Spindle Neurons of the Human Anterior Cingulate Cortex

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ABSTRACT

The human anterior cingulate cortex is distinguished by the presence of an unusual cell type, a large spindle neuron in layer Vb. This cell has been noted numerous times in the historical literature but has not been studied with modern neuroanatomic techniques. For instance, details regarding the neuronal class to which these cells belong and regarding their precise distribution along both ventrodorsal and anteroposterior axes of the cingulate gyrus are still lacking. In the present study, morphological features and the anatomic distribution of this cell type were studied using computer-assisted mapping and immunocytochemical techniques. Spindle neurons are restricted to the subfields of the anterior cingulate cortex (Brodmann's area 24), exhibiting a greater density in anterior portions of this area than in posterior portions, and tapering off in the transition zone between anterior and posterior cingulate cortex. Furthermore, a majority of the spindle cells at any level is located in subarea 24b on the gyral surface. Immunocytochemical analysis revealed that the neurofilament protein triplet was present in a large percentage of these neurons and that they did not contain calcium-binding proteins. Injections of the carbocyanine dye DiI into the cingulum bundle revealed that these cells are projection neurons. Finally, spindle cells were consistently affected in Alzheimer's disease cases, with an overall loss of about 60%. Taken together, these observations indicate that the spindle cells of the human cingulate cortex represent a morphological subpopulation of pyramidal neurons whose restricted distribution may be associated with functionally distinct areas. © 1995 Wiley-Liss, Inc.

Indexing terms: cerebral cortex, cytoarchitecture, human brain, neurofilament protein, pyramidal neuron

In recent years, the human cingulate cortex, especially its anterior portion, has been the subject of heightened interest. This development stands in stark contrast to many decades of neglect in large part due to its inaccessibility. The relatively few physiological studies that were performed in the monkey generally focused on the role of the anterior cingulate cortex in autonomic control (Kaada, 1951; Showers and Crosby, 1958; Dua and MacLean, 1964; Robinson and Mishkin, 1968). These complemented the handful of studies performed in human subjects that involved stimulation of this area during neurosurgical procedures (Pool and Ransohoff, 1949; Pool, 1954). The results of these experiments confirmed the role of the anterior cingulate gyrus in autonomic regulation and supported the notion that the monkey anterior cingulate cortex was functionally homologous to that of the human. However, studies of patients with anterior cingulate lesions, organically or surgically induced, hinted at a broader role for this area involving speech initiation (Nielsen and Ja-

cobs, 1951; Barris and Schuman, 1953; Nakasu et al., 1991), attentiveness (Watson et al., 1973), and expression of emotion (Arroyo et al., 1993). With the advent of functional imaging techniques such as positron emission tomography, this region has been implicated in manual and oculomotor control (Petit et al., 1993), memory (Perani et al., 1993), attention (Pardo et al., 1990; Corbetta et al., 1991, 1993; Bench et al., 1993), and the experience of pain (Jones et al., 1991; Talbot et al., 1991; Pagni and Canavero, 1993). Although some of these functions, such as the processing of pain or painful stimuli, were originally described in nonprimate species (Sikes and Vogt, 1992), some of them seem distinctly human, such as the expression of the emotional content of speech (Nielsen and Jacobs, 1951)

and the experience of auditory hallucinations in schizophrenia (McGuire et al., 1993). Finally, recent detailed studies of the cytoarchitecture of the cingulate gyrus in the macaque monkey (Vogt, 1976, 1993; Vogt et al., 1987) and human (Vogt et al., in preparation) demonstrate that, although there are many similarities, substantive differences exist between the cingulate cortices of these two species. As the capacity for functional studies in the human increases, so does the appreciation of the physiological significance of the anterior cingulate gyrus and the interest in identifying structural features unique to the anterior cingulate cortex of humans.

In his 1881 series of articles on the cytoarchitecture of the human cerebral cortex, Betz described a distinctive neuron in the human cingulate gyrus (Betz, 1881). What was so striking was not merely its shape—vertical (spindle-shaped neurons can readily be encountered in layer VI of most cortical areas; Tömböl, 1984)—but its size, its abundance, and especially its location in layer V. Almost two decades later, Cajal, who credited both Betz and Hammarberg with the initial descriptions (Betz, 1881; Hammarberg, 1895), reported these neurons in the insular cortex of a newborn human and mentioned their presence in the cingulate gyrus, referring to their location as the "layer of spindle cells" (Cajal, 1899). He reported that he was unable to apply successfully the Golgi stain to interhemispheric cortex in any animal but rodents and other small mammals. The Golgi-impregnated spindle neurons he drew were taken from the insular cortex and showed neurons with a long, tapered soma with a basal dendrite that formed a tuft of spiny dendrites and an apical dendrite that usually branched in layer IV but occasionally branched shortly after its origin. The axon descended toward the white matter after giving off numerous collaterals. In 1926, Von Economo undertook a study of these cells, which he first thought were pathological structures, because he saw them initially in the brains of patients with encephalitis lethargica (Von Economo, 1926). Rose made mention of them in his 1927 monograph and made the interesting observation that these neurons can also be observed in the cingulate cortex of the chimpanzee (Rose, 1927). In his 1932 description, Ngowyang mentioned that these cells frequently form clusters (Ngowyang, 1932). Most recently, Braak (1980) noted these neurons' distinctive pigment pattern.

Despite more than a century of periodic interest, however, the above-mentioned studies leave many questions unanswered. First, morphological details are lacking. All of these studies except Cajal's were based solely on the Nissl stain, and Cajal's Golgi analysis was based on neurons in the insular, not in the cingulate, cortex. Second, it is uncertain what type of neurons these are. Cajal's report of an axon heading toward the white matter is suggestive of a projection neuron, but, again, it is from a different cortical area and could form mainly, or only, recurrent collaterals. Third, the precise location of these neurons is unclear. In view of recent advances in the anatomic parcellation of the cingulate gyrus, and, in view of the understanding of the connectivity of its subregions, the knowledge of exactly which areas contain distinctive cell types can contribute greatly to an understanding of how the cingulate gyrus is organized and, perhaps, what the functional role of these neurons might be. Finally, it is unclear what happens to these cells in disease processes. Current views of neurodegenerative disorders recognize a degree of selective neuronal vulnerability among neuronal populations (Morrison et

al., 1987; Hof and Morrison, 1990, 1991; Hof et al., 1990, 1991, 1993b,c). The susceptibility or resistance of a defined cell type to degeneration can provide information about both the cell type and the pathological process itself. The present study constitutes a more detailed analysis of these neurons and their distribution in order to characterize further one of the features that sets the human anterior cingulate cortex apart from other cortical areas.

