

The neostriatal mosaic: Compartmental distribution of calcium-binding protein and parvalbumin in the basal ganglia of the rat and monkey

(striatal compartments/substantia nigra/dopamine)

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ABSTRACT Calcium-binding protein (CaBP) and parvalbumin are two proteins that are expressed in brain and bind calcium in the micromolar range. The immunohistochemical distribution of these two proteins was examined in the basal ganglia of rats and rhesus monkeys. In the striatum, CaBP immunoreactivity is localized to a subset of striatonigral projection neurons; CaBP-positive neurons are distributed in areas containing somatostatin-immunoreactive fibers and not in the complementary areas containing dense μ opiate-receptor binding. These biochemical labels mark, respectively, the matrix and patch compartments of the striatum. Previous studies have shown that striatal matrix neurons project to the substantia nigra pars reticulata, whereas striatal patch neurons project to the substantia nigra pars compacta. Consistent with the restricted localization of CaBP in the matrix projection neurons is the confinement of CaBP-immunoreactive afferent fibers to the pars reticulata. CaBP is also localized to a portion of dopaminergic and a few nondopaminergic neurons in the substantia nigra pars compacta and in most dopaminergic neurons in the ventral tegmental area. Parvalbumin immunoreactivity is localized to a subset of substantia nigra pars reticulata neurons and their axons. In the lateral striatum, some medium-sized aspiny interneurons are also parvalbumin immunoreactive. The distinct distributions of CaBP and parvalbumin in the basal ganglia are discussed in terms of their possible roles as intracellular calcium buffer systems related to the physiologic response properties of the neurons in which they are contained.

A calcium-binding protein (CaBP) isolated from human cerebellum (1, 2), similar to vitamin D-induced calcium-binding protein isolated from chicken intestine (1, 3), has been immunohistochemically localized in select subsets of neurons distributed heterogeneously in the brain (2). Another calcium-binding protein, parvalbumin, isolated from muscle, has also been found in brain but with a distribution that is distinct, for the most part, from that of CaBP (4). Aside from their capacity to bind calcium in the micromolar range, the neuronal functions of CaBP and parvalbumin are unknown (5). In muscle, parvalbumin has been suggested to serve as an intracellular calcium buffer (6), and its discrete distribution in fast- but not slow-twitch muscle fibers (7) suggests a relationship to the physiologic response properties of these fibers. In an effort to determine whether the distribution of these proteins in the brain may be compatible with similar functions, their distributions were examined and compared in the basal ganglia of the rat and monkey by using immunohistochemical techniques.

One of the major parts of the basal ganglia is the striatum, and within the striatum two neurochemically distinct com-

partments, the patches and matrix, are arranged in a mosaic fashion. Opiate receptors are concentrated in patches (8, 9) that are complementary to a matrix marked by staining for acetylcholinesterase (9, 10) and a somatostatin-immunoreactive fiber plexus (11, 12). The patches and matrix receive different afferents from the cortex and midbrain. For instance, the rat prelimbic cortex preferentially innervates the striatal patches (11, 13), whereas other prefrontal, motor, and sensory cortical areas in the rat, cat, and monkey provide inputs to the striatal matrix (13–15). A portion of dopamine-containing neurons in the ventral tegmental area (VTA) and substantia nigra and some nondopaminergic nigral neurons project to the striatal matrix, whereas other dopaminergic substantia nigra neurons innervate the striatal patches in the rat (16–18). In addition, the two striatal compartments give rise to separate projection systems to the substantia nigra; patch neurons project to the pars compacta (SNc, location of neurons containing dopamine), whereas matrix neurons project to the pars reticulata [SNr, location of neurons containing γ -aminobutyric acid (GABA)] (11, 12). To determine whether biochemical markers, which may be related to the physiologic response properties of neurons, might differentially characterize these striatal subsystems, the immunohistochemical localization of CaBP and parvalbumin was examined in the basal ganglia.

