Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 6526–6530, October 1984
Neurobiology

Neuropeptide-containing neurons of the cerebral cortex are also GABAergic

(immunocytochemistry/somatostatin/cholecystokinin/nonpyramidal cell)

S. H. C. HENDRY*†, E. G. JONES*†, J. DEFELIPE*†, D. SCHMECHEL‡, C. BRANDON§, AND P. C. EMSON¶

*James L. O'Leary Division of Experimental Neurology and McDonnell Center for Study of Higher Brain Function, Washington University Medical School, 660 South Euclid Avenue, Saint Louis, MO 63110; ‡Division of Neurology, Duke University Medical School, Durham, NC 27710; §Department of Biological Chemistry and Structure, Chicago Medical School, 3333 Green Bay Road, North Chicago, IL 60064; and ¶Neurochemical Pharmacology Unit, Medical Research Council Centre, Hills Road, Cambridge, England

Communicated by W. Maxwell Cowan, June 25, 1984

ABSTRACT **Neurons in the cat and monkey cerebral cortex were stained immunocytochemically for glutamic acid decarboxylase (GluDCase; L-glutamate 1-carboxy-lyase, EC 4.1.1.15), somatostatin (SRIF), neuropeptide Y (NPY), and cholecystokinin octapeptide (CCK). In all areas of cortex examined (somatic sensory, motor, parietal and visual areas), neurons displaying immunoreactivity for each of these molecules were nonpyramidal cells. Co-localization of GluDCase immunoreactivity with peptide immunoreactivity in the same cells was demonstrated by (*i*) the antibody elution method, staining the same cells by immunofluorescence, first for a peptide and then for GluDCase; (*ii*) double staining of the same sections with sheep anti-GluDCase and rabbit anti-peptide antisera, the bound antibodies being localized by rhodamine-conjugated donkey anti-sheep and fluorescein-conjugated swine anti-rabbit secondary antisera. With both procedures, cell bodies immunoreactive for GluDCase and for each of the peptides were found in all areas of cortex examined. With double labeling on single sections, it was found that all CCK-, SRIF-, and NPY-immunoreactive cells in cat cortex and 90%–95% in monkey cortex are also GluDCase positive. Many more cells, however, are immunoreactive for GluDCase alone. GluDCase was co-localized with CCK, SRIF, or NPY not only in cell somata, but also in small punctate structures, which are likely to be axon terminals. From the data gained in previous electron microscopic studies, we postulate that neurons displaying GluDCase- and CCK-like immunoreactivity are a class separate from those displaying GluDCase- and SRIF-like immunoreactivity. NPY, however, is co-localized with SRIF immunoreactivity. These results imply that classes of cortical interneuron contain a conventional neurotransmitter (γ-aminobutyric acid) and a neuromodulator (one of the peptides).**

Many neurons in the mammalian cerebral cortex appear to use γ-aminobutyric acid (GABA) as a neurotransmitter (1). Neurons displaying immunoreactivity for the GABA synthetic enzyme, glutamic acid decarboxylase (GluDCase; L-glutamate 1-carboxy-lyase, EC 4.1.1.15), have been identified in all layers of rat (2, 3), cat (4), and monkey (5) visual cortex and monkey sensory-motor cortex (6). The GluDCase-positive neurons all belong to the broad class of nonpyramidal cells but can be subdivided into several types based on the size and laminar distribution of their cell bodies and the morphology and sites of termination of their axons. Previous studies have conclusively shown that two morphological types of nonpyramidal cells (basket cells and chandelier cells) are GluDCase-positive and have suggested that certain other types may also be GluDCase-positive (4, 6, 7).

Immunocytochemical studies have also shown that cortical cells displaying immunoreactivity for one or more neuropeptides also belong to the nonpyramidal group (8). Our studies (9) of the monkey sensory-motor cortex indicate that many of these cells show co-localization of immunoreactivity for at least two neuropeptides: somatostatin (SRIF) and neuropeptide Y (NPY). At least 25% of the SRIF- and NPY-positive cells in the monkey cortex (9) and an undetermined number in rat (10) and human cortex (11) are immunoreactive for both peptides.‖ Cells displaying immunoreactivity for cholecystokinin octapeptide (CCK) (12–14) and for vasoactive intestinal polypeptide (15, 16) are, similarly, nonpyramidal in form but differences in sites of termination of their axons, as revealed by electron microscopy (14, 17), suggests that they may belong to a class separate from those with SRIF and NPY-like immunoreactivity.

