Regional Heterogeneity in the Distribution of Somatostatin-28- and Somatostatin-28₁₋₁₂-Immunoreactive Profiles in Monkey Neocortex

Michael J. Campbell,¹ David A. Lewis,^{1,a} Robert Benoit,² and John H. Morrison¹

¹Scripps Clinic and Research Foundation, La Jolla, California, and ²Department of Medicine, McGill University, Montreal General Hospital Research Institute, Montreal H3G 1A4, Canada

The distribution of the prosomatostatin-derived peptides (PSDP), somatostatin-28 and somatostatin-28₁₋₁₂, in the cynomolgus monkey (*Macaca fascicularis*) neocortex was characterized in quantitative immunohistochemical studies of 3 visual areas (V1, primary visual cortex; V2, the adjacent visual association area; and AIT, a visual association area in anterior inferior temporal cortex), 2 auditory areas (AI, primary auditory cortex; and T1, an adjacent auditory association area) and anterior cingulate cortex (Area 24). The results of similar quantitative analyses in 3 homologous areas in rat neocortex (primary visual, primary auditory, and anterior cingulate) are also presented.

Primate cortical areas differed significantly in both density and laminar distribution of PSDP-immunoreactive profiles. Area 24, the most densely labeled area, had nearly 6 times as many PSDP-immunoreactive neurons as V1. Both auditory areas contained approximately two-thirds the number of PSDP-immunoreactive neurons found in Area 24; however, both had nearly 4 times as many immunoreactive neurons as V1. The 3 visual areas showed incremental increases in the number of PSDP-immunoreactive neurons; V2 contained nearly twice and AIT nearly 3 times the number of immunoreactive neurons present in V1. Both the supra- and infragranular layers were densely labeled in Area 24 and Area T1, however, in AI, V1, V2, and AIT the infragranular layers were relatively sparsely labeled. In contrast to the regional heterogeneity found in the primate neocortex, the distribution of immunoreactive neurons was quite uniform across the 3 rat cortical areas. The rat cortical areas contained substantially fewer immunoreactive neurons than most of the monkey cortical areas, and a majority of these immunoreactive neurons were located in the infragranular layers.

These findings suggest that the regional specialization of primate neocortex involves the selective distribution of

PSDP-immunoreactive neurons. They also suggest that chemically specified intrinsic organization of neocortex is not likely to be uniform across species or across cortical areas in the primate. The distinctive regional distribution patterns of PSDP-immunoreactive profiles appear to parallel that of the long corticocortical projections (contralateral and distant ipsilateral projections), suggesting an association between these presumed inhibitory interneurons and this important extrinsic system.

Although it is clear that different cortical areas possess distinctive sets of extrinsic connections, the degree to which intrinsic cell populations and intrinsic circuitry vary across cortical areas has not been clearly established. The ubiquity of the nonpyramidal morphologic cell classes demonstrated by Golgi studies (for review, see Fairen et al., 1984) and the columnar properties of cortex revealed in electrophysiologic studies (for review, see Mountcastle, 1979) suggested that the intrinsic organization of neocortex might possess a basic pattern that exhibits minimal regional variation. This concept has been supported by the reports of Powell and his colleagues (Rockel et al., 1980; Winfield et al., 1980; Powell, 1981) that the total number of neurons and the relative proportion of pyramidal and nonpyramidal cells within a given cortical traverse from pia to white matter are invariant across cortical regions and species, with the exception of the primate primary visual cortex. However, these data have not been fully substantiated in recent studies by Peters and colleagues (Peters et al., 1985) and Beaulieu and Colonnier (1984). Furthermore, only the relative number of the general cell classes of pyramidal versus nonpyramidal neurons has been reported; quantitative variations in the distribution of morphological subclasses have not been investigated.

The development of chemically specific anatomical methods, such as immunohistochemistry, has made it possible to indicate the neuroactive substance used by the labeled neurons. This has allowed for the definition of chemically identified subsets of intrinsic neurons. Using immunohistochemical methods, we have examined the distribution of the prosomatostatin-derived peptides (PSDP), somatostatin 28 (SS-28) and somatostatin 28₁₋₁₂ (SS-28₁₋₁₂) in the neocortex of cynomolgus monkeys (*Macaca fascicularis*). SS-28, a 28 amino acid peptide, is an N-terminal extension of the first PSDP fragment discovered, somatostatin-14 (SS-14). SS-28₁₋₁₂ is the N-terminal dodecapeptide of SS-28. Although less is known about the physiological actions of SS-28₁₋₁₂, it is clear that SS-14, SS-28, and SS-28₁₋₁₂ are neuroactive. SS-14 and SS-28 are released from nervous tissue *in vitro* (Iversen et al., 1978; Kewley et al., 1981; Peterfreund and Vale, 1982)

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Correspondence should be addressed to John H. Morrison, Ph.D. (BCR-1), Scripps Clinic and Research Foundation, 10666 North Torrey Pines Road, La Jolla, CA 92037.