MATERIALS AND METHODS
Staining procedures

The brains from three neurologically normal patients (mean age 77.3 ± 5.0 years, range 72–84) and four patients clinically and pathologically diagnosed with Alzheimer's disease (AD; mean age 73.5 ± 10.6 years, range 60–89) were obtained at autopsy. In addition, the brain of a 72-year-old patient with large striatal infarcts was obtained, and, because it showed no pathological alterations in the cingulate cortex, it was utilized as a control case. All of the AD cases had numerous senile plaques and neurofibrillary tangles throughout the cerebral cortex. The neuropathologic evaluation of the control cases revealed rare neurofibrillary tangles exclusively located in the entorhinal cortex. All brains were collected within 1 and 10 hours of death and fixed by immersion for 48–72 hours in fresh 4% paraformaldehyde. The brains were collected according to appropriate ethical guidelines, and all protocols were reviewed and approved by the relevant institutional ethics committees. The entire cingulate gyrus was dissected out of the hemispheres and removed intact. The brain sample was placed in fresh 4% paraformaldehyde and postfixed for up to 48 hours longer. The tissue was then dissected either into 1-cm-thick coronal blocks or parasagittally, separating the medial face containing largely areas 24b and 23b from the rest of the gyrus. The blocks were then cryoprotected by immersion in solutions of increasing concentrations of sucrose, from 12% to 30%, in phosphate-buffered saline (PBS). Blocks were frozen on dry ice and cut at 40 μm on a cryostat or sliding microtome. Frozen sections were utilized in preference to plastic- or paraffin-embedded sections, because neurofilament immunoreactivity is best preserved in frozen material, especially in human brain. For the neurologically normal cases, five consecutive series were taken every 25 sections, with the result that the entire gyrus was sampled once every 1 mm, and all sections stained at each level were no more than 200 μm apart. The sections stained with thionin and those processed for immunohistochemistry were always immediately adjacent to each other. In the remaining cases, the anterior cingulate gyrus was sampled at the level of the genu, and sections were taken in a nonserial fashion.

After sectioning, the sections were rinsed thoroughly in PBS, treated in a solution of 0.75% hydrogen peroxide in 75% methanol for 20 minutes to eliminate endogenous peroxidase activity, rinsed again, and then placed into a solution of primary antibody in a diluent containing 0.3% Triton X-100 and 0.5 mg/ml bovine serum albumin. One series of sections was incubated in an antibody that recognizes a nonphosphorylated epitope on the neurofilament protein triplet (SMI32; Sternberger Monoclonals, Baltimore, MD; Sternberger and Sternberger, 1983; Lee et al., 1988) at a dilution of 1:10,000. In addition, series from four cases were stained with antibodies to the calcium-binding proteins parvalbumin, calbindin, and calretinin (Swant,

Bellinzona, Switzerland) at dilutions of 1:3,000, 1:800, and 1:3,000, respectively. Tissue was incubated for a minimum of 40 hours on a rotating table at 4°C. Following incubation, sections were processed by the avidin-biotin method using a Vectastain ABC kit (Vector Laboratories, Burlingame, CA) and intensified in 0.0067% osmium tetroxide.

Tract tracing with the carbocyanine dye DiI was performed in accordance with previously described protocols (Mufson et al., 1990; Supprian et al., 1993). Crystals of DiI were mixed into a paste with methanol and placed in the cingulum bundle with a 23-gauge needle. The deposits were made 2–3 mm below the cortex, and care was taken to prevent stray crystals from dislodging and settling on other parts of the tissue. The block was then placed in phosphate buffer with 0.1% sodium azide at room temperature and protected from light for 2–5 months. One hundred-micrometer-thick sections were cut on a vibratome and viewed under rhodamine narrow-pass filters at ×40 to visualize the dye.

Quantitative analysis

All quantitative analyses were performed on a computer-assisted image analysis system consisting of a Zeiss Axiophot photomicroscope equipped with a Zeiss MSP65 computer-controlled motorized stage, a high sensitivity Zeiss ZVS-47E video camera, a DEC 5000 workstation and Macintosh II microcomputer, and custom software developed in collaboration with the Scripps Research Institute (La Jolla, CA). For each section, cortical fields were captured with a Zeiss ×10 PlanApochromat objective and digitized, and the cingulate cortex was mapped using a Zeiss ×20 PlanApochromat objective to confirm cellular details. All labeled cells that fit the morphologic criteria described below were mapped, as were the pial surface and the corpus callosum. Adjacent Nissl-stained sections were also mapped for areal and laminar boundaries using the criteria of Vogt et al. (1987, submitted). For the display of the maps themselves, the Nissl-based laminar maps were superimposed on the adjacent immunostained maps.

For all the analyses in this study, due to the peculiar shape of the neurons being described, only sections that were cut perpendicularly to the pial surface were used. To determine this, SMI32-immunostained sections were examined, and the corresponding level was used only if apical dendrites could be followed from layer V through layer III. A neuron was considered a spindle cell on Nissl-stained material if it had an ovoid nucleus with a visible nucleolus and if it had a basal dendrite that was at least as thick as its apical dendrite. In SMI32-immunostained material, a neuron was counted as a spindle neuron if it had an immunoreactive ovoid soma and immunoreactive dendrites, again, with the basal dendrite as thick as or thicker than the proximal dendrite. For both Nissl and SMI32 analyses, neurons whose apical and basal dendrites were much thinner than the soma were excluded. The purpose of these requirements was to eliminate any neurons that could be defined as inverted pyramidal cells or large interneurons. As a result of these criteria, neurons whose somata were not labeled—a situation commonly encountered in SMI32-immunostained material—were not counted. In addition, neurons not oriented precisely parallel to the angle of cut could not be considered. Therefore, the numbers obtained are likely to be underestimates of the actual numbers. Optical density measurements were obtained on a single section to circumvent variability in staining intensity. The

first 30 pyramidal and fusiform neurons encountered in the section were sampled using a Zeiss ×63 NeoFluar objective. A box measuring 3 × 3 μm was generated, and the mean transmittance of this area was obtained. Five to eight measurements were taken of each neuron, depending on the size of the cell. Care was taken to avoid the nucleus, to sample both polar ends of the neuron, as well as to sample equally both more and less intensely immunoreactive portions of the neuron, because the neurofilament protein triplet is not homogeneously distributed throughout the soma. A mean of these observations was obtained for each neuron. In addition, a number of measurements were made of the unstained neuropil between immunoreactive neurons. A mean was obtained for these values, and all transmittance values were divided by this background mean to obtain a standardized transmittance value. Optical density was defined as the reciprocal of this transmittance value. Statistical analysis was performed using a two-tailed Student's t test.

