholaminergic organization: (i) all pyramidal neurons examined have TH-ir appositions on the dendritic arbor; (ii) the density of these appositions within a given layer is similar in the apical and basal dendritic arbors; (iii) pyramidal cells from layers II, V and VI display a higher density of TH-ir appositions than layers IIIa and IIIb; and (iv) the distribution of TH-ir appositions along the apical and basal dendritic arbors of pyramidal cells is evenly distributed in all cortical layers, except for layer V, where a lower density exists in the distal dendritic compartments.

In general, pyramidal cells of the supragranular layers are the main origin of callosal and ipsilateral corticocortical connections, whilst cells located in the infragranular layers project to either subcortical nuclei or to other cortical areas. Indeed, the deeper the cells are in the supragranular layers, the further away the cortical fields to which they tend to project. In addition, there are considerable variations in the local axonal collaterals of pyramidal cells located in different layers (Jones, 1984; White, 1989; Lund, 1990; Felleman and Van Essen, 1991). The results presented here show that in the human neocortex, all pyramidal neurons appear to be recipients of catecholaminergic afferents, irrespective of the layer in which they are located. In turn, this suggests that catecholaminergic afferents may modify the function of a larger variety of extrinsic and intrinsic circuits than previously thought, consistent with the idea of the general modulatory role of the catecholaminergic innervation (reviewed in Goldman-Rakic, 1996; Sesack *et al.*, 2003).

In the human temporal neocortex the density of TH-ir appositions in the apical dendrites was similar to that found in the basal dendrites in any given cortical layer. Similarly, a study performed in the monkey prefrontal cortex failed to detect differences between the apical and basal regions of pyramidal cells in any layer (Krimer *et al.*, 1997). However, in this study a higher density of TH-ir appositions was found in all layers of the monkey prefrontal cortex (0.75–1.65 TH-ir appositions per 100 µm of dendritic length) when compared with the human temporal neocortex (0.35–0.66; present study). Since there is a higher density of catecholaminergic fibers in the prefrontal cortex than in the temporal cortex (reviewed in Lewis, 1992), the differences found in the macaque prefrontal cortex and human temporal cortex might be attributed to differences between cortical areas and/or species. Indeed, layer II in the macaque presented the highest TH-ir apposition density whereas in the human cortex TH-ir appositions were most dense in layers II, V and VI. Nevertheless, in both cases the

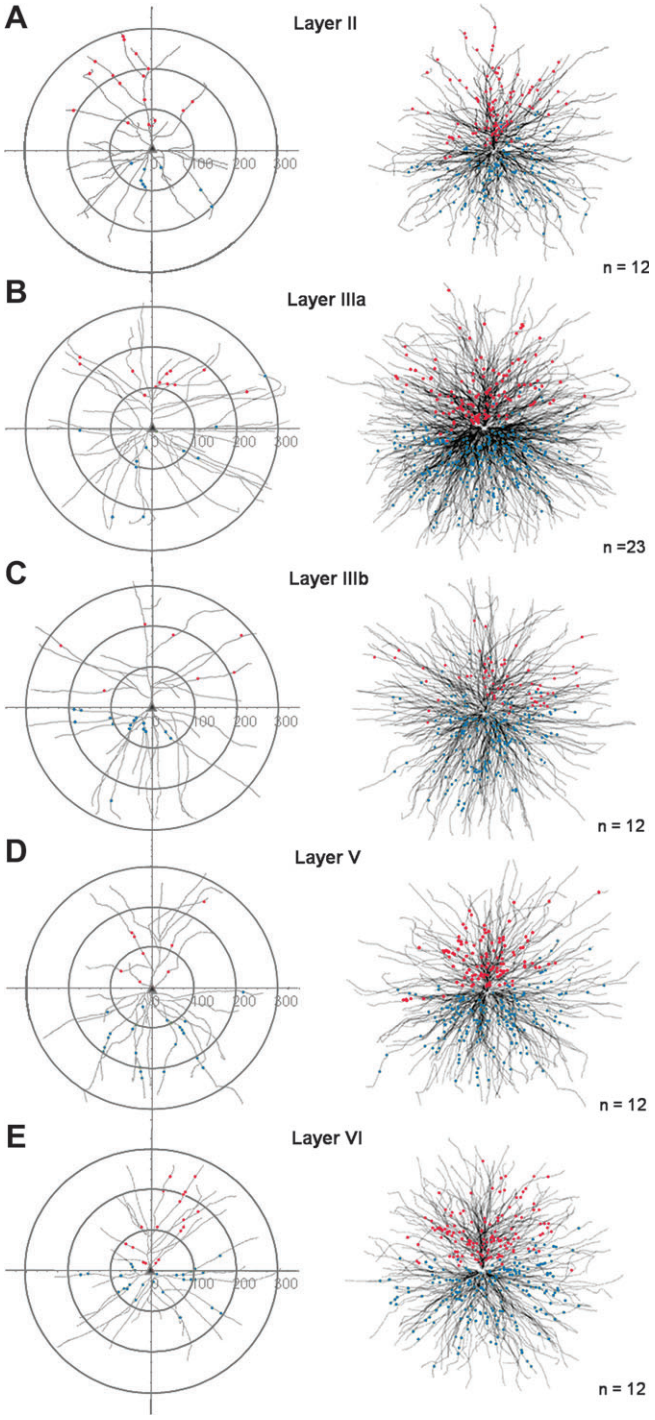

Figure 3. The distribution of TH-ir inputs along the length of apical (red) and basal (blue) dendrites of reconstructed pyramidal cells from layers II (*A*), IIIa (*B*), IIIb (*C*), V (*D*) and VI (*E*) of the human temporal cortex. Left column, examples of single reconstructed neurons in each layer. Right column, superimposition of all the reconstructed neurons per layer.