METHODS

To determine the immunohistochemical distribution of CaBP and parvalbumin in the brain, adult female albino rats were anesthetized and perfused transcardially with 100 ml of physiologic saline (4°C) followed by 500 ml of 4% formaldehyde (from paraformaldehyde)/1% calcium acetate/100 mM NaCl (20°C). The brains were post-fixed for 48 hr, transferred to a solution of 20% sucrose in physiologic saline overnight, and then cut frozen into 30- μ m-thick sections, which were collected in 20 mM potassium phosphate-buffered physiologic saline (pH 7.4; KPBS). Four sets of serial sections through the striatum were processed in the following manner. Series A was processed for autoradiographic demonstration of μ opiate-receptor binding by the procedure of Herkenham and Pert (19). Slide-mounted sections were incubated in a solution of 2.5 nM [³H]naloxone (specific activity, 44.4 Ci/mmol; 1 Ci = 37 GBq) in 50 mM Tris buffer (pH 7.4) and 100 mM NaCl at 4°C for 90 min, rinsed, dried, fixed, defatted, and processed for emulsion autoradiography. Series B–D were processed to localize CaBP, somatostatin, and parvalbumin by using rabbit primary antisera each diluted in KPBS to which 0.3% Triton X-100 and 2% normal goat serum

Abbreviations: CaBP, calcium-binding protein; GABA, γ -aminobutyric acid; SNc, substantia nigra pars compacta; SNr, substantia nigra pars reticulata; TH, tyrosine hydroxylase; VTA, ventral tegmental area.

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had been added. Series B was incubated in a 1:50 dilution of rabbit antiserum directed against CaBP isolated from human cerebellum (previously characterized in ref. 1). Series C was incubated in rabbit antisera directed against somatostatin [SS28(1–12), 1:5000; SS28, 1:2000; characterized in ref. 20; a gift of R. Benoit]. Series D was incubated in a 1:200 dilution of rabbit antiserum directed against mouse muscle parvalbumin (a gift of M. Johnson). Incubation of these series was carried through 48 hr and then processed with an immunoperoxidase method using the avidin/biotin/peroxidase method (21) supplied by Vector Laboratories, Burlingame, CA, using diaminobenzidine as a chromogen. After the diaminobenzidine reaction, sections were transferred to a 10% formalin solution for 1 hr, rinsed in KPBS, mounted onto chrom-alum-coated slides, air dried, defatted, and then intensified in a 0.005% solution of osmium tetroxide for 2–3 hr according to the method described (12).

Four series of sections through the substantia nigra were also collected and processed for immunohistochemical localization of parvalbumin (series A), CaBP (series B), tyrosine hydroxylase [TH; series C (22), rabbit antiserum used at 1:1500; a gift of J. Thibault], and substance P (series D, rabbit antiserum used at a dilution of 1:2000; a gift of M. Brown). These sections were processed as described above.

Some sections through the substantia nigra were also processed to examine the possible coexistence of CaBP and TH immunoreactivity in the same cells. Sections were incubated in antiserum directed against CaBP (1:100) for 8 hr at 4°C and then processed by the immunoperoxidase method using the avidin biotin peroxidase system as described above, except that 4-chloro-1-naphthol was used as a chromogen. Sections were rinsed and mounted directly out of KPBS and covered with buffered glycerol and a coverslip. After the sections were photographed, the coverslips were removed and the slides were rinsed thoroughly in KPBS for 15 min, followed by an overnight incubation in 1.0 M NaCl in 0.1 M acetate buffer (pH 3.5). The slides were then rinsed in water, dehydrated in an ascending series of alcohols, and left in ethanol for 2 hr, which removed the 4-chloro-1-naphthol reaction product. Slides were then rehydrated, and half were incubated in rabbit antiserum directed against TH (1:1000) for 24 hr and half were incubated in KPBS alone. Both sets of sections were rinsed and underwent reaction according to the immunoperoxidase protocol using diaminobenzidine as a chromogen, as described above. Sections not incubated in antiserum directed against TH showed no immunoreactive labeling. Those sections incubated in antiserum directed against TH were realigned in the microscope, and the areas photographed previously were rephotographed. The absence of immunoreactivity in lateral midbrain tegmental areas that contain CaBP, following the elution of CaBP immunolabeling and restaining for TH immunoreactivity, served as an internal control for the removal of label from CaBP-immunoreactive cells.