RESULTS
Spindle cell distribution

In Nissl-stained material, spindle neurons were found almost exclusively in layer Vb among the groups of small to medium-sized pyramidal cells that characterize this layer (Fig. 1). They often occurred in clusters of two or three, separate from the pyramidal cell clusters, and were more prevalent at the edges of the gyrus. At all rostrocaudal levels examined, two-thirds of the total number of spindle cells were located in subarea 24b on the gyral surface (Table 1). Most of the remaining third was located in area 24a. Although area 24b had a relatively high overall density of spindle cells, the cortex in dimples within area 24b had a low density of spindle neurons. The predominance of area 24b was not due solely to the large size of this subarea. The density of these cells was highest in area 24b, and area 24a had the second highest density (Table 1). Furthermore, their numbers decreased steadily from anterior to posterior portions of the anterior cingulate gyrus (Fig. 2a–d). This latter gradient was confirmed in a horizontal series as well. The density of fusiform neurons was greater anteriorly than posteriorly and dropped abruptly at the transition zone between anterior and posterior cingulate cortex (Vogt's area 24'; Fig. 3). This distribution pattern was observed both in Nissl-stained sections and in those immunostained with SMI32 (Fig. 2e–h).

Spindle cell morphology

On Nissl-stained materials, spindle neurons were readily distinguishable from pyramidal neurons and exhibited a variety of morphologies (Fig. 4a–d). Some were very slender and elongate, with apical and basal dendrites nearly as thick as the soma at its widest point. Others were shorter, more stout, and usually curved. Occasionally, neurons were encountered with a bifid basal dendrite or a third major dendrite emerging from the soma. In addition, lipofuscin deposits were common and were occasionally so large that they distorted the shape of an otherwise very slender neuron. "Corkscrew" cells, as described by Von Economo (Von Economo, 1926), were not encountered in the control brains.

Immunostaining with SMI32 revealed further morphological details. In the most slender neurons, the shape of the neuron resembled that observed in Nissl-stained material
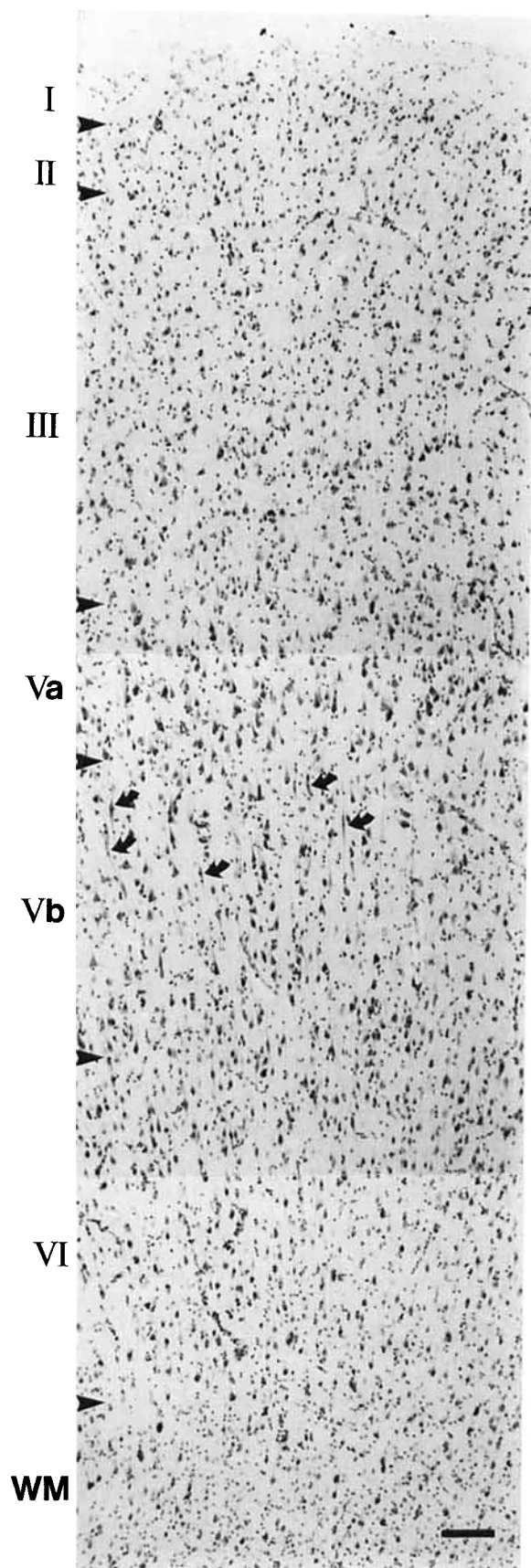
Fig. 1. Photomontage of a traverse through area 24b in the anterior cingulate cortex. Note the lack of a granular layer IV and the spindle cells (curved arrows) limited to layer Vb. Scale bar = 100 μm.

TABLE 1. Distribution and Density of Spindle Neurons in the Subareas of the Human Anterior Cingulate Cortex[1]

Area	Percentage of total number	Mean density (per mm²)
24a	13.0 ± 4.4	1.4 ± 0.7
24b	68.0 ± 1.8	19.6 ± 9.9
24c	19.0 ± 5.8	5.9 ± 3.1

[1]Proportions of the total number of spindle cells counted that were observed in each of the cingulate subareas (Percentage of total number) and the areal density of spindle cells in each subarea (Mean density) in Nissl-stained material are shown. Values represent means ± S.E.M. from four levels in the anterior cingulate cortex. Note that no attempt was made to account for tissue shrinkage, and no other correction factor was introduced.