highest numbers of appositions corresponded to the layers with the highest density of TH-ir fibers. Furthermore, in the human neocortex, the catecholaminegic inputs to pyramidal cells may originate both from neurons located in subcortical nuclei (locus coeruleus, substantia nigra, ventral tegmental area) and intrinsic cortical neurons, and hence in all cortical layers these connections could originate from both sources (see Benavides-Piccione and DeFelipe, 2003). However, there are no or very few (depending on the cortical area) TH-ir cortical interneurons in the macaque monkey (Kohler *et al.*, 1983; Lewis *et al.*, 1988; R. Benavides-Piccione and J. DeFelipe, unpublished observations), suggesting that the pattern of TH-ir fiber in monkeys may better reflect the extrinsic inputs from the midbrain than in humans.

The distribution of dendritic spines, and therefore of glutamatergic inputs, along the dendrites of pyramidal cells, has been shown not to be uniform in different cortical areas and species (reviewed in Elston and DeFelipe, 2002). While the proximal portions of pyramidal cell dendrites are devoid of spines (~10–15 μm from the soma), there is a progressive increase in the density of spines. The highest densities are found at variable distances from the soma, depending on the cortical area and species. In the human temporal cortex, the highest density is found at a distance of 75–125 μm from the soma. Thereafter, there is a progressive decrease towards the distal tips of dendrites where the density is again very low (Elston *et al.*, 2001). The examination of the dendritic segment with the highest density of spines showed a similar density of TH-ir appositions than in the other dendritic segments. Hence, the distribution of TH-ir appositions does not appear to be correlated with the density of spines and, thus, with the density of glutamatergic inputs. In general, the regular distribution of TH-ir appositions throughout the apical and basal dendritic arbors of pyramidal neurons in all cortical layers suggests that, in principle, all afferent systems of pyramidal neurons might be equally affected. Furthermore, since no significant differences in the distribution of TH-ir appositions were observed by branch order (except for the basal dendrites of pyramidal cells of layer VI and IIIa), the effect of TH inputs appears to depend mainly on the complexity of the pyramidal cell dendritic arbor. Nevertheless, TH-ir appositions were sometimes found in small clusters, and thus we cannot ignore the possibility that a particular set of inputs in certain dendritic segments are more affected than others. These results are similar to those reported by Krimer *et al.* (1997) in the monkey prefrontal cortex, where TH-ir appositions to pyramidal cells were also relatively evenly distributed. However, these authors did not study the density of appositions all along the dentritic length from the soma. Therefore, the limited and qualitative nature of the observations of Krimer *et al.* prevent us from contrasting both studies in more detail.

Considering that subcortical afferent catecholaminergic fibers are widespread (Hökfelt *et al.*, 1976, 1977; Fuxe and Agnati, 1991), it is hard to believe that target specificity exists in this system. This is also consistent with the rather even distribution of the TH-ir appositions along the dendritic arbors of all pyramidal cells and with the higher density of appositions in the layers with higher density of fibers. However, it is also true that the variability in the innervation of individual pyramidal neurons indicates that some pyramidal neurons could be preferentially innervated, as has been shown for certain GABAergic interneurons (Sesack *et al.*, 2003). A possible source of this differential targeting are the axons that arise from the numerous TH-ir interneurons present in layers V–VI of the human neocortex (Gaspar *et al.*, 1987; Hornung *et al.*, 1989; Kuljis *et al.*, 1989; Lewis, 1992; Benavides-Piccione and DeFelipe, 2003). These neurons have local axons and some of them have been identified as Martinotti cells or neurons with ascending axons (Benavides-Piccione and DeFelipe, 2003). We

Table 1
Density of TH-ir appositions (mean ± sem) in the apical, basal and total dendritic regions of pyramidal cells in layers II, IIIa, IIIb, V and VI of the human temporal cortex