The method of Sawchenko and Swanson (23) was used to determine whether striatonigral neurons contain either CaBP or parvalbumin immunoreactivity. Ten rats received an injection of 400 nl of fast blue (2.5% in saline) into the substantia nigra ($n = 5$) or entopeduncular nucleus ($n = 5$). After a 10-day survival period, striatal sections were processed for immunofluorescent localization of CaBP and parvalbumin. The procedure described above was followed except that affinity-purified goat anti-rabbit IgG conjugated with fluorescein [GaR-fluorescein isothiocyanate (FITC), 1:200] was used as the labeled secondary antiserum. Sections were then mounted directly out of KPBS, air-dried, covered with buffered glycerol and a coverslip, and examined with epifluorescent filters that allow the separate visualization of fast blue (excitation filter, 330–380 nm; barrier filter, 420 nm)

and FITC (excitation filter, 460–485 nm; barrier filter, 515–545 nm).

Sections from two adult rhesus monkeys were also examined for CaBP immunoreactivity. The same protocol as that used for the rat brain tissue was followed. Adjacent sections from the caudate and putamen were examined for CaBP and somatostatin immunoreactivity and serial sections through the substantia nigra were examined for CaBP, TH, substance P, enkephalin, and dynorphin immunoreactivity.

RESULTS

CaBP in the Matrix Striatonigral System. CaBP immunoreactivity labels most of the projection system from the striatal matrix compartment to the substantia nigra pars reticulata (SNr). In the striatum, CaBP is present in the neuropil and in medium-sized cell bodies of the matrix, except in the dorsolateral area where there is relatively little label. In Fig. 1, three serial coronal sections through the rat striatum show that the patches, marked by [³H]naloxone binding to μ opiate receptors (Fig. 1A), contain little CaBP immunoreactivity (Fig. 1B), whereas the distributions of CaBP and somatostatin-fiber immunoreactivities (Fig. 1C) are coextensive in the matrix. Experimental evidence that CaBP-containing striatal cells project to the substantia nigra is provided by the colocalization of CaBP immunoreactivity in cells that are retrogradely labeled by fast blue injections into the substantia nigra (Fig. 2 A and A'). Whereas almost every retrogradely labeled striatonigral neuron in the matrix contains CaBP, very few retrogradely labeled neurons in the patches contain CaBP. Similar labeling patterns were obtained with injections of fast blue into the entopeduncular nucleus.

CaBP immunoreactivity is also present in fibers distributed in the globus pallidus, entopeduncular nucleus, and substantia nigra. Such labeling corresponds to the distribution of striatal projection fibers (12) and is decreased by kainic acid lesions of the striatum (data not shown). This is consistent with the retrograde data, suggesting that the CaBP-immunoreactive neuropil in the substantia nigra represents labeled afferents from the striatal matrix. Such CaBP-labeled terminals are distributed preferentially in the SNr (Fig. 3B) where GABAergic neurons are located (24) and are absent in the substantia nigra pars compacta (SNc) where dopaminergic neurons, marked by TH immunoreactivity (Fig. 3C), are located. CaBP-labeled terminals are densest in the medial SNr. Although present, CaBP is less dense in the lateral SNr, which most likely reflects the sparse CaBP labeling of projection neurons in the dorsolateral quadrant of the striatum.

These data suggest that neurons in the striatal matrix project selectively to the SNr. On the other hand, substance P, which is contained in both striatal patch and matrix projection neurons (25, 26), is localized in afferents in both the SNr and SNc (27). This is seen in Fig. 3D, which shows the distribution of substance P terminal immunoreactivity in a section adjacent to one labeled for CaBP immunoreactivity (Fig. 3C). While substance P is densely distributed throughout the SNr, there are also dense areas of labeling in the ventral SNc, a zone that contains dopaminergic neurons (Fig. 3B) but no CaBP-labeled terminals (Fig. 3C).

CaBP in the Nigrostriatal System. Within the rat midbrain, CaBP-containing neurons are located in the ventral tegmental area, substantia nigra, and retrorubral area. Sequential localization of CaBP and TH immunoreactivity in sections through the midbrain allow the determination of which CaBP-containing neurons are dopaminergic. In the ventral tegmental area, a high proportion of TH-immunoreactive cells also stain for CaBP. In the SNc, CaBP is colocalized in only a subpopulation of TH-immunoreactive cells (Fig. 4), such that CaBP/TH coreactive cells are interspersed among