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Figure 1. Bright-field photomicrograph of SS-28₁₋₁₂-immunoreactive fibers in Area V1 (primary visual cortex) of the monkey. The cortical layers in Figures 1–6 were determined from adjacent Nissl-stained sections. Calibration bar, 200 μ m.

and bind to high-affinity binding sites in rat brain membranes (Reubi et al., 1981; Srikant and Patel, 1981; Tapia-Arancibia et al., 1981). Both SS-14 and SS-28 influence the release (Tanaka and Tsujimoto, 1981; Chesselet and Reisine, 1983) and the synaptic effects (Delfs and Dichter, 1983) of other neurotransmitters, as well as spontaneous neuronal activity in the neocortex and in the hippocampal formation (Dodd and Kelly, 1978; Olpe et al., 1980; Phillis and Kirkpatrick, 1980; Pittman and Siggins, 1981). In preliminary studies, SS-28₁₋₁₂ depressed spontaneous activity of rat cortical and hippocampal neurons *in vivo* (Siggins and French, 1984).

The extensive expansion and differentiation of the primate neocortex reflect the development of a greater number of specialized cortical areas than are present in the rodent brain. Immunohistochemical and histochemical studies have revealed that extrathalamic monoaminergic and cholinergic afferents exhibit substantial regional heterogeneity in the monkey neocor-

tex, findings that could not have been anticipated from rodent studies (Morrison et al., 1982, 1986; Takeuchi and Sano, 1983; Levitt et al., 1984; Mesulam et al., 1984; Lewis et al., 1987). In a previous study of presumably intrinsic PSDP-immunoreactive neurons in monkey prefrontal cortex, we observed that the laminar distribution of immunoreactive neurons and fibers was consistent across prefrontal regions. However, there were substantial variations in density (Lewis et al., 1986). Our studies of prefrontal cortex (Lewis et al., 1986), as well as preliminary studies of other cortical regions (Campbell et al., 1985) suggested that the differentiation of primate neocortex involves the variation of this chemically identified intrinsic neuronal population across cortical areas. In order to pursue this issue further, we made qualitative and quantitative comparisons of the distribution of PSDP-labeled profiles in 3 visual areas, 2 auditory areas, and the anterior cingulate area in monkey neocortex. Quantitative studies were also done in 3 homologous areas in the rat neocortex: primary visual, primary auditory, and anterior cingulate cortex. These analyses revealed a dense, morphologically diverse system of PSDP-immunoreactive neurons with a high degree of regional heterogeneity in its distribution across the monkey neocortex. In contrast, the distribution of PSDP-immunoreactive neurons was quite uniform across rat cortical areas.

Materials and Methods

Seven Old World cynomolgus monkeys (*Macaca fascicularis*) and 3 albino rats were used in these studies. Rats were deeply anesthetized with chloral hydrate [350 mg/kg, intraperitoneally (i.p.)] and monkeys with ketamine hydrochloride (25 mg/kg, i.m.) and pentobarbital sodium (10 mg/kg, i.p.). Each monkey was intubated with a 3.0 or 3.5 pediatric size endotracheal tube and mechanically ventilated with 100% oxygen. After the chest was opened and the heart exposed, 1.0–2.0 ml of 1% aqueous sodium nitrite was injected into the left ventricle. In both rats and monkeys, the descending aorta was clamped and the animals perfused transcardially with cold 1% paraformaldehyde in PBS for 30–60 sec followed by perfusion with cold 4% paraformaldehyde in PBS for 8–10 min. The brains were removed, cut into 3- to 5-mm-thick blocks, and placed in cold 4% paraformaldehyde in PBS for 6–8 hr. These blocks were washed in a series of cold, graded sucrose solutions, then frozen and coronally sectioned in a cryostat. Adjacent 40 μ m sections were collected for immunohistochemical studies and Nissl staining.

For immunohistochemical studies, sections were incubated overnight at 4°C with antisera S320 or S309 diluted 1:4000 and 1:3000, respectively, in PBS containing 0.3% Triton X-100 and 0.5 mg/ml of BSA. The sections were then processed by the avidin–biotin method (Hsu et al., 1981) using Vectastain ABC kits (Vector Laboratories, Burlingame, CA) and diaminobenzidine (DAB). They were then mounted on gelatin-coated slides and air-dried. In some sections, the DAB reaction product was intensified by serial immersions in 0.005% osmium tetroxide (6–7 min), 0.5% thiocarbonylhydrazide (10 min), and 0.005% osmium tetroxide (2 min). Other sections were counterstained with either cresyl violet or with a modification of the Giemsa method (Iniguez et al., 1985).