Up to the present, there has been no indication that the cortical neurons expressing peptide immunoreactivity also express immunoreactivity for markers of a conventional transmitter such as GluDCase. In the present study, we have used two methods to determine that immunoreactivity for CCK, SRIF, or NPY is virtually always co-localized with GluDCase immunoreactivity in local circuit neurons of the cat and monkey cerebral cortex.

MATERIALS AND METHODS

Four cats and two monkeys (*Macaca fascicularis*) were used in this study. The cats and one monkey were normal. In the second monkey, 120 μl of colchicine (10 μg/μl) were injected into the lateral ventricles 2 days before sacrifice. All animals were perfused with 2% paraformaldehyde/0.1 M phosphate buffer with either 0.03% or 0.05% glutaraldehyde added. Perfusion was in two steps: 1 liter of fixative (pH 6.5) was perfused first, followed by 2.5–3 liters of the same fixative (pH 8.5). The brains were removed and stored in 2% paraformaldehyde (pH 8.5) at 4°C for 4, 12, or 24 hr. Some blocks of cortex were soaked in 30% (vol/vol) sucrose/0.1 M phosphate buffer until they had sunk, and they were then cut in 15-μm sections on a cryostat; others were cut immediately in 20-μm sections, using a Vibratome.

Sections of both kinds from two cats and one monkey were processed sequentially, first with a rabbit anti-peptide antiserum in dilutions of 1:500 (anti-CCK), 1:1000 (anti-SRIF), or 1:1500 (anti-NPY). The sections were incubated for 18–24

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: GABA, γ-aminobutyric acid; GluDCase, glutamic acid decarboxylase; SRIF, somatostatin; NPY, neuropeptide Y; CCK, cholecystokinin octapeptide.

†Present address: Department of Anatomy, University of California College of Medicine, Irvine, CA 92717.

‖Neurons in rat (10) and human cortex (11) were identified as displaying avian pancreatic polypeptide-like (APP) immunoreactivity. The immunoreactive material recognized by anti-APP antisera is now considered to be NPY or a NPY-like molecule (for discussion, see ref. 9).