(Fig. 4e–g). In many instances, additional, very thin dendrites were visible emerging from the soma in close proximity to the apical dendrite. In others, the basal dendrite bifurcated shortly after its origin. In some examples, the soma was not immunoreactive, but its apical and basal dendrites were immunoreactive. In others, the neurofilament protein manifested as a single, thick rod coursing through the neuron parallel to its long axis and extending outward from the soma well into the apical and basal dendrites with little loss of thickness before abruptly disappearing. A common observation was a neuron whose thick, tapered apical dendrite was intensely immunoreactive and could be followed through layer III and whose basal dendrite, equally broad and intensely immunoreactive, extended for 25–50 μm before terminating in a spray of branches in layer VI (Fig. 4e,f).

In order to determine whether there was a substantive difference in the mean intensity of SMI32 immunoreactivity in these neurons when compared with neurofilament protein-immunoreactive pyramidal neurons, optical density measurements were obtained for a sample of each population (Fig. 5). The mean relative optical density for the fusiform neurons (22% greater than background staining) was significantly higher than that for pyramidal neurons (19% greater than background staining; $P < 0.05$). This result suggests a slightly greater content of neurofilament protein in the somatic compartment of the fusiform neurons than of pyramidal neurons. It should be noted that the fusiform neurons demonstrated a somewhat greater degree of intracellular heterogeneity in neurofilament protein distribution. Spindle cells constituted from 16.4% of the total neurofilament protein-immunoreactive neuronal population of layer V anteriorly to 3.9% of the total population posteriorly. This reflects the progressive overall increase of neurofilament protein-immunoreactive neurons as one proceeds posteriorly in the cingulate gyrus as well as the progressive decrease in the number of fusiform neurons. In contrast to this steady trend, the ratio of the number of neurofilament protein-immunoreactive fusiform neurons to that of the total population of spindle cells in immediately adjacent Nissl-stained sections ranged in one serial case from 28% to 80% with no evidence of a clear anterior-to-posterior trend. Samples from other cases yielded percentages within this range. In addition, when immunostained sections counterstained with thionin were examined, the percentages obtained were also within this range, but they tended to be clustered toward the higher end of the range, probably because, in Nissl-stained material, faint or only dendritic immunoreactivity can more easily be identified as belonging to a cell, whereas, in the absence of the Nissl stain, continuity between immunoreactive profiles can be difficult to establish.

In order to confirm that spindle cells belong to the class of cortical projection neurons, the lipophilic dye DiI was placed into the white matter. These injections into the
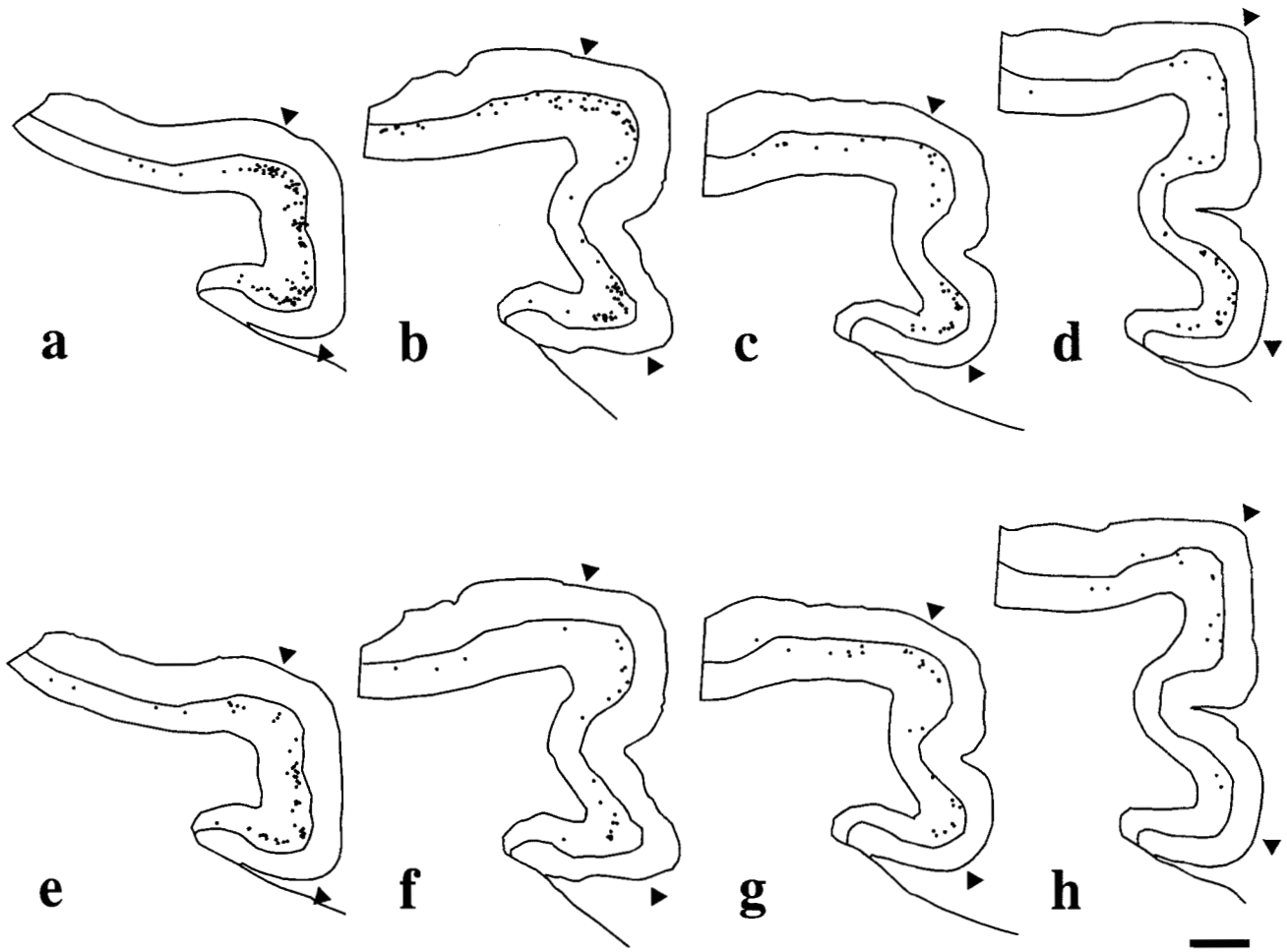
Fig. 2. Computer-generated maps showing the distribution of spindle cells in coronal sections through the anterior cingulate cortex of a representative control case. Sections are 1 cm apart. The most anterior sections are on the left. Maps showing the distribution as observed in Nissl-stained material (a–d) are of sections adjacent to those showing the locations of SMI32-immunoreactive spindle cells (e–h). Borders shown indicate the pia, the layer III/V border, and the layer VI/white matter border. Arrowheads indicate the borders between (ventrally to dorsally) areas 24a and 24b and areas 24b and 24c. Scale bar = 2 mm.

cingulum bundle labeled some fusiform neurons as well as numerous pyramidal neurons. The proximal portions of their apical and basal dendrites lacked spines, but more distal regions were spiny. Some examples are illustrated in Figure 6. Finally, immunostaining with antibodies to the calcium-binding proteins revealed that no spindle cells were immunoreactive for parvalbumin, calbindin, or calretinin.