Antiserum S320 is directed against the C-terminus of SS-28₁₋₁₂ and does not recognize SS-28, whereas S309 is directed against the N-terminal 11 amino acids of SS-28 and recognizes primarily SS-28 with 10% cross-reactivity with SS-28₁₋₁₂ (Benoit et al., 1982, 1985). Using these antisera, we previously demonstrated that SS-28 is located primarily in the perinuclear region of the neuronal soma and SS-28₁₋₁₂ primarily in distal processes (Morrison et al., 1983; Lewis et al., 1986). Thus, sections reacted with S320 reveal a much greater density of fibers than those reacted with S309. In contrast, S309 labels a substantially larger number of neuronal cell bodies than S320. For this report, the distribution of SS-28₁₋₁₂-immunoreactive fibers was studied in the monkey primary auditory cortex (Area AI), adjacent auditory association cortex (Area T1), primary visual cortex (Area V1), adjacent visual association cortex (Area V2), visual association cortex in the anterior inferior temporal lobe (Area AIT), and anterior cingulate cortex (Area 24). Cytoarchitectonic areas were identified according to published criteria (Walker, 1940; Lund, 1973; Jones and Burton, 1976; Desimone

Figure 2. Bright-field photomicrograph of SS-28₁₋₁₂-immunoreactive fibers in Area V2 (visual association cortex adjacent to primary visual cortex) of the monkey. Note the increased density in the supragranular layers (particularly the increased number of radially oriented fibers) compared to V1 (Fig. 1). Calibration bar, 200 μ m.

and Gross, 1979). Regional and laminar distribution patterns of PSDP immunoreactivity were determined by comparing immunohistochemical sections with adjacent Nissl-stained sections and by the examination of counterstained sections.

Sections reacted with S309 and Giesma counterstained were used for the quantitative studies. The sections were examined at a magnification of $250\times$ with a Zeiss inverted microscope. This microscope was equipped with Burleigh optical position sensors to detect stage position. The microscopist made the determination whether a neuron was immunoreactive, centered the ocular reticle on the neuron, and the x - y position of the cell was entered into a Digital Equipment Corp. LSI 11/73 microcomputer via the sensors. Laminar boundaries were entered in a similar fashion at a magnification of $63\times$. Two-dimensional maps were constructed and the number of immunoreactive neurons per cortical lamina were tabulated by the computer system (Young et al., 1985). A majority of the soma of S309-immunoreactive neurons were incompletely stained. In these cases, immunoreactivity had to be contained within a Giemsa counterstained soma to be considered a PSDP-labeled neuron (Fig. 9). In monkey cortical areas, labeled cells in a 500- μ m-wide cortical traverse from the pia to a depth of 400 μ m into the white matter were counted from 5 sections for each cortical area. The results were expressed as the number of labeled cells per lamina per 1.0 mm cortical traverse. Cells were counted in monkey cortical Areas AI, T1, V1, V2, and AIT. Cell counts for anterior cingulate (Area 24) were adapted from our study of the prefrontal cortex (Lewis et al., 1986). In rat cortex, quantitative analyses were performed in a similar fashion on 7 sections for each cortical area. However, labeled cells in the white matter were not counted, and the results were expressed as cells in layers II–IV and layers V–VI per 1.0 mm cortical traverse. Rat cortical areas were identified according to published criteria (Zilles, 1985). Differences

Figure 3. Bright-field photomicrograph of SS-28₁₋₁₂-immunoreactive fibers in Area AIT (visual association cortex in anterior inferior temporal lobe) of the monkey. Compared to V2 (Fig. 2), there are increases in the number of radial fibers and in the density of the fiber plexus in layers II and superficial layer III. As in both V1 (Fig. 1) and V2 (Fig. 2), there is a relative paucity of immunoreactive fibers in the infragranular layers. Calibration bar, 200 μ m.

in the number of S309-labeled cell bodies among cortical areas were compared with a one-way analysis of variance. Duncan's method (α level = 0.05) was used for between-group comparisons (Kleinbaum and Kupper, 1978).