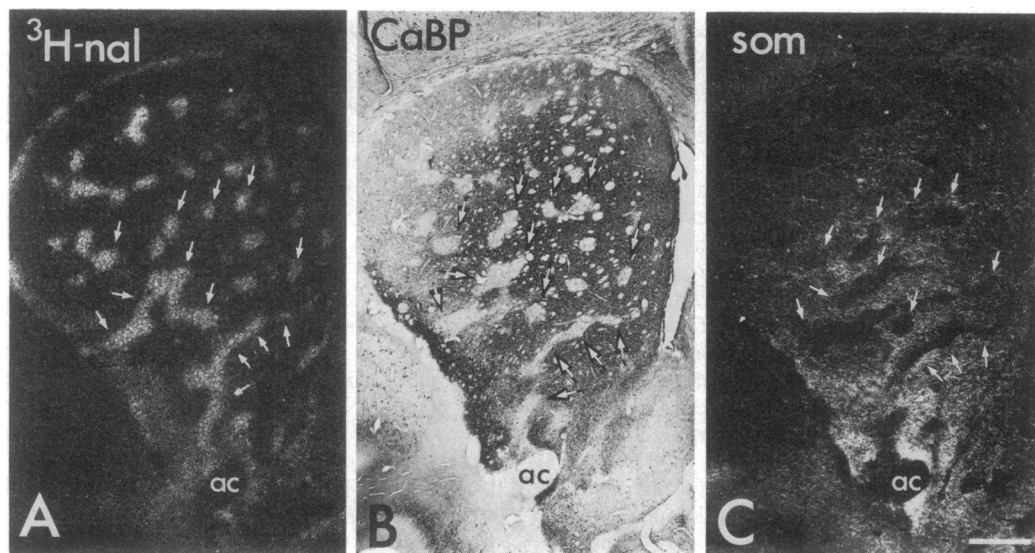


FIG. 1. Photomicrographs of three serial coronal sections from the rat striatum show (A) μ opiate receptor binding as marked by [3 H]naloxone binding (3 H-nal), (B) CaBP, and (C) somatostatin immunoreactivity (som). A and C are viewed with dark-field illumination so that labeled areas are light, whereas B is viewed with bright-field illumination so that labeled structures are dark. Arrows in each section mark striatal patches, showing that CaBP immunoreactive cells are distributed in the matrix compartment as marked by somatostatin fibers that are distributed complementary to opiate receptor-rich patches. Both CaBP and somatostatin fiber immunoreactivity are relatively sparse in the dorsolateral quadrant of the striatum. (Bar = 500 μ m.)

cells displaying only TH. Most CaBP neurons in the substantia nigra pars lateralis are not labeled for TH. CaBP and TH are usually colocalized in cells in the retrorubral area.

CaBP Immunoreactivity in the Monkey. Fig. 5 shows the distribution of CaBP immunoreactivity in the brain of the rhesus monkey. Within the caudate and putamen (Fig. 5A),

a pattern similar to that in the rat striatum is seen. Immunoreactive cells and neuropil are distributed heterogeneously, with distinct patches in which labeling is absent. This pattern is observed in both the caudate and putamen; however, in the dorsolateral quadrant of both nuclei, CaBP staining is relatively sparse. As in the rat striatum, CaBP-labeled cells are distributed in areas containing somatostatin-

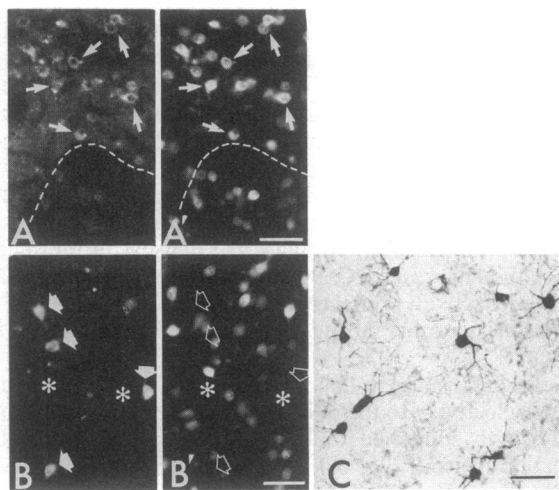


FIG. 2. (A, A', B, and B') Photomicrographs showing labeling in the striatum in a rat in which fast blue was injected into the substantia nigra. (A) CaBP immunoreactivity is revealed with a fluorescent filter that shows fluorescein isothiocyanate to be distributed in the neuropil and neurons of the matrix (area above the dashed line). Viewed with the fluorescent filter to show fast blue (A'), all of the retrogradely labeled neurons in the matrix are seen to also contain CaBP (A), whereas retrogradely labeled neurons in the patch (below dashed line) do not. Arrows mark five such double-labeled neurons. (B) Parvalbumin immunoreactivity is observed in four neurons (arrows) that are not retrogradely labeled by fast blue injections into the substantia nigra (B'). Open arrows in B' mark the location of parvalbumin-containing neurons. Asterisks mark fiber fascicles used for alignment. The morphological form of parvalbumin-containing neurons is shown in C with the immunoperoxidase method. The perikarya of these neurons are of medium size and the dendrites lack labeled spines. (Bar = 50 μ m.)