Alzheimer's disease

Analysis of the anterior cingulate cortex from the brains of patients diagnosed with AD produced variable results. Because entire gyri were not available from these cases, serial analyses were not possible. In one of the four AD cases, there was severe, almost complete loss of spindle cells and pyramidal neurons. However, in the other three cases, there was more than a 60% loss on the basis of the Nissl stain (27.8 ± 3.3 spindle neurons per section compared with 72.5 ± 8.5 for control cases). In the first of these, from a patient suffering from severe AD associated with Balint's syndrome (reported in Hof et al., 1993a), the anterior cingulate cortex contained numerous neurofibrillary tangles in all cortical layers. In the other two cases, there was severe cell loss, particularly in layer III, although far fewer

neurofibrillary tangles were present. In all of these cases, there were morphological alterations in the spindle neurons, such as more dendritic processes per cell than found in the normal brains, and bizarrely twisted apical and basal dendrites that often assumed a corkscrew-like appearance. It should be noted that these features were also observed in the pyramidal neurons in the same area and layer. Tissue suitable for immunocytochemistry was available from one of these brains. In this brain, 70% of the spindle neurons were SMI32-immunoreactive, well within the range obtained for normal brains.

DISCUSSION

The spindle neurons appear to represent a neuronal subclass with a highly restricted distribution and considerable vulnerability in AD cases. These neurons were observed in restricted domains of the human cingulate cortex, with higher densities in the anterior area 24b and fewer in areas 24a and 24c. They were not found in the posterior cingulate cortex. A subpopulation of spindle cells was strongly immunoreactive for the neurofilament protein triplet. Although neurofilament immunostaining did not
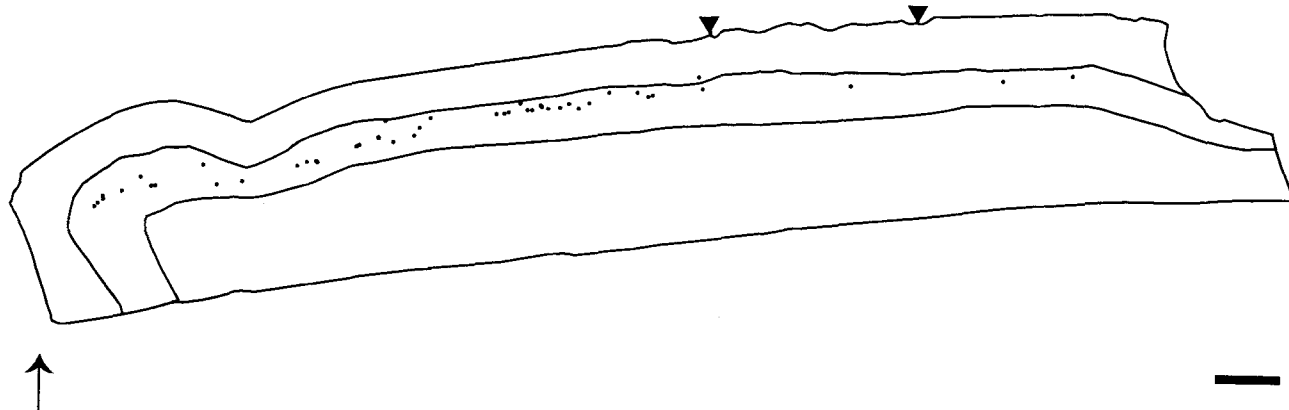
Fig. 3. Computer-generated map showing the distribution of spindle cells in a horizontal section through ventral area 24b. Arrowheads indicate the zone of transition between anterior and posterior cingulate cortex. Anterior is to the left; the medial wall of the hemisphere is up. Arrow indicates the caudal bank of a vertically oriented dimple. Scale bar = 2 mm.

label the entire neuron, it did allow certain comparisons with Cajal's Golgi preparations in the insula. Specifically, the appearance of the SMI32-immunoreactive spindle cells as elongate, tapered somata with a basal dendrite that, after emerging from the soma, gives rise to a spray of dendrites closely parallels Cajal's description and drawings (Cajal, 1899). On this basis, it may be inferred that the spindle cells in the insula and in the anterior cingulate cortex are the same cell type.

The general cell type to which the spindle cell belongs is a difficult issue. Although every attempt was made to avoid counting large interneurons as spindle cells, it is likely that some of the smaller cells counted with the Nissl stain were in fact large bitufted or even basket cells. However, given the fact that γ-aminobutyric acid-containing (GABAergic) interneurons constitute only about 20–25% of cortical neurons overall and only 15–17% in layer V (Hendry et al., 1987; Hornung and De Tribolet, 1994), and given the precautions taken in the counting process, the number of cells thus erroneously counted is likely to be very small. The fact that no layer Vb spindle neurons were found to contain any of the three calcium-binding proteins, parvalbumin, calbindin, and calretinin, suggests that these cells may be considered modified pyramidal neurons. These three calcium-binding proteins are found in separate populations of cortical GABAergic interneurons (Hendry et al., 1989; Celio, 1990; Jacobowitz and Winsky, 1991; Hof and Nimchinsky, 1992; Hof et al., 1993b), and, although it is not clear that these proteins taken together label all interneurons, the absence of immunoreactivity at the very least limits the possibility that these neurons constitute a discrete population of GABAergic cells. The probable pyramidal cell-like nature of these neurons is further evidenced by their frequent content of neurofilament protein, which is found in subpopulations of pyramidal neurons (Campbell and Morrison, 1989; Hof and Morrison, 1990; Hof et al., 1990; Campbell et al., 1991) but has not been demonstrated in local circuit neurons (Vickers and Costa, 1992). Finally, the labeling of these neurons after placement of DiI in the subcortical white matter provides the strongest evidence that at least some these neurons project into the cingulum bundle, as suggested by Cajal for the spindle cells of the insula (Cajal, 1899).