Results

Distribution of SS-28₁₋₁₂-immunoreactive fibers in monkey neocortex

There were striking regional differences in the distribution of SS-28₁₋₁₂-immunoreactive fibers in monkey cortex. However,

Figure 4. Bright-field photomicrograph of SS-28₁₋₁₂-immunoreactive fibers in area AI (primary auditory cortex) in the monkey. The layer III–IV border is not particularly distinct in the primary auditory cortex, so it was not demarcated. Note the high density of labeled fibers as compared to V1 (Fig. 1). However, both primary visual and primary auditory cortices have a sparse infragranular innervation. Calibration bar, 200 μ m.

all regions shared the following general characteristics. The superficial half of layer I invariably contained a very dense, highly arborized terminal plexus of immunoreactive fibers. In general, there was a sharp decrease in the density of this terminal plexus in the deep half of layer I. The density increased slightly in layer II, and even more in superficial layer III, although in both layers the density of immunoreactive fibers was substantially less than that of the superficial portion of layer I. The majority of immunoreactive fibers in layers I through superficial III were highly branched and varicose. Deep layer III and superficial layer IV

were characterized by largely unbranched, thick, radially oriented fibers. Far fewer small varicose fibers were present here compared to the more superficial layers. In some cortical areas the density remained low throughout layers IV–VI, whereas in others the density increased in V and VI relative to IV. In these cortical areas, layers V and VI contained both radial fibers and a plexus of small oblique and horizontal fibers.

Area VI (Fig. 1) was the least densely labeled cortical area. The low density in V1 was most apparent in the paucity of radially oriented fibers in deep III and superficial IV compared to other areas. Although area V1 was the least densely labeled cortical area, the distribution of immunoreactive fibers was more heterogeneous than other cortical areas. For example, layer III contained patches of immunoreactive radial fibers, and in the most rostral areas of V1, the V1–V2 border region contained a moderately dense terminal plexus at the IV C β –VA border. The V1–V2 border was sharply defined due to the increased density of immunoreactive fibers in V2. Although V2 (Fig. 2) had a substantially higher density of immunoreactive fibers than V1, it was not as densely labeled as AIT (Fig. 3). These increases in V2 and AIT occurred predominantly in the supragranular layers; the infragranular layers were very sparsely labeled in all 3 of these areas. AI (Fig. 4) was characterized by a dense system of fibers in layers I–IV; however, like the visual areas, the infragranular layers contained few immunoreactive fibers. T1 (Fig. 5), in contrast to AI, possessed a high density of immunoreactive fibers throughout all layers. The A1–T1 border, like the V1–V2 border, was sharply defined. Area 24 (Fig. 6) was densely labeled in both supra- and infragranular layers. Layer I in Area 24 contained a dense terminal plexus throughout its entire extent, in contrast to the other cortical areas, where this dense terminal plexus was attenuated in the deep half of layer I.

Distribution of PSDP-immunoreactive neurons in monkey neocortex

PSDP-immunoreactive neurons were also distributed heterogeneously across cortical areas in the monkey neocortex. Figure 7 shows the mean ($\pm$ SD) of S309-labeled neurons in the 6 cortical layers and the subcortical white matter in five 1.0 mm cortical traverses in each of 6 areas of monkey cortex. Statistical analyses of these data and the percentage of total labeled cells found in each layer are presented in Table 1 (layers II–VI only; i.e., labeled neurons in the white matter were not included in the totals). Area V1 contained the fewest number of immunoreactive neurons (58 ± 16). The majority of these, 54.9%, occupied layers II and III. Layers IV (6.1%) and V (5.2%) contained few cells compared to layer VI (33.8%). Area V2 (115 ± 15) showed a significant increase in number of immunoreactive neurons compared to Area V1. In Area V2, 76.8% of the immunoreactive neurons were in layers II and III, whereas layers V and VI contained only 22%. AIT (161 ± 5) contained significantly more immunoreactive neurons than V2, although the laminar distribution was similar in both areas. In AIT, 73.8% of the immunoreactive neurons were distributed in layers II and III and 25.7% in layers V and VI. Auditory areas, AI (215 ± 11) and T1 (222 ± 17), had significantly fewer immunoreactive neurons than the anterior cingulate cortex (322 ± 68) but substantially more than the 3 visual areas. Although similar in total number, the 2 auditory areas differed significantly from each other in the laminar distribution of immunoreactive neurons. Layers II–III contained 80.9 and 56.2% and layers V–VI 18.4 and 42.9% in AI and T1, respectively. Area 24, which contained the highest

Figure 5. Bright-field photomicrograph of SS-28₁₋₁₂-immunoreactive fibers in area TI (auditory association cortex adjacent laterally to primary auditory cortex) of the monkey. Note that both supra- and infragranular layers have a dense system of labeled fibers. Calibration bar, 200 μ m.

density of PSDP-immunoreactive neurons of any cortical area examined, had 54.9% of labeled neurons in layers II-III and 45.1% in layers V-VI.