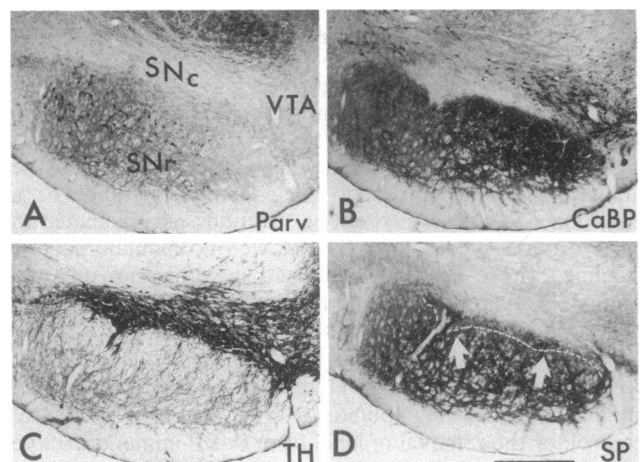


FIG. 3. Photomicrographs of serial coronal sections through the rat substantia nigra show the distribution of (A) parvalbumin immunoreactivity (Parv), (B) CaBP immunoreactivity, (C) TH immunoreactivity to mark the location of dopaminergic cells, and (D) substance P immunoreactivity (SP). The relative distribution of these markers to the SNc, SNr, and VTA can be compared. (A) Parvalbumin is localized in the majority of GABAergic neurons in the lateral three-fourths of the SNr. (B) CaBP is contained in a subpopulation of SNc and VTA neurons and is also distributed in terminals in the SNr. (C) The distribution of CaBP-labeled terminals is complementary to the areas containing dopaminergic neurons. Substance P is localized to terminals distributed throughout the SNr and in the ventral SNc. In the area above the dashed line in D, SP-labeled terminals are distributed in areas where dopaminergic neurons are located but that are devoid of CaBP-labeled terminals. Arrows mark two particularly dense areas of substance P labeling in this area. (Bar = 500 μ m.)

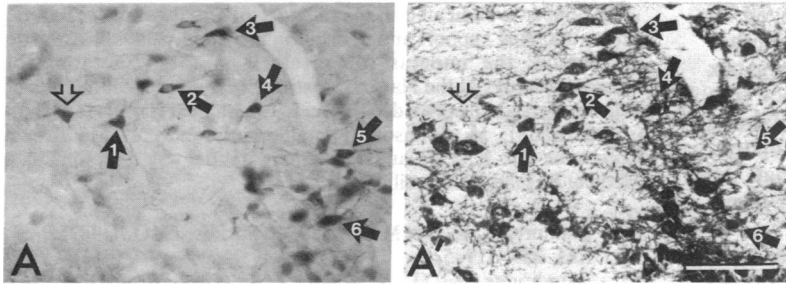


FIG. 4. Photomicrographs from a section through the substantia nigra processed to show colocalization of CaBP and TH immunoreactivity in SNc and VTA cells. A shows CaBP immunoreactivity and A' shows the same area after CaBP immunolabeling had been removed and the section was restained for TH immunoreactivity. Many of the CaBP-immunoreactive cells are seen to also contain TH immunoreactivity. Six examples of cells in which CaBP and TH are colocalized are marked. One CaBP-labeled neuron in this field (open arrow) does not also contain TH. (Bar = 100 μm .)

immunoreactive fibers, which mark the matrix (Fig. 5B). Also, similar to the rat, CaBP-containing terminals in the substantia nigra are densely distributed in the SNr (Fig. 5C) and sparsely distributed in areas that contain dopaminergic cells (Fig. 5D). In the monkey, there are islands of dopaminergic neurons in the pars reticulata and, notably, these islands are devoid of CaBP-terminal labeling. Substance P-, [Met]enkephalin-, and dynorphin-immunoreactive fibers within the substantia nigra each overlap, in part, areas containing dopaminergic cells (data not shown; see ref. 27). CaBP is also localized in a subpopulation of SNc neurons and in a large number of VTA neurons.