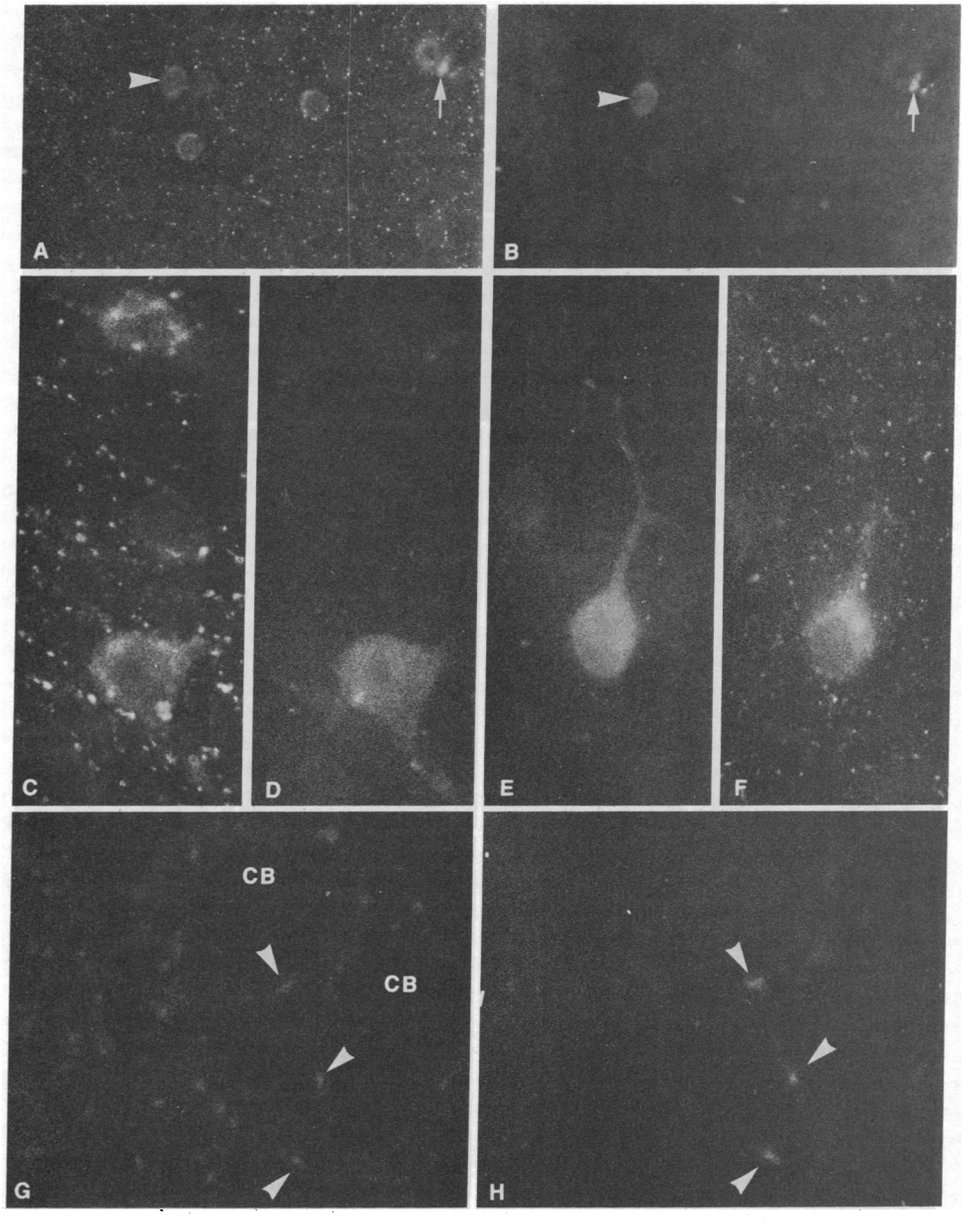

FIG. 1. Pairs of fluorescence micrographs showing co-localization of GluDCase and CCK-like immunoreactivity in neurons of cat and monkey cerebral cortex. (*A* and *B*) Cat motor cortex (area 4) stained with sheep anti-GluDCase and rabbit anti-CCK. Several GluDCase-positive cells are present in layers II and III (*A*) and one of these (arrowheads) also displays CCK-like immunoreactivity (*B*). Arrows point to autofluorescent granules. (×440.) (*C* and *D*) Layer VI of monkey first somatic sensory area (area 3b) stained with sheep anti-GluDCase (*C*). Cell in lower part of the picture is also immunoreactive for CCK (*D*). (×1300.) (*E* and *F*) Cat visual cortex; section was incubated in rabbit anti-CCK, photographed (*E*), and then eluted, incubated in rabbit anti-GluDCase and rephotographed (*F*). The cell body is in layer II. (×1200.) (*G* and *H*) Numerous punctate profiles (arrowheads), which are probably axon terminals, are stained with sheep anti-GluDCase (*G*); some of them surround two cell bodies (CB) in layer II of cat parietal cortex (area 5). Three of the profiles are also CCK-positive (*H*). (×2100.)

hr in primary antiserum at 4°C, washed thoroughly in buffer for 4 hr at 4°C, and incubated in rhodamine-conjugated swine anti-rabbit antiserum (Dako, Santa Barbara, CA) diluted 1:100 for 2 hr at room temperature. Stained cells were examined (see below) and photographed. The antisera were then eluted (18), and the sections were restained with rabbit anti-GluDCase antiserum and diluted 1:1000 at 4°C for 24 hr, washed, and incubated in fluorescein-conjugated swine anti-rabbit antiserum for 2 hr. Cells stained for GluDCase were then photographed again. Control sections were examined directly after elution or after incubating in fluorescein-conjugated secondary antiserum without prior incubation in anti-GluDCase antiserum. No stained cells were seen in either case.

Additional sections from each animal were incubated simultaneously in sheep anti-GluDCase antiserum (diluted 1:1500) and rabbit anti-CCK, anti-SRIF, or anti-NPY antiserum. After incubating for 18 hr at 4°C, the sections were washed in buffer for 4 hr at 4°C and then incubated in a phosphate-buffered mixture of rhodamine-conjugated donkey anti-sheep (Pel-Freez) secondary antiserum and fluorescein-conjugated swine anti-rabbit secondary antiserum (Dako) for 2 hr at room temperature. The sections were washed in buffer for 1 hr, mounted on glass slides, and covered in a 1:3 solution of phosphate-buffered saline and glycerol. They were examined in a fluorescence microscope with exciting filters for wavelengths of 450–490 nm and 530–560 nm.