Distribution of PSDP-immunoreactive neurons in rat neocortex

In a previous study we reported the distribution of SS-28- and SS-28₁₋₁₂-immunoreactive neuronal profiles in the rat primary somatosensory cortex (Morrison et al., 1983). Here, we add to that report with cell counts (Fig. 8) of PSDP-immunoreactive

Figure 6. Bright-field photomicrograph of SS-28₁₋₁₂-immunoreactive fibers in area 24 (anterior cingulate cortex) of the monkey. Labeled fibers are very dense in both the supra- and infragranular layers. Note the dense terminal plexus that extends through most of layer I in this cortical area compared to the other cortical areas (Figs. 1-5). Calibration bar, 200 μ m.

Figure 7. Laminar distribution of PSDP-immunoreactive neurons in monkey neocortex: visual areas V1, V2, and AIT; auditory areas AI and T1 and anterior cingulate, area 24. Each bar represents the mean number of S309-labeled neurons from 5 coronal sections, counted in a 500- μ m-wide traverse but expressed as the number of labeled neurons per layer per 1.0 mm cortical traverse. For the statistical analysis of these data see Table 1. Roman numerals indicate the cortical layers and WM indicates the white matter. Labeled neurons were counted to a depth of 400 μ m into the white matter.

neurons in primary visual cortex (129 ± 19), primary auditory cortex (109 ± 18), and anterior cingulate cortex (124 ± 18). There were no statistical differences among these 3 cortical areas in the total cell counts. In addition, the percentage of labeled cells in layers II–IV (32%) and layers V–VI (68%) was the same for all 3 areas.

Morphology of PSDP-immunoreactive neurons in the monkey neocortex

PSDP-immunoreactive neurons were morphologically diverse. Although the entire neuron was not visualized by either S309 or S320 (particularly in the case of S309, which in a majority

Table 1. Relative¹ laminar distribution of S309-immunoreactive neurons in 6 cortical areas of the monkey

Layer	Cortical areas						Difference ³
	V1	V2	AIT	AI	T1	24 ²	
II	17.2 ^a (3.7)	19.6 ^a (3.5)	13.5 ^b (0.8)	19.2 ^a (2.5)	17.0 ^a (1.7)	2.3 ^c (2.4)	$F_{3,24} = 30.5, p < 0.001$
III	37.7 ^a (11.3)	57.2 ^{b,c} (5.8)	60.3 ^{b,c} (1.8)	62.7 ^{b,c} (3.8)	39.2 ^a (2.6)	52.6 ^b (7.6)	$F_{3,24} = 13.6, p < 0.001$
IV	6.1 ^a (3.6)	1.2 ^b (1.8)	0.5 ^b (1.2)	0.7 ^b (1.2)	0.9 ^b (0.6)	—	$F_{4,20} = 7.3, p < 0.001$
V	5.2 ^a (4.5)	10.6 ^b (4.7)	12.2 ^b (0.8)	9.1 ^{a,b} (1.7)	23.3 ^c (3.6)	27.5 ^d	$F_{3,24} = 37.6, p < 0.001$
VI	33.8 ^a (4.9)	11.4 ^{b,c} (2.6)	13.5 ^{b,c} (0.9)	9.3 ^b (2.1)	19.6 ^d (1.1)	17.6 ^d (3.2)	$F_{3,24} = 48.3, p < 0.001$
Total neurons ⁴	55 ^a (16)	112 ^b (13)	151 ^c (5)	208 ^d (9)	212 ^d (15)	303 ^c (64)	$F_{3,24} = 46.6, p < 0.001$

¹ The value for each layer is the mean ($\pm$ SD) percentage of the total number of S309-immunoreactive neurons ($n = 5$).

² Area 24 (anterior cingulate) is agranular.

³ Within each row, values not sharing the same superscript are significantly different ($p < 0.05$).

⁴ The mean number of S309-immunoreactive neurons in layers II–VI in a 1.0 mm cortical traverse ($n = 5$ for each cortical area).

of cases revealed a perinuclear staining pattern; Fig. 9), several distinct morphological classes could be distinguished based on the size and shape of the soma, and the orientation of the proximal processes (Fig. 10). Both large and small multipolar cells were labeled. The small multipolar cells had soma ranging from 10 to 12 μ m in diameter, whereas the large multipolar cells had somal diameters that were more than 16 μ m. Both vertically oriented and horizontally oriented bitufted neurons also were labeled. These bitufted neurons had a long axis that varied in length from 18 μ m to over 30 μ m. The short axis length was in the 8 to 12 μ m range. The horizontally oriented bitufted neurons were most often located in deep layer VI, whereas the vertically oriented bitufted and multipolar cells were more widely distributed.