Parvalbumin Immunoreactivity in the Rat. In the striatum, parvalbumin immunoreactivity is localized in medium-sized cells (perikaryal width/length, 11 μm /14 μm to 12 μm /20 μm) that possess long apparently spine-free dendrites (Fig. 2C). These cells are present in greatest numbers in the lateral striatum. The inability to retrogradely label parvalbumin-immunoreactive neurons with fast blue injections into the substantia nigra (Fig. 2B and B') or entopeduncular nucleus suggests that these are striatal interneurons.

In the substantia nigra, parvalbumin immunoreactivity is localized to cell bodies in the SNr (Fig. 3A). These SNr neurons have previously been shown to be GABAergic (24). The number of parvalbumin-immunoreactive neurons is greatest in the lateral SNr. Parvalbumin is also localized in fibers in the mediodorsal, ventromedial, and parafascicular thalamic nuclei, and in the superior colliculus and pedunculopontine nucleus, regions that receive inputs from the SNr (28).

DISCUSSION

The present series of experiments shows that CaBP immunoreactivity is localized in specific subsets of both striatonigral and nigrostriatal projection neurons. The pattern of distribution of CaBP in both the monkey and rat suggests that CaBP marks, in each species, most of the striatonigral system originating from the striatal matrix, whose cells selectively project to the SNr. The distribution of CaBP is consistent with previous tract tracing studies that demonstrated, in the rat, the distinct striatal projections of the patches to the SNc and the matrix to the SNr (11, 12). The similarity of CaBP distribution in the rat and monkey allows for the tentative conclusion that separate matrix and patch striatonigral systems are also present in the monkey.

A number of peptides, including enkephalin (26, 29), dynorphin (30), and substance P (25), have been shown to be present in striatonigral neurons. In some areas of the striatum, specific peptidergic cell types are preferentially distributed in either the patch or matrix, while in other areas each type of peptide neuron is located in both compartments (25, 26). Consistent with the reported dichotomy of striatonigral systems arising from the patch and matrix, these peptides are not only distributed in both matrix and patch neurons but are also contained in afferents to both the SNr and SNc in the rat and monkey (27).

The distribution of substance P in the striatonigral system provides a particularly good example of the distribution of a peptide in both striatonigral systems. In the rat, substance P

is contained in striatonigral neurons that are localized primarily in patches in the rostral-dorsal striatum, but in the ventral and caudal striatum they are located in both compartments (25). Substance P is contained in nigral afferents distributed throughout the SNr and in the ventral SNc (Fig. 3D). A similar pattern of substance P terminal distribution in the monkey has been described (27). The distribution of substance P-immunoreactive terminals in the SNr and ventral SNc of the rat is consistent with previous studies showing that nigral afferents from the dorsal striatum are split, with part going to the ventral SNc, from the patches, and the remainder, from the matrix, going to the SNr (12). Although various peptides, such as substance P, dynorphin, and enkephalin, mark subsets of the striatonigral system, none appears solely restricted to either the patch or matrix subsystems (26). On the other hand, CaBP is selectively localized in nearly the entire striatonigral system arising from the matrix. This finding strengthens the concept that there are distinct patch and matrix striatal projection systems.

Whereas the common feature of the mix of striatonigral cells that contain CaBP is that they are distributed in the matrix, the common feature of VTA and nigral cells that express CaBP is that they appear to project to the striatal matrix. Previous studies have shown that most VTA dopaminergic and a subpopulation of SNc dopaminergic and nondopaminergic neurons project to the striatal matrix, while other SNc dopaminergic neurons project to the striatal patches (16–18). The absence of CaBP in terminals in the striatal patches is consistent with CaBP selectively marking midbrain neurons that project only to the striatal matrix and