The primary antisera used were as follows: rabbit anti-GluDCase (19), sheep anti-GluDCase (20), rabbit anti-CCK

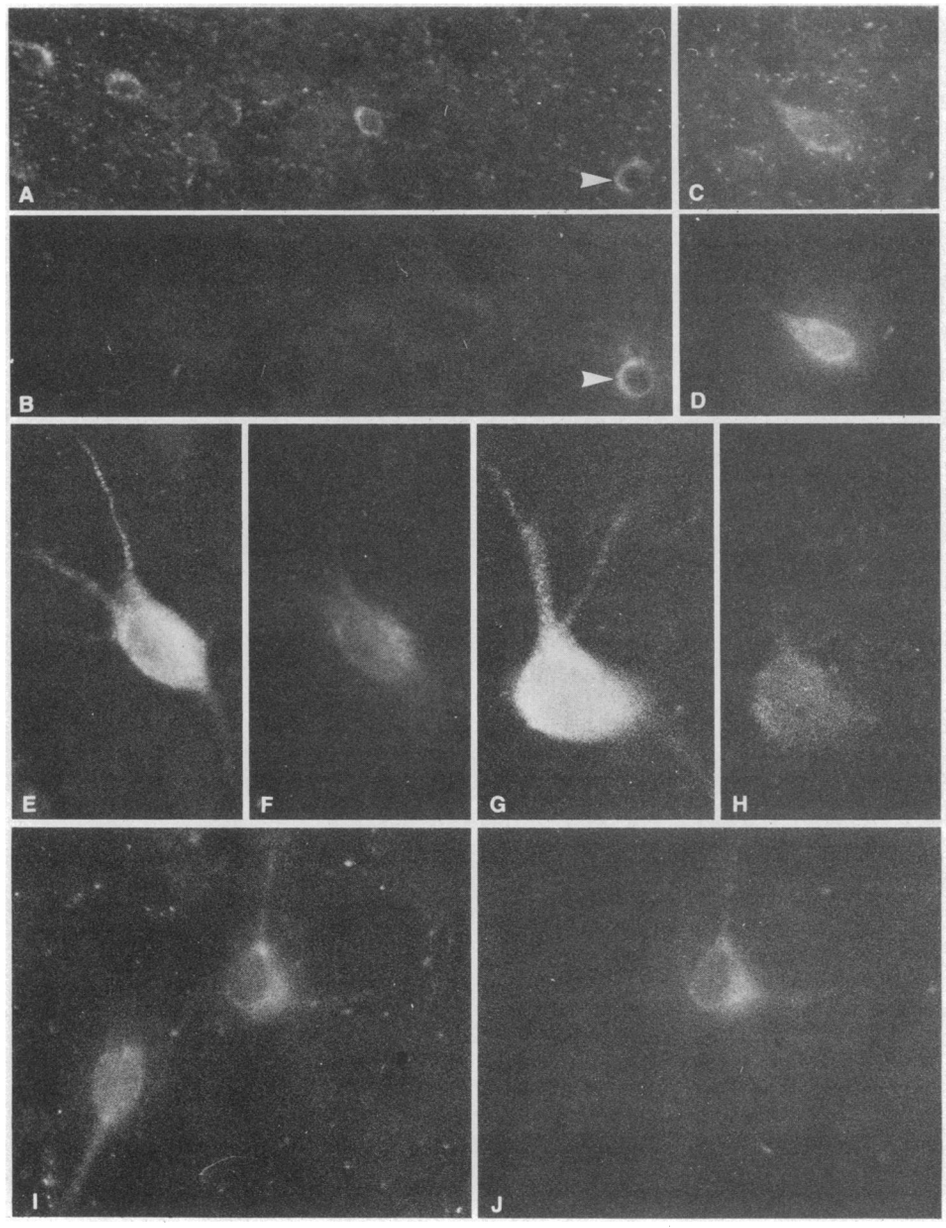

FIG. 2. Pairs of fluorescence micrographs showing coexistence of GluDCase and SRIF-like (*A–H*) or NPY-like (*I* and *J*) immunoreactivity. (*A* and *B*) Layer II of monkey visual cortex (area 17) incubated in sheep anti-GluDCase (*A*) and rabbit anti-SRIF (*B*). One of the GluDCase-positive neurons in *A* (arrowheads) is also SRIF-positive (*B*). (×440.) (*C* and *D*) Cell in layer III of cat motor cortex (area 4) stained first with rabbit anti-SRIF (*D*) and after elution, stained with rabbit anti-GluDCase (*C*). (×1200.) (*E* and *F*) Cell in layer VI of monkey visual cortex (area 17) stained with sheep anti-GluDCase (*E*) and rabbit anti-SRIF (*F*). (×1200.) (*G* and *H*) Cell in layer III of monkey motor cortex (area 4) stained with sheep anti-GluDCase (*G*) and rabbit anti-SRIF (*H*). (×1300.) (*I* and *J*) Two neurons stained with sheep anti-GluDCase (*I*) in layer III of monkey first somatic sensory cortex (areas 1–2). Neuron in upper right is also SRIF-positive (*J*). (×900.)