Discussion

There were significant differences in both density and laminar distribution of SS-28₁₋₁₂-immunoreactive fibers and PSDP-immunoreactive neurons across monkey cortical areas. Area 24 (anterior cingulate) was the most densely labeled area. Although the laminar distribution in area 24, with 55% of immunoreactive neurons in the supragranular layers and 45% in the infragranular layers, was characteristic of areas in the prefrontal cortex (Lewis et al., 1986), T1 (an auditory association area) was the only other area to share this feature in the present study. SS-28₁₋₁₂-immunoreactive processes showed a similar laminar distribution in Areas 24 and T1. In addition, in Area 24, there was a dense terminal plexus that extended through the full thickness of layer I. This may be a feature of cortical areas that receive polymodal sensory input, and it contrasts with the modality-specific areas in this study, in which the dense terminal plexus was attenuated in the deep half of layer I.

Comparison of the data from the 2 auditory areas (AI, primary auditory cortex; and T1, an adjacent auditory association area) with the 3 visual areas (V1, primary visual cortex; V2, the adjacent visual association area; and AIT, a visual association

area in anterior inferior temporal cortex) reveals some remarkable differences between these 2 sensory systems in the monkey. In general, auditory areas had greater density of PSDP-immunoreactive neurons than the visual areas. AI had more than 3.5 times as many immunoreactive neurons in the same volume of cortex as V1. This difference assumes additional significance in view of the reports (Rockel et al., 1980) that V1 has more than 2.5 times the number of total neurons as other cortical areas. Although not as dramatic, T1 had 104% more immunoreactive neurons than V2 and 38% more than AIT. Another important difference in the 2 systems was apparent in the contrasting laminar profiles. For example, in T-1, layers V and VI contained 44% of the immunoreactive neurons as compared with 25% in

Figure 8. Laminar distribution of PSDP-immunoreactive neurons in rat neocortex. Each bar represents the mean number of S309-labeled neurons from 7 coronal sections counted in a 500- μ m-wide traverse but expressed as the number of labeled neurons per layer II–IV or V–VI per 1.0 mm cortical traverse (anterior cingulate cortex is agranular). There were no statistically significant differences between the total number of labeled neurons in these 3 areas. The percentage of labeled cells in layers II–IV (32%) and layers V–VI (68%) was the same for all 3 areas.

Figure 9. PSDP-immunoreactive neurons in Giemsa-counterstained sections. In the microscope, immunoreactivity in Giemsa counterstained sections is black and Nissl substance is blue. *A*, Bright-field photomicrograph of $250\times$ field in layer VI of area T1 in the monkey. *B*, Same field as *A*; however, the photomicrograph was taken with a Kodak No. 47 color filter. This filters out the blue Nissl staining, leaving the black immunoreactive product unfiltered. Labeled cells that are indicated by arrows suggest the range of the perinuclear labeling seen in sections reacted with S309. Only immunoreactivity that was clearly contained within a counterstained soma was considered to be a PSDP-immunoreactive neuron in quantitative determinations. Calibration bar, $50\ \mu\text{m}$.

the same cortical layers in V2 and AIT. In the visual areas there were increases in the number of immunoreactive profiles from V1 to V2 and V2 to AIT that paralleled their position in the proposed hierarchical organization of the visual system (Van Essen, 1985). In contrast, there was no significant difference in the total number of immunoreactive cells between the 2 auditory areas.

The notable differences in the density and laminar distribution of PSDP-immunoreactive neurons across cytoarchitectonic areas in the monkey contrasted sharply with their homogenous distribution in the rat neocortex. Both total number and relative laminar distribution of PSDP-immunoreactive neurons were essentially the same in all 3 of these functionally distinct cortical areas in the rat. Anterior cingulate cortex and primary auditory cortex in the rat contained substantially fewer numbers of immunoreactive cells than their homologous areas in the monkey, whereas the primary visual cortex contained more. These 3 areas in the rat also revealed a substantially different laminar distribution than their counterpart in the monkey. All 3 areas in the rat had a majority (68%) of the labeled neurons in the infragranular layers, whereas in the homologous areas of monkey cortex, the majority of labeled cells were in the supragranular layers.