Although the above evidence suggests that the spindle neurons are pyramidal neurons, the fairly high proportion of the total spindle cell population that contains neurofilament protein sets these neurons aside from pyramidal cells as a group. Neurofilament protein is estimated to be present at immunohistochemically detectable levels in approximately 10–20% of cortical pyramidal neurons (Hof and Morrison, 1990; Hof et al., 1990), although it is present in roughly half of the layer Vb spindle neurons that may also have, on average, more neurofilament protein per cell, as indicated by their higher average optical density. This is especially intriguing in view of the degree of involvement of these cingulate neurons in the degenerative process in AD. Neurofilament protein-immunoreactive neurons have been shown to be especially vulnerable to degeneration in AD (Morrison et al., 1987; Hof and Morrison, 1990; Hof et al., 1990), and it has been postulated that there is a gradual transformation of the neurofilamentous component of the cell into neurofibrillary tangles (Vickers et al., 1992, in press). A population of pyramidal neurons with a high likelihood of neurofilament protein content would be expected to be especially vulnerable. Nevertheless, despite a loss of spindle neurons overall, there seems to be no preferential loss of the neurofilament-immunoreactive spindle cells in AD, because the percentage of spindle cells containing neurofilament protein is comparable in control brains and in AD. Therefore, at least in this particular cell population, the degenerative process appears to affect neurofilament-immunoreactive and nonneurofilament-immunoreactive neurons to the same degree.