(21), rabbit anti-NPY (22), and rabbit anti-SRIF directed against the tetradecapeptide (Immuno Nuclear, Stillwater, MN). Adsorption controls were carried out with each group of incubations. Staining by the sheep and rabbit anti-GluDCase antisera was not affected by adsorption of the antisera with an excess of synthetic SRIF, CCK, or NPY. Staining with the peptide antisera was only affected when the primary antiserum was adsorbed with the relevant synthetic peptide (SRIF for anti-SRIF, CCK octapeptide for anti-CCK, and NPY for anti-NPY). Staining was not affected by prior adsorption with vasoactive intestinal polypeptide, peptide HI, secretin, motilin, or [Met]enkephalin.

For quantitation, stained neurons in radial strips ≈85 μm wide and extending across the cortex from the pia mater to a level in the white matter 1 mm deep to layer VI were counted and determined to be single- or double-labeled, either by comparing fluorescence micrographs of sections subjected to the elution method or directly by switching filters in the co-incubated sections. All single- and double-labeled cells in layers II and VI of 15 sections from cat and monkey sensory-motor cortex were counted.

RESULTS

Co-Localization of Peptide and GluDCase Immunoreactivity. Neurons throughout the cat and monkey cerebral cortex were stained immunocytochemically for GluDCase, CCK, SRIF, or NPY. Descriptions of the sizes, laminar distributions, and morphologies of the stained cells in the somatic sensory, motor, parietal, and visual areas of the monkey have already been published (6, 9, 14). In all areas and in

both species, the number of GluDCase immunoreactive cells is far greater than the combined numbers of CCK, NPY, and SRIF immunoreactive cells.

With both staining procedures, a proportion of the GluDCase-positive cells in cat and monkey cortex were also immunoreactive for CCK, SRIF, or NPY. Fig. 1 shows stained cells in sections incubated either simultaneously (Fig. 1 *A–D*, *G* and *H*) or sequentially (Fig. 1 *E* and *F*) in anti-GluDCase and anti-CCK antisera. Not all GluDCase-positive cells are CCK positive (Fig. 1*A*), but all CCK-positive cells in the cat cortex (Fig. 1 *B* and *E*) and the vast majority in the monkey cortex (Fig. 1*D*) are also GluDCase-positive. Double-labeled cell bodies are found in all layers of cortex and in the subcortical white matter, but they are concentrated in layers II and III and in layer VI. In layers II and III, numerous punctate GluDCase-positive profiles surround the cell bodies of both stained and unstained small neurons (Fig. 1*G*) and a few CCK-positive punctate profiles are present around some of the same cells (Fig. 1*H*). Close examination (Fig. 1 *G* and *H*) reveals that some of the CCK-positive puncta are the same as those that are positive for GluDCase.

All SRIF- and NPY-positive cells in cat cortex and the majority in monkey cortex are also GluDCase-positive (Fig. 2). As with neurons in which CCK and GluDCase immunoreactivity are co-localized, neurons immunoreactive for SRIF or NPY and for GluDCase are present in all cortical layers and in the subcortical white matter.

Most of the neurons displaying both GluDCase and peptide immunoreactivity possess small somata (major diameters, 8–12 μm), give off vertically oriented processes (e.g., Figs. 1 *E* and *F* and 2 *E–J*), and conform to previous morphological descriptions of CCK (12–14), SRIF (9), and NPY (9) immunoreactive cells and of the smaller GluDCase (6) immunoreactive cells. The largest double-labeled cells are a few GluDCase- and CCK-positive neurons in layer III of the monkey motor cortex, but the diameters of their somata never exceed 15 μm. All immunoreactive somata larger than this are GluDCase-positive only (Fig. 3 *A* and *B*).

With both staining procedures, a small proportion of cells in monkey cortex display immunoreactivity for CCK, SRIF, or NPY, but not for GluDCase. These neurons are present in all areas examined. With simultaneous staining, they make up ≈5% of the peptide-positive cells in the cortex of the colchicine-injected monkey and 10% in the untreated monkey. Approximately 15% of the peptide-positive cells in the subcortical white matter of both monkeys are not stained for GluDCase.