These data differ in some respects from other immunohistochemical studies of PSDP fragments. In an examination of rat and monkey cortices with antisera directed against SS-14, Hendry et al. (1984a) reported that the rat neocortex had more

PSDP-immunoreactive neurons than that of the monkey. However, they described only areas in the pre- and postcentral gyrus and the primary visual cortex in the monkey, so regional variability in the monkey neocortex may account for some of the differences. Furthermore, they may have visualized fewer cells than we did in our analysis. For example, they reported an average immunoreactive cell density of $3.2/\text{mm}^2$ ($40\ \mu\text{m}$ section) in layers II and III of Brodmann area 4 in the monkey in contrast to the $57/\text{mm}^2$ ($40\ \mu\text{m}$ section) reported in our previous study (Lewis et al., 1986). In addition they counted $28.7\ \text{cells}/\text{mm}^2$ ($40\ \mu\text{m}$ section) in layers II and III in sensory motor regions in the rat cortex compared to the $90\ \text{cells}/\text{mm}^2$ ($40\ \mu\text{m}$ section) found in the 3 areas counted in the present study. Although the laminar distribution of labeled neurons in the rat cortex found in this study is similar to that of the somatosensory cortex in our previous report (Morrison et al., 1983), it differs substantially from other reports that did not present quantitative data (Bennett-Clarke et al., 1980; Takatsuki et al., 1981; McDonald et al., 1982).

PSDP-immunoreactive neurons constitute a morphologically diverse cell class. This diversity was revealed by differences in somal size and shape, as well as differences in the number and orientation of proximal processes. The degree to which this morphologic heterogeneity represents the involvement of multiple classical cell types as described in Golgi studies is not clear. In fact, Jones and Hendry (1986) have suggested that all peptide-containing neurons may fall into a single broad class of cortical

Figure 10. Morphological diversity of PSDP-labeled neurons in monkey cortex. *A*, Vertically oriented bitufted neuron with a long, descending fine process (arrows) located in layer III of AIT. *B*, Small multipolar neuron in layer III of area V1. *C*, Large multipolar neuron with a descending fine process (arrow) located over a blood vessel in layer III of area T1. *D*, Vertically oriented bitufted neuron with a long, horizontally oriented fine process with a vertically oriented branch (arrows) located in layer III of area V1. *E*, Horizontally oriented bitufted neuron in layer VI of area T1. The fine caliber of the processes seen in *A*, *C*, and *D* is suggestive of axons. *A*, *C*, and *E* from sections reacted with S309; *B* and *D*, sections reacted with S320. Calibration bar, 50 μ m.

neurons. Further ultrastructural and light-microscopic analyses will be necessary to determine the degree to which PSDP-containing neurons can be subdivided and assigned to previously defined cell classes.

In general, PSDP-immunoreactive neurons are assumed to be intrinsic interneurons. This assumption is largely based on their nonpyramidal morphology but is further supported by the co-localization of PSDP fragments with GABA (Hendry et al., 1984b; Schmechel et al., 1984; Somogyi et al., 1984). Recent reports have provided evidence that some nonpyramidal neurons have long corticocortical projections (Jacobson and Trojanowski, 1974; Hedreen and Yin, 1981; Schwartz and Goldman-Rakic, 1984; Caminiti et al., 1985; Code and Winer, 1985). These cells have a morphology and laminar location that is similar to some of the PSDP-immunoreactive neurons we observed. Since antiserum to SS-28₁₋₁₂ preferentially labels fibers, it is difficult to determine the cell bodies of origin of most of these fibers. Thus, it is possible that some SS-28₁₋₁₂-immunoreactive fibers represent corticocortical afferents. Undercut studies of layer VI in the rat cortex (Morrison et al., 1985b) resulted in no discernible decrease in SS-28₁₋₁₂-immunoreactive fibers superficial to the undercut, suggesting that the major source of the SS-28₁₋₁₂-immunoreactive fibers was intrinsic. However, be-

cause the monkey has a much higher density of SS-28₁₋₁₂-immunoreactive fibers than the rat, the undercut results in the rat may not be applicable to the monkey. Thus, it is possible that some of the nonpyramidal neurons furnishing corticocortical projections may contain PSDP.

Implications for cortical organization

Anatomical (Lorente de No, 1938) and physiological studies (Mountcastle, 1957; Hubel and Wiesel, 1963) of neocortex have fostered the notion that the basic unit of information processing in the neocortex is a vertically arranged set of cells that are heavily interconnected in the vertical axis with minimal horizontal connections (Mountcastle, 1979). This columnar organization has been demonstrated in several cortical regions and is thought to exist throughout the cortex. Even though elaborate models of the cellular constituents of the column have been developed (Szentagothai, 1979), the definitive anatomic substrate of functional columns remains elusive. However, the assumption that columnar organization is a general feature of neocortex has led to the suggestion that there is a high degree of regional uniformity in the complement of interneurons constituting the column (Mountcastle, 1979; Szentagothai, 1979). As noted earlier, this concept of regional uniformity of intrinsic

organization was strengthened by the quantitative cell distribution studies of Powell and his colleagues (Rockel et al., 1980; Winfield et al., 1980; Powell, 1981).