The degree of spindle cell loss observed is higher than the 40% pyramidal cell loss reported in the prefrontal cortex in AD (Terry et al., 1981). It is, however, considerably greater than the relatively mild degree previously described for pyramidal neurons in the anterior cingulate cortex by various authors (Brun and Englund, 1981; Mountjoy et al., 1983). These results suggest that, although the anterior cingulate cortex may remain relatively unaffected in AD, at least one conspicuous cell population is affected and may represent a more sensitive indicator of the presence of a degenerative process. An alternative interpretation is that AD cases with involvement of the anterior cingulate cortex tend to belong to a subgroup of AD cases characterized by
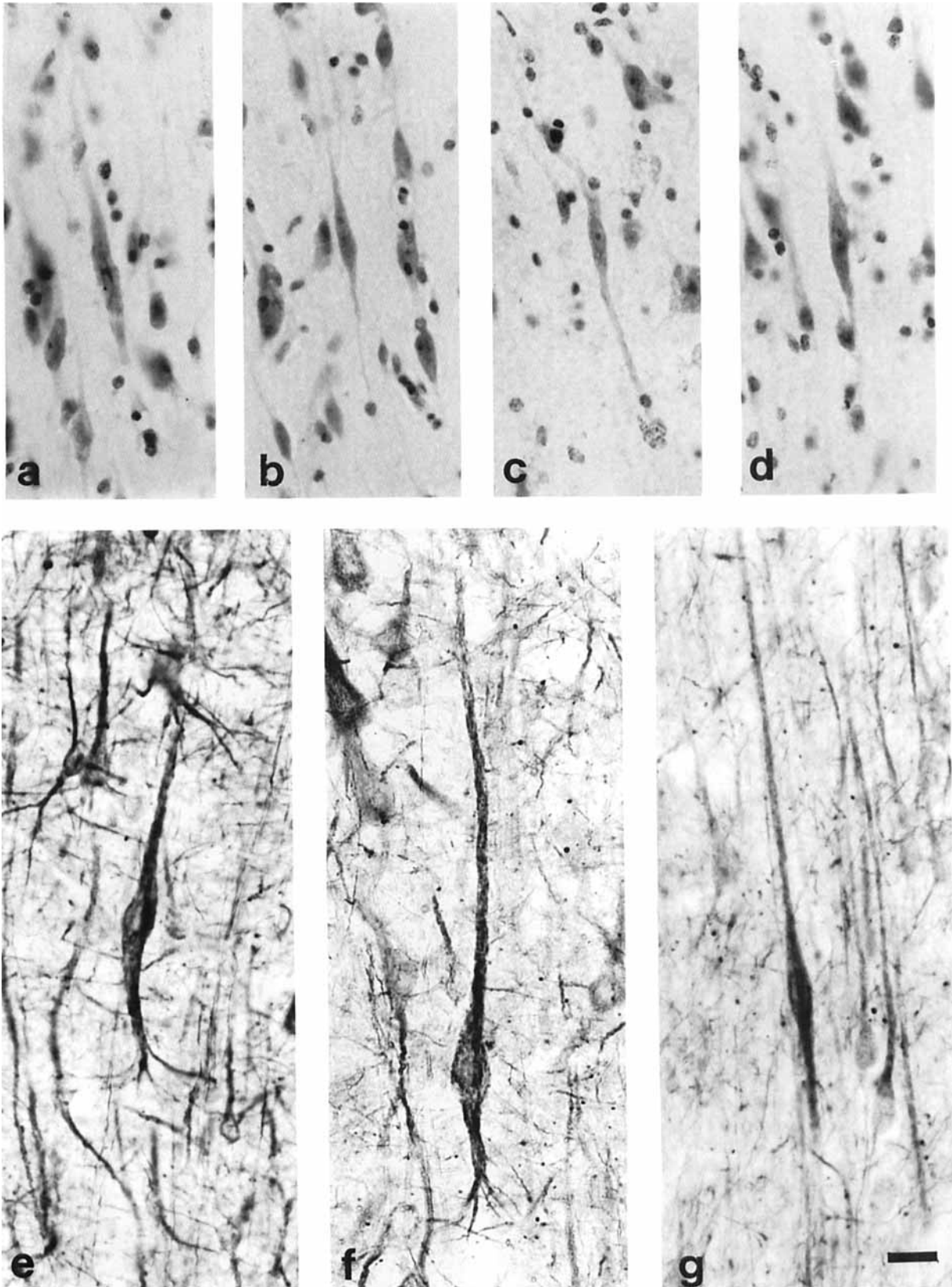
Fig. 4.   Examples of spindle cells as seen with the Nissl stain (**a–d**) and with SMI32 immunohistochemistry (**e–g**). Note variations in somal thickness and in the degree of tapering toward the apical and basal dendrites. In e and f, note the abrupt branching of the basal dendrite into numerous secondary dendrites. Scale bar = 25 μm.
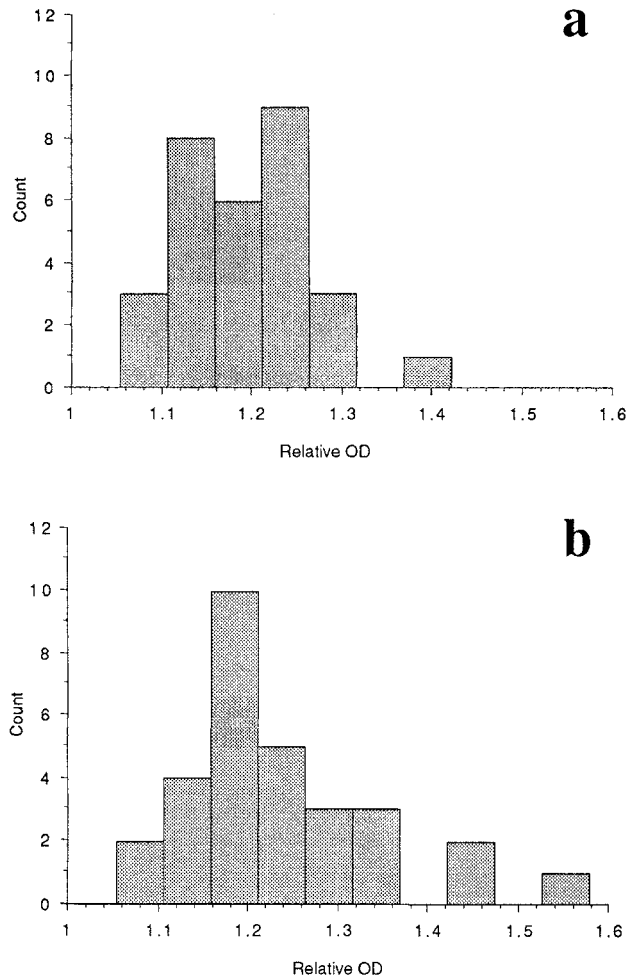
Fig. 5. Frequency distributions of the relative optical densities (OD) of samples of SMI32-immunoreactive pyramidal neurons (a) and SMI32-immunoreactive spindle cells (b). Values represent means from four levels in the anterior cingulate cortex.
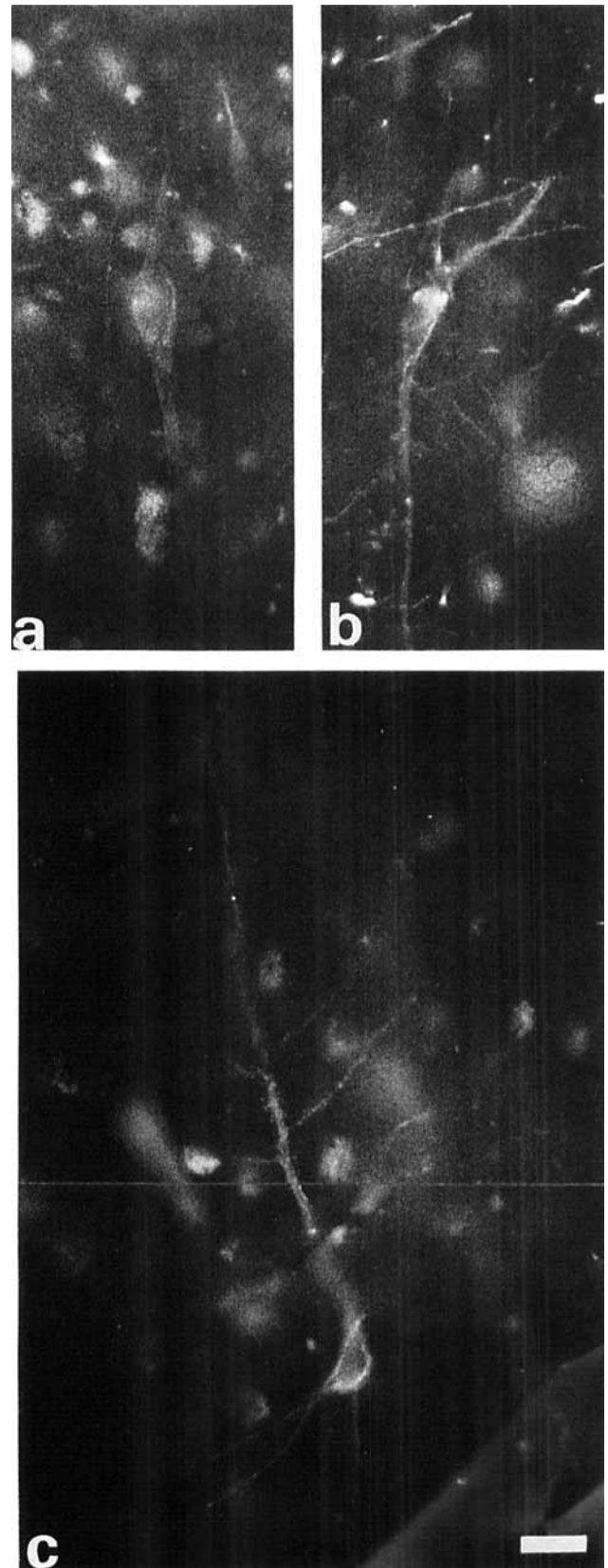

Fig. 6. Spindle cells (a,b) in area 24b stained after placement of crystals of the carbocyanine dye DiI into the cingulum bundle. Note the labeling of two distinct morphological types; one with a stout soma and apical and basal dendrites (a) and one with much narrower apical and basal dendrites (b). A classical pyramidal neuron (c) is shown for comparison. The pial surface is up. Scale bar = 50 μm.

cell loss mostly in layers V and VI (corresponding to Vogt's class 4; Vogt et al., 1990). If this is true, then such cases might not necessarily show overall pyramidal cell loss, because the degree of cell loss, although considerable for the relatively cell-sparse layer V, would be obscured by the preservation of the very high neuronal density in layers II and III.