Layers	Dendritic region	Density per 100 μm of dendritic length	Average number of appositions per neuron (range)	Average dendritic length per neuron (μm)	Total number of appositions per layer	Total dendritic length per layer (μm)	Number of neurons analyzed
II	Apical	0.59 ± 0.07	8.3 ± 1.6	1377 ± 146	100	16519	12
	Basal	0.52 ± 0.06	10.5 ± 1.2	2101 ± 189	126	25209	12
	Total	0.55 ± 0.04	18.8 ± 1.6 (12–29)	3477 ± 205	226	41728	12
IIIa	Apical	0.35 ± 0.03	5.4 ± 0.7	1697 ± 213	119	37323	22
	Basal	0.34 ± 0.03	13.4 ± 1.3	4052 ± 218	309	93198	22
	Total	0.33 ± 0.05	18.6 ± 1.4 (9–32)	5675 ± 333	428	130521	23
IIIb	Apical	0.25 ± 0.04	6.1 ± 1.3	2454 ± 511	67	26997	11
	Basal	0.44 ± 0.04	16.8 ± 2.2	3795 ± 299	185	41740	12
	Total	0.35 ± 0.03	21.0 ± 2.6 (11–40)	5728 ± 435	252	68737	12
V	Apical	0.59 ± 0.07	9 ± 1.1	1586 ± 194	108	19030	12
	Basal	0.55 ± 0.05	14.5 ± 2	2532 ± 183	174	30390	12
	Total	0.57 ± 0.04	23.5 ± 2.1 (11–33)	4118 ± 302	282	49420	12
VI	Apical	0.62 ± 0.06	11 ± 1.3	1781 ± 118	132	21372	12
	Basal	0.70 ± 0.07	15.3 ± 1.8	2246 ± 183	183	26952	12
	Total	0.66 ± 0.05	26.3 ± 2.6 (16–48)	4027 ± 186	315	48324	12

Table 2
Statistical comparisons of the density of TH-ir appositions in the apical, basal and total dendritic regions of pyramidal cells of the human temporal cortex between layers II, IIIa, IIIb, V and VI

		II	IIIa	IIIb	V	VI
Apical, one-way ANOVA, $F(4,68) = 9.99$, $P < 0.001$	II		*	**		
	IIIa	*			*	**
	IIIb	**			**	***
	V		*	**		
	VI		**	***		
Basal, one-way ANOVA, $F(4,70) = 9.49$, $P < 0.001$	II		*			
	IIIa	*			*	***
	IIIb					*
	V		*			
	VI		***	*		
Total, one-way ANOVA, $F(4,70) = 15.89$, $P < 0.001$	II		***	*		
	IIIa	***			***	***
	IIIb	*			*	***
	V		***	*		
	VI		***	***		

*Post-hoc Bonferroni analysis, $P < 0.05$.
**Post-hoc Bonferroni analysis, $P < 0.005$.
***Post-hoc Bonferroni analysis, $P < 0.0005$.

have also observed that most TH-ir boutons (94%) are located in the neuropil, indicating that these neurons most probably target dendrites. If these interneurons preferentially innervate certain pyramidal cells, as has been shown to occur in the human neocortex with calretinin-positive interneurons, for example (del Río and DeFelipe, 1997), this could be related to the differences in the density of TH-ir appositions to pyramidal cells found here. TH-ir cortical neurons have been shown to be neurochemically heterogeneous (Benavides-Piccione and DeFelipe, 2003). These neurons appear to synthesize only DOPA, as they do not express aromatic amino acid decarboxylase and dopamine-β-hydroxylase necessary for the synthesis of dopamine and noradrenaline, respectively (Gaspar *et al.*, 1987; Lewis, 1992; Ikemoto *et al.*, 1999). Nevertheless, the levels of one or both of these enzymes may be below the sensitivity of the standard immunocytochemical procedures in these cells. Another possibility is that TH might be inactive in cortical neurons. Hence, further studies will be necessary to elucidate the neurochemical characteristics and connections made by intrinsic TH-ir neurons and their convergence with subcortical cathecolaminergic systems.

Electron microscopical analysis of the cathecolaminergic innervation of the neocortex in both monkeys and humans has shown that catecholamines can act directly through synapses with dendritic shafts and spines of pyramidal cells (Goldman-Rakic *et al.*, 1989; Smiley *et al.*, 1992; Smiley and Goldman-Rakic, 1993; Cowan *et al.*, 1994; Carr and Sesack, 1996; Carr *et al.*, 1999; Erickson *et al.*, 2000). Since not all TH-ir terminals are engaged in synapses and some dopamine receptors appears to be extrasynaptic, it is thought that catecholamines may also exert their modulatory effect on target neurons through a non-synaptic and volume transmission mechanism (reviewed in Goldman-Rakic, 1996; Sesack *et al.*, 2003). The present results are consistent with these electron microscope studies and with those of the distribution of dopaminergic receptors. These receptors have been preferentially localized in dendritic shafts and spines of pyramidal cells (Smiley *et al.*, 1994; Bergson *et al.*, 1995). In particular, the D1 receptor is prevalent in dendritic spines whereas D5 is predominately found on dendritic shafts of pyramidal cells (reviewed in Goldman-Rakic, 1996; Sesack *et al.*, 2003).

Finally, it has been suggested that the basis of certain psychiatric disorders, such as schizophrenia, may be due to aberrant connections between cortical neurons and the monoaminergic system (Desimone, 1995; Benes *et al.*, 1997, 2000). Since the intracellular injection methodology can also be applied successfully to material from human autopsies (Elston *et al.*, 2001), future studies on the distribution of catecholaminergic innervation to pyramidal cells in these subjects might help better understanding the alterations of catecholaminergic circuits in these disorders.

Notes

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