The data presented in this report clearly demonstrate that the quantitative representation of a chemically identified group of intrinsic neurons may exhibit a high degree of regional variation in primate cortex, and these regional differences are not apparent in rodent cortex. In addition, the regional variation in the distribution of SS-28₁₋₁₂-immunoreactive fibers suggests significant differences in intrinsic circuitry across the monkey cortex. Consequently, if there is a fundamental cortical unit that is represented in all cortical regions, its chemically specific intrinsic organization is not likely to be uniform across species or across cortical areas in the primate. An important remaining question, however, is whether this variation in PSDP-immunoreactivity across primate cortical areas actually represents differences in the structural or morphological intrinsic organization or only a biochemical shift in neurotransmitter representation.

A number of salient features that characterize the distribution of PSDP-immunoreactivity across the primate neocortex suggests a possible organizing principle for this dense system of chemically identified neurons. These features include: the increases in density of immunoreactive structures from V1 to V2 and V2 to AIT, and the limited infragranular representation in these 3 visual areas; the higher density of immunoreactive neuronal profiles in the primary auditory cortex compared to the primary visual cortex; the dense infragranular labeling in T1 compared to V2 and AIT; and the high density of immunoreactivity in both the supra- and infragranular layers of the anterior cingulate cortex. The available literature suggests that the distribution of the long corticocortical projections (i.e., contralateral and distant ipsilateral projections) parallels these differences in density and laminar distribution. V1 has a very limited distant ipsilateral projection and even more restricted contralateral projection (Tigges et al., 1981; and for review, see Swadlow, 1983). Both types of projections increase significantly in V2 and still more in AIT (Cragg, 1969; Zeki, 1969; Jones and Powell, 1970). The vast majority of the projections from occipital cortex to the temporal lobe originate in layer III (Rockland and Pandya, 1979; Desimone et al., 1980). Projections to the PFC from both occipital and inferior temporal cortex have been shown to originate primarily in layer III with less than 10% in V2 and less than 20% in the inferior temporal areas originating in the infragranular layers (Barbas and Mesulam, 1981, 1985). The contralateral projections have a similar layer III distribution (Van Essen et al., 1982). A major difference between primary auditory cortex and primary visual cortex is that primary auditory cortex has a much more substantial and more widely distributed commissural projection (Pandya et al., 1969; Karol and Pandya, 1971; Fitzpatrick and Imig, 1980; Cipolloni and Pandya, 1985). In auditory association cortex, more than 40% of the neurons projecting to areas in the PFC are located in the infragranular layers (Barbas and Mesulam, 1981, 1985). The anterior cingulate and areas in PFC have numerous long corticocortical projections. The anterior cingulate has an even higher percentage of its projections to PFC originating in the infragranular layers than that seen in the auditory association cortex (Barbas and Mesulam, 1981, 1985).

This parallel distribution suggests that shifts in the density and laminar origin of the long corticocortical projections may be accompanied by a corresponding shift in the population of

the PSDP-immunoreactive intrinsic neuronal class. Since the vast majority of PSDP-immunoreactive neurons have also been shown to be immunoreactive for the GABA or GAD (the synthetic enzyme for GABA) (Hendry et al., 1984b; Schmechel et al., 1984; Somogyi et al., 1984), this parallel distribution suggests a relationship between the density and laminar origin of long corticocortical projections and a subset of inhibitory interneurons. Thus, PSDP-containing neurons may well have an important role in the modulation of the long corticocortical systems.

Conclusions

The expansion and differentiation of the primate neocortex reflect the development of a far greater number of specialized cortical areas than are present in the rodent. This specialization appears to have been accomplished, in part, by selective extrinsic circuitry. The data presented here suggest that this specialization also involves the selective distribution of PSDP-immunoreactive neurons. PSDP are unlikely to be an exception and other neuroactive substances associated with intrinsic neurons may also show this regional heterogeneity in the primate neocortex. In addition, these immunohistochemical analyses suggest that the chemically specific intrinsic organization of cortex is not uniform in the primate and it is not uniform across species. The parallel in the distribution of this dense system of PSDP neurons and the long corticocortical systems suggests an association between these presumed inhibitory interneurons and an important set of extrinsic projections.

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