With respect to their possible function, Braak (1980) regards spindle cells, on the basis of the laminar position and pattern of lipofuscin pigmentation, as belonging to the same class as the giant layer V cells of Betz in the primary motor cortex. This suggestion is provocative, because it allows one to begin to hypothesize a possible role for these neurons. A role in motor control would not be very surprising in view of the numerous motor functions, autonomic and somatic, that are subserved by anterior cingulate cortex (Delgado and Livingston, 1948; Pool and Ransohoff, 1949; Kaada, 1951; Pool, 1954; Showers and Crosby, 1958; Dua and MacLean, 1964; Robinson and Mishkin, 1968; Terreberry and Neafsey, 1983; Luppino et al., 1991; Shima et al., 1991; Dum and Strick, 1992, 1993; Buchanan and Powell, 1993; Grafton et al., 1993). Furthermore, both Betz cells and spindle cells contain high levels of neurofilament protein (Nimchinsky et al., 1992). However, this is true of

virtually all Betz cells but only a subpopulation of spindle cells. In addition, a subpopulation of human Betz cells has been shown to contain the calcium-binding protein calretinin (Nimchinsky et al., 1992), and, as mentioned above, no layer Vb spindle neurons were found to contain any of the three calcium-binding proteins used in the study. These facts do not rule out a motor role for these neurons but do indicate that they are a population separate from the Betz cells. Irrespective of their functions, however, it is possible that large or otherwise unusual neuronal types in layer Vb are a consistent feature of certain agranular cortical areas such as anterior cingulate, anterior insula, and primary motor cortex.

The limitation of spindle cells to the subareas of the anterior cingulate cortex is sufficiently complete as to recommend them as markers of these cortical areas (when found on the cingulate gyrus). In fact, these neurons have a regional and laminar distribution that is as restricted as that of Meynert cells in the primary visual cortex. The presence of spindle cells in all subfields of the anterior cingulate cortex and their absence from any cortical areas lying dorsally to the cingulate sulcus supports the use of this sulcus as an indicator of a cytoarchitectonic border. The marked reduction in the density of these cells from anterior to posterior cingulate cortex occurs precisely in the area of transition between anterior cingulate cortex (area 24) and posterior cingulate cortex (area 23), referred to as area 24' (Vogt, 1993). This is one of the areas on the cingulate gyrus where, in the depths of the cingulate sulcus, motor areas have been described in the macaque (Muakkassa and Strick, 1979; Hutchins et al., 1988; Luppino et al., 1991; Matelli et al., 1991; Shima et al., 1991; Dum and Strick, 1992, 1993; Morecraft and Van Hoesen, 1992). It has been suggested that the gigantopyramidal field described by Braak (1976) represents at least one or part of one of these motor areas in the human (Morecraft and Van Hoesen, 1992). Due to the decreased density of spindle cells in the dorsal- and lateralmost portions of the cingulate gyrus and to their absence in its caudal portion, they were seldom found alongside cells belonging to the gigantopyramidal field, suggesting that these cells are separate from the cingulate motor areas as well.

Although only neurons with a truly spindle-like morphology were considered for this study, it is probable that these represent one end of a spectrum ranging in morphology from the classical pyramid to the most narrow spindle cell. Many layer Vb cells were encountered that had stout basal dendrites oriented at a sharp angle to the axis of the apical dendrite (described in layer VI throughout the cortex by both De Crinis and Braak as "a pair of compass cells"; De Crinis, 1933; Braak, 1980). In addition, a large number of pyramidal neurons were observed that, although possessing the classic pyramidal somal morphology, had one vertically oriented basal dendrite that was much thicker than all the others and often as thick as the apical dendrite. This variation of the standard pyramidal morphology, in addition to numerous inverted pyramidal neurons, was especially common in the more dorsal portions of the anterior cingulate gyrus, and all these alternative shapes were rarely if ever observed in layer Vb elsewhere in the cortex. Such cells, in addition to a few fusiform neurons, were occasionally seen in layer III in the anterior cingulate cortex, especially in its more caudal portions. Thus, the anterior cingulate cortex is an area that contains many cell types with unusual morphologies. The significance of this

cellular variability is not clear, but it might be related to the cytoarchitectonic variability in this region. Far from being a homogeneous structure, the cingulate gyrus has been proposed as an area whose progressive differentiation is evident from the least differentiated anteroventral portions to the most differentiated dorsal and posterior portions (Sanides, 1962; Vogt et al., 1987; Hof and Nimchinsky, 1992). All the areas considered in the present study are agranular, lacking the internal granular layer IV, or dysgranular, possessing an incipient or poorly defined layer IV. They are described as "periallocortex" or "proisocortex" (Braak, 1980; Vogt et al., 1987), not as the homotypical isocortex found in the most caudal and dorsal portions of the gyrus and in association cortices throughout the brain. In this context, it is noteworthy that the only other cortical area known to contain large layer Vb fusiform neurons is the anterior insula (Cajal, 1899; Von Economo, 1926; Ngowyang, 1932). This area too, is proisocortical and ranges from agranular to dysgranular. Both these areas are considered "limbic" cortices because of their poorly differentiated cytoarchitecture and their strong connections with other components of the limbic system, most notably the amygdala (Mufson et al., 1981; Pandya et al., 1981; Barbas and Pandya, 1989; Van Hoesen et al., 1993). Large layer Vb spindle cells may, therefore, be characteristic of certain limbic cortical areas.

It is interesting that areas such as the anterior cingulate and agranular insular cortex, whose cytoarchitecture is regarded as relatively archetypal, are the preferential loci for a neuronal type that appears to be found only in primates. In his comprehensive survey of the anterior cingulate and retrosplenial cortices in a wide range of mammals, Rose (1927) reported the presence of layer Vb spindle neurons in small numbers in the ring-tailed lemur. They are much more abundant in the common chimpanzee, mostly in the area corresponding to 24b and, to a lesser degree, in area 24a (Rose, 1927). In *Macaca* sp., spindle neurons are occasionally encountered in anterior cingulate cortex (Nimchinsky, unpublished observations). These observations attest to the fact that the anterior cingulate cortex has continued to evolve structurally. It is likely, therefore, that this cortex has also evolved functionally, developing roles that are now emerging from functional imaging studies.

ACKNOWLEDGMENTS

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