GluDCase-Positive Neurons Displaying No Peptide Immunoreactivity. Neurons displaying immunoreactivity only for GluDCase are present in every layer of all areas of cat and monkey cortex examined. In one experiment in which sections were incubated in a mixture containing antisera to GluDCase and to all three peptides, less than half the GluDCase-positive cells in layers II, III, and VI of the cat and monkey first somatic sensory area were double-labeled, and an even larger proportion of GluDCase-positive cells displaying no peptide immunoreactivity were found in layers IV and V. In the monkey first somatic sensory and motor areas, as few as 4 and no more than 11 out of every 100 GluDCase-positive cells in layer IV display immunoreactivity for one of the peptides.

Large (15–25 μm diameter) GluDCase-positive somata surrounded by GluDCase-positive puncta are present in layers IIIB–VI of all areas examined (Fig. 3 *A* and *B*). Of >300 such cells in cat and monkey cortex, none was also CCK-, SRIF, or NPY-positive. Large pyramidal cells in these same layers are also surrounded by GluDCase-positive puncta (Fig. 3*C*), but no GluDCase-positive puncta surrounding the large pyramidal cells were found to be immunoreactive for one of the peptides (Fig. 3*D*).

DISCUSSION

In the present study, we have demonstrated CCK-like, SRIF-like, and NPY-like immunoreactivity in GluDCase-immunoreactive nonpyramidal neurons of the cat and monkey cerebral cortex. Immunoreactivity to GluDCase and the peptides could be co-localized not only in somata but also in certain punctate structures that correlative electron microscopy (2–4, 9, 14) suggests are axon terminals. Our data indicate that all CCK-, SRIF-, and NPY-positive cells in cat cortex and the vast majority in monkey cortex are also GluDCase-positive. It is important to note that the total number of double-labeled cells is far exceeded by the number of cells immunoreactive for GluDCase alone. Firm conclusions as to the relative proportions of single- and double-labeled cells are difficult to draw because of technical considerations,

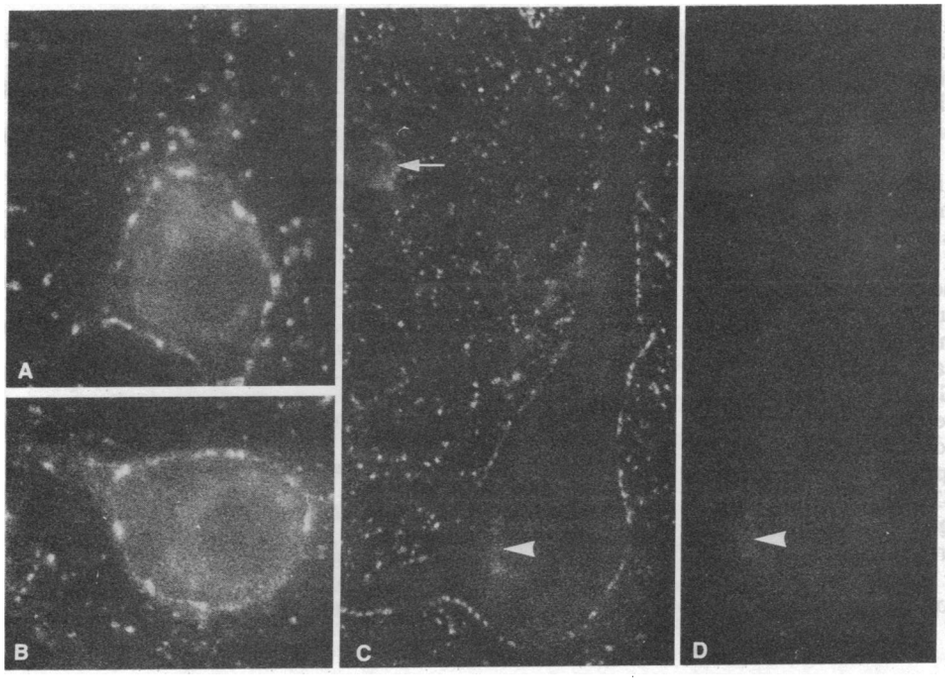

FIG. 3. (*A* and *B*) Two large neurons in layer V of motor cortex (area 4) of a monkey (*A*) and a cat (*B*) both stained with sheep anti-GluDCase. The cell bodies are surrounded by GluDCase-positive puncta, which are probably axon terminals. Cells of this type are probably basket cells; none were found to display additional immunoreactivity for CCK, SRIF, or NPY. (×1300.) (*C* and *D*) Unstained cell body and proximal dendrites of a pyramidal cell in layer V of cat motor cortex (area 4) surrounded by puncta stained with sheep anti-GluDCase (*C*). A GluDCase-positive cell body (arrow) is also present. None of the puncta (terminals) is immunoreactive for CCK (*D*). Arrowheads point to the same autofluorescent granule in the pyramidal cell. (×700.)

some of which relate to the sensitivity of the immunocytochemical method, and others, more importantly, to the difficulty of optimizing conditions for localizing two antigens simultaneously.