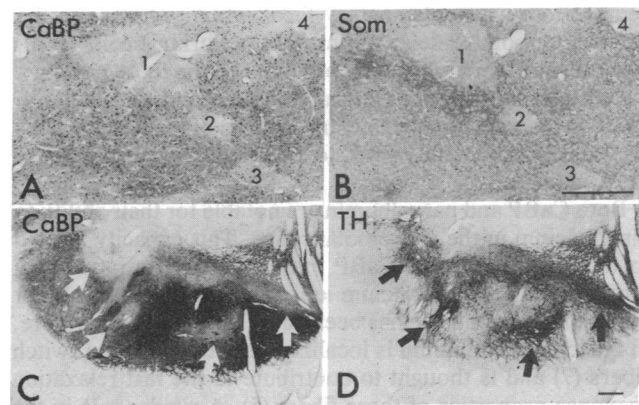


FIG. 5. Photomicrographs of brain sections from a rhesus monkey show that in the caudate CaBP immunoreactivity (A) is contained in neurons distributed in the matrix, which is marked by the distribution of somatostatin immunoreactive terminals (Som) (B). Four patches that contain little CaBP or somatostatin label are marked. In the substantia nigra, CaBP (C) is localized in neurons in the pars compacta and VTA and is contained in terminals that are distributed in the SNr but avoid areas containing dopaminergic neurons labeled for TH immunoreactivity (D). Arrows in C and D mark some of the areas that are devoid of CaBP terminals (C) in which dopaminergic neurons are distributed (D). (Bar = 500 μm .)

not to the patches. Furthermore, CaBP is localized in dopaminergic neurons in the retrorubral area, another midbrain cell group that projects preferentially to the matrix (unpublished observations). Interestingly, the absence of a projection from the striatal matrix to CaBP-containing midbrain neurons, which provide inputs to the striatal matrix, is consistent with previous studies suggesting that the striatonigral and nigrostriatal systems are not under direct reciprocal control (31).

Parvalbumin immunoreactivity is present in medium-sized aspiny interneurons in the lateral striatum. Three major types of striatal interneurons have been described, including the large aspiny cholinergic neuron (32), the medium aspiny somatostatin neuron (33, 34), and another medium aspiny cell type that accumulates GABA (35). Preliminary colocalization studies suggest that parvalbumin immunoreactivity is not colocalized in striatal cells with either choline acetyltransferase or somatostatin immunoreactivity (unpublished observation). Whether parvalbumin-immunoreactive neurons correspond to GABA-accumulating striatal interneurons remains to be determined. In the substantia nigra, parvalbumin is contained in SNr neurons. As in the striatum, there is a gradient of density of neurons expressing parvalbumin, which increases from medial to lateral. Not only are CaBP and parvalbumin contained in different striatal and nigral cells, but their distribution gradients are also complementary in these structures.

No particular morphologically or biochemically defined neuronal cell type appears to exclusively contain either parvalbumin or CaBP. In the basal ganglia, parvalbumin is colocalized in some but not all GABAergic neurons, being absent in the striatonigral GABAergic system (36) and in some nigral GABAergic neurons. In other brain areas including the cortex, hippocampus, and thalamic reticular nucleus, parvalbumin immunoreactivity is contained in neurons that most likely are GABAergic, but again it does not label the entire population of such neurons (ref. 4; unpublished observations). There is no clear association of CaBP with any particular neurotransmitter. As shown in the substantia nigra, CaBP is localized in both dopaminergic and nondopaminergic neurons, and in the striatum it is localized in neurons that express a number of different peptides or transmitters. In the basal ganglia, parvalbumin and CaBP demonstrate a complementary localization, whereas in the cerebellum both proteins are present in Purkinje cells (1, 4). Despite the diversity of cell types that contain these proteins, the distinct distributions of parvalbumin and, particularly, CaBP in discrete compartmental systems of the basal ganglia suggest a possible role related to the physiologic properties of these neurons.

Both CaBP and parvalbumin are notable for their ability to bind calcium in the micromolar range. This capacity has led to the suggestion that CaBP may act as an intraneuronal buffering system for calcium ions (1), and a similar role for parvalbumin in muscle has been suggested (6). Furthermore, in muscle, parvalbumin is localized selectively in fast twitch fibers (7) and is thought to contribute to the fast relaxation response properties of these fibers (5). A similar role in the brain for parvalbumin and, perhaps, CaBP as well may be related to the physiologic response properties of the neurons in which they are contained. The present results suggest the intriguing possibility that CaBP-containing striatonigral matrix neurons, which are shown to have distinct connections, may also have physiologic characteristics that distinguish them from their patch counterparts.