Two morphologically characterized classes of cortical nonpyramidal cells have already been shown to be GluDCase-positive (4, 6, 7). One is the large basket cell of layers IIIB–VI, the somata of which are large (15–25 μm diameter), and frequently surrounded by GluDCase-positive terminals (6). Their axons terminate in pericellular nests around the somata and proximal dendrites of pyramidal neurons in the deep cortical layers (23, 24). We found no large GluDCase-positive soma to be immunoreactive for CCK, SRIF, or NPY, nor could we find GluDCase-positive terminations on pyramidal cells displaying peptide-like immunoreactivity in layers IIIB–VI. A second class of GluDCase-positive neuron, the chandelier cell, is characterized by vertical rows of axon terminations that synapse on the initial segments of pyramidal cell axons (3, 4). Previous electron microscopic studies have failed to show CCK-, SRIF-, or NPY-positive terminals synapsing on initial segments (9, 14). Our data suggest that large basket cells and chandelier cells, although GABAergic, do not show co-localization of any of the three peptides examined.

Both SRIF-like and NPY-like immunoreactivity can be co-localized in some neurons of rat (10), monkey (9), and human (11) cerebral cortex. It is very likely, then, that a number of GluDCase-positive neurons would display immunoreactivity for both these peptides. All previous attempts to co-localize CCK with either SRIF or NPY in neurons of rat, cat, and monkey cerebral cortex have failed (unpublished observations), a finding that is consistent with the electron microscopic observations that axon terminals immunoreactive for CCK synapse at sites different from those contacted by SRIF- or NPY-positive terminals (9, 14). Hence, it is unlikely that individual GluDCase-positive cortical neurons display immunoreactivity for all three peptides.

Other transmitter substances or their related enzymes have also been identified immunocytochemically in cortical neurons. Neurons immunoreactive for vasoactive intestinal polypeptide are similar in morphology and laminar distribution to the CCK-, SRIF-, and NPY-immunoreactive neurons (15), suggesting vasoactive intestinal polypeptide-like immunoreactivity may coexist either alone or along with one or more other neuropeptides in the population of GluDCase-positive cortical neurons. The biosynthetic enzyme of acetylcholine, choline acetyltransferase, has also been localized in cortical nonpyramidal cells resembling the peptide-positive neurons (25, 26). Whether these cholinergic cells also display neuropeptide-like or GluDCase immunoreactivity is now worth exploring.

The weight of available evidence indicates that CCK and SRIF are released from axon terminals in a calcium-dependent manner (27, 28), and they act as excitatory agents in the mammalian cerebral cortex (29–31). There is little information available on NPY. GABA, on the other hand, appears to be the major cortical inhibitory neurotransmitter (32). It is intriguing to consider the significance of neuropeptide coexistence in GABAergic cell bodies and axon terminals. It is conceivable that in these neurons GABA acts as a conventional transmitter agent and the peptides, released either simultaneously with GABA or at different times, exert a modulatory action on the postsynaptic neuron, perhaps by an action on a second messenger (33, 34).

The present results lead us to suggest that many, if not all, of the smaller GluDCase-positive cortical nonpyramidal cells may be immunoreactive for one or more other transmitter substances. Our evidence is based on the co-localization of GluDCase with only three of the currently recognized peptide neurotransmitter candidates present in the cerebral cortex. Apart from determining whether other transmitter candidates also coexist in GluDCase-positive cortical neurons, it will be important to consider if different cotransmitters can be expressed by the same neuron at different times and to determine the conditions under which the two or more transmitters are normally released.

1. Emson, P. C. & Lindvall, O. (1979) *Neuroscience* **4**, 1–30.
2. Ribak, C. E. (1978) *J. Neurocytol.* **7**, 461–478.
3. Peters, A., Proskauer, C. C. & Ribak, C. E. (1982) *J. Comp. Neurol.* **206**, 397–416.
4. Freund, T. F., Martin, K. A. C., Smith, A. D. & Somogyi, P. (1983) *J. Comp. Neurol.* **221**, 263–278.
5. Hendrickson, A. E., Hunt, S. P. & Wu, J-Y. (1981) *Nature (London)* **292**, 605–607.
6. Houser, C. R., Hendry, S. H. C., Jones, E. G. & Vaughn, J. E. (1983) *J. Neurocytol.* **12**, 617–638.
7. Houser, C. R., Vaughn, J. E., Hendry, S. H. C., Jones, E. G. & Peters, A. (1984) in *The Cerebral Cortex*, eds. Jones, E. G. & Peters, A. (Plenum, New York), Vol. 2, in press.
8. Emson, P. C. & Hunt, S. P. (1984) in *The Cerebral Cortex*, eds. Jones, E. G. & Peters, A. (Plenum, New York), Vol. 2, in press.
9. Hendry, S. H. C., Jones, E. G. & Emson, P. C. (1984) *J. Neurosci.*, in press.
10. Vincent, S. R., Skirboll, L., Hökfelt, T., Johansson, O., Lundberg, J. M., Elde, R. P., Terenius, L. & Kimmel, J. (1982) *Neuroscience* **7**, 439–446.
11. Vincent, S. R., Johansson, O., Hökfelt, T., Meyerson, B., Sachs, C., Elde, R. P., Terenius, L. & Kimmel, J. (1982) *Nature (London)* **298**, 65–67.
12. McDonald, J. K., Parnavelas, J. G., Karamanlidis, A., Brecha, N. & Rosenquist, G. (1982) *J. Neurocytol.* **11**, 881–895.
13. Peters, A., Miller, M. & Kimerer, L. M. (1983) *Neuroscience* **8**, 431–448.
14. Hendry, S. H. C., Jones, E. G. & Beinfeld, M. C. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 2400–2404.
15. McDonald, J. K., Parnavelas, J. C., Karamanlidis, A. N. & Brecha, N. (1982) *J. Neurocytol.* **11**, 825–837.
16. Emson, P. C., Hunt, S. P., Rehfeld, J. F., Golterman, N. & Fahrenkrug, J. (1980) in *Neural Peptides and Neuronal Communication*, eds. Costa, E. & Trabucchi, M. (Raven, New York), pp. 63–74.
17. Peters, A. & Connor, J. R. (1983) *Soc. Neurosci. Abstr.* **9**, 1181.
18. Tramu, G., Pillez, A. & Leonardell, J. (1978) *J. Histochem. Cytochem.* **26**, 322–324.
19. Brandon, C. (1984) *Brain Res.*, in press.
20. Oertel, W. H., Schmechel, D. E., Tappaz, M. L. & Kopin, J. (1981) *Neuroscience* **6**, 2689–2700.
21. Beinfeld, M. C., Meyer, D. K., Eskay, R. L., Jensen, R. T. & Brownstein, M. J. (1981) *Brain Res.* **212**, 51–57.
22. Sundler, F., Moghimzadeh, E., Hakanson, R., Ekelund, M. & Emson, P. C. (1983) *Cell Tissue Res.* **230**, 487–493.
23. Marin-Padilla, M. (1969) *Brain Res.* **14**, 633–646.
24. Jones, E. G. (1975) *J. Comp. Neurol.* **160**, 205–268.
25. Houser, C. R., Crawford, G. D., Barber, R. P., Salvaterra, P. M. & Vaughn, J. E. (1983) *Brain Res.* **266**, 97–119.
26. Houser, C. R., Crawford, G. D., Salvaterra, P. M. & Vaughn, J. E. (1983) *Soc. Neurosci. Abstr.* **9**, 80.
27. Iversen, L. L., Iversen, S. D., Bloom, F., Douglas, C., Brown, M. & Vale, W. (1978) *Nature (London)* **273**, 161–163.
28. Emson, P. C., Lee, C. M. & Rehfeld, J. F. (1980) *Life Sci.* **26**, 2157–2163.
29. Phillis, J. N. & Kirkpatrick, J. R. (1980) *Can. J. Physiol. Pharmacol.* **58**, 612–623.
30. Kelly, J. S. (1982) *Br. Med. Bull.* **38**, 283–290.
31. Delfs, J. R. & Dichter, M. A. (1983) *J. Neurosci.* **3**, 1176–1188.
32. Krnjević, K. (1974) *Physiol. Rev.* **54**, 318–450.
33. Dokas, L. A., Zwiers, H., Coy, D. H. & Gispen, W. H. (1983) *Eur. J. Pharmacol.* **88**, 185–193.
34. Catalán, R. E., Martinez, A. M. & Aragones, M. D. (1983) *Neuropharmacology* **22**, 641–645.