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1. Baimbridge, K. G., Miller, J. J. & Parkes, C. O. (1982) *Brain Res.* **239**, 519–525.
2. Baimbridge, K. G. & Miller, J. J. (1982) *Brain Res.* **245**, 223–229.
3. Wasserman, R. H. & Taylor, A. N. (1966) *Science* **156**, 791–793.
4. Celio, M. R. & Heizmann, C. W. (1981) *Nature (London)* **293**, 300–302.
5. Heizmann, C. W. (1984) *Experientia* **40**, 910–921.
6. Pechere, J.-F., Derancourt, J. & Haiech, J. (1977) *FEBS Lett.* **75**, 111–114.
7. Celio, M. R. & Heizmann, C. W. (1982) *Nature (London)* **297**, 504–506.
8. Pert, C. B., Kuhar, M. J. & Snyder, S. H. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 3729–3733.
9. Herkenham, M. & Pert, C. B. (1981) *Nature (London)* **291**, 415–418.
10. Graybiel, A. M. & Ragsdale, C. W., Jr. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 5723–5726.
11. Gerfen, C. R. (1984) *Nature (London)* **311**, 461–464.
12. Gerfen, C. R. (1985) *J. Comp. Neurol.* **236**, 454–476.
13. Donoghue, J. P. & Herkenham, M. (1983) *Soc. Neurosci. Abstr.* **9**, 15 (abstr.).
14. Ragsdale, C. W., Jr., & Graybiel, A. M. (1981) *Brain Res.* **208**, 259–266.
15. Goldman-Rakic, P. S. (1982) *J. Comp. Neurol.* **205**, 398–413.
16. Herkenham, M., Moon Edley, S. & Stuart, J. (1984) *Neuroscience* **11**, 561–593.
17. Wright, A. K. & Arbuthnott, G. W. (1981) *Neuroscience* **6**, 2063–2067.
18. Gerfen, C. R. (1984) *Soc. Neurosci. Abstr.* **10**, 9 (abstr.).
19. Herkenham, M. & Pert, C. B. (1982) *J. Neurosci.* **2**, 1129–1149.
20. Morrison, J. H., Benoit, R., Magistretti, P. J., Ling, N. & Bloom, F. E. (1983) *Brain Res.* **262**, 344–351.
21. Hsu, S. M., Raine, L. & Fanger, H. (1981) *J. Histochem. Cytochem.* **29**, 577–580.
22. Aruilson, M., Dietl, M. & Thibault, J. (1984) *Brain Res. Bull.* **13**, 269–285.
23. Sawchenko, P. E. & Swanson, L. W. (1981) *Brain Res.* **210**, 31–51.
24. Oertel, W. H., Mugnaini, E., Nitsch, C., Schmechel, D. E. & Kopin, I. J. (1982) *Brain Res. Bull.* **9**, 463–474.
25. Kohno, J., Shiosaka, S., Shinoda, K., Inagaki, S. & Tohyama, M. (1984) *Brain Res.* **308**, 309–317.
26. Graybiel, A. M. & Chesselet, M. F. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 7980–7984.
27. Inagaki, S. & Parent, A. (1984) *Brain Res. Bull.* **13**, 319–329.
28. Gerfen, C. R., Staines, W. A., Arbuthnott, G. W. & Fibiger, H. C. (1982) *J. Comp. Neurol.* **207**, 283–303.
29. Aronin, N., DiFiglia, M., Graveland, G. A., Schwartz, W. J. & Wu, J.-Y. (1984) *Brain Res.* **300**, 376–380.
30. Vincent, S., Hökfelt, T., Christensson, I. & Terenius, L. (1982) *Eur. J. Pharmacol.* **85**, 251–252.
31. Kitai, S. T., Wagner, A., Precht, W. & Ohno, T. (1975) *Brain Res.* **85**, 44–48.
32. Bolam, J. P., Wainer, B. H. & Smith, A. D. (1984) *Neuroscience* **12**, 711–718.
33. DiFiglia, M. & Aronin, N. (1982) *J. Neurosci.* **2**, 1267–1274.
34. Takagi, H., Somogyi, P., Somogyi, J. & Smith, A. D. (1983) *J. Comp. Neurol.* **214**, 1–16.
35. Bolam, J. P., Clarke, D. J., Smith, A. D. & Somogyi, P. (1983) *J. Comp. Neurol.* **213**, 121–134.
36. Staines, W. A., Nagy, J. I., Vincent, S. R. & Fibiger, H. C. (1980) *Brain Res.* **194**, 391–402.