Neurotransmitters in the Mammalian Striatum: Neuronal Circuits and Heterogeneity

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ABSTRACT: The major input and output pathways of the mammalian striatum have been well established. Recent studies have identified a number of neurotransmitters used by these pathways as well as by striatal interneurons, and have begun to unravel their synaptic connections. The major output neurons have been identified as medium spiny neurons which contain γ-aminobutyric acid (GABA), endogeneous opioids, and substance P. These neurons project to the pallidum and substantia nigra in a topographic and probably chemically organized manner. The major striatal afferents from the cerebral cortex, thalamus, and substantia nigra terminate, at least in part, on these striatal projection neurons. Striatal interneurons contain acetylcholine, GABA, and somatostatin plus neuropeptide Y, and appear to synapse on striatal projection neurons. In recent years, much activity has been directed to the neurochemical and hodological heterogeneities which occur at a macroscopic level in the striatum. This has led to the concept of a patch-matrix organization in the striatum.

The striatum contains a variety of neurotransmitters, some of which have been associated with neurological disorders including Parkinson’s and Huntington’s diseases. The purpose of the present review is to provide an updated, brief summary of the recent advances in our knowledge of the biochemical anatomy of the striatum, with particular emphasis on: 1) the synaptic connections among striatal afferents, projection neurons, and interneurons, and 2) the neurochemical and hodological heterogeneities in the striatum. The reader is referred to the following reviews for more comprehensive treatment of the literature on the anatomy and neurochemistry of the striatum.1-6

1. STRIATAL INPUTS

The cortical afferents

The major striatal input arises in the cerebral cortex which projects topographically to the striatum. At a gross level, the putamen receives primarily sensorimotor information, while the caudate nucleus receives major inputs from the limbic and associational cortical areas. The corticostriatal input has been suggested to arise from both supragranular and infragranular cortical layers, and some corticostriatal cells may also project to other subcortical regions.7-9 Although most of the corticostriatal...
projection arises from the ipsilateral cortex, as much as a third of the cortical projections to the striatum arises in the opposite hemisphere. The corticostriatal afferents terminate principally in asymmetric contacts on the spines of the medium spiny striatal neurons. There is much circumstantial evidence to suggest that the corticostriatal fibers use an excitatory amino acid, perhaps glutamate, as a transmitter.

The thalamic afferents

The second major striatal input arises in the thalamus. Although the major sources are the intralaminar nuclei, some striatal afferents also arise in "specific" thalamic nuclei including the ventral anterior, ventral lateral, lateral posterior and supra-geniculate nuclei. As with the corticostriatal projection, the thalamostriatal pathway is topographically organized. Some of the thalamostriatal fibers send collaterals to the cerebral cortex. Again, like the cortical afferents, terminals arising from thalamic neurons appear to form primarily asymmetric contacts with the dendritic spines of the medium spiny neurons.

Although the thalamostriatal projection appears to be excitatory, the transmitters contained in this pathway are not known. There was a suggestion that this pathway is cholinergic, but this does not appear to be the case. Some neuropeptides have recently been detected in thalamostriatal neurons. Cells in the centromedian-parafascicular complex in the cat projecting to the caudate nucleus have been shown to contain substance P, vasoactive intestinal polypeptide (VIP)-, cholecystokinin (CCK)- and neurotensin-immunoreactivities. Some of the enkephalin in the cat striatum could also arise in the intralaminar thalamus where met-enkephalin-immunoreactive cell bodies are present.

The nigral afferents

The well known nigrostriatal dopaminergic projection provides a third major input to the striatum. In addition, a small non-dopaminergic projection from the substantia nigra also appears to exist. The nigrostriatal projection is topographically organized (e.g.,). The dopaminergic terminals tend to form symmetric contacts with dendrites and with the spines of the medium spiny neurons. Many of these spines also receive asymmetric inputs, probably arising in the cortex and thalamus. Thus dopaminergic terminals may be well placed to modulate the actions of other inputs on the striatal neurons.

Some nigrostriatal dopaminergic neurons also contain neuropeptides. CCK-immunoreactivity is found in many of the ventral tegmental dopamine neurons projecting to ventral striatal regions including the nucleus accumbens and olfactory tubercle. Neurotensin may also be present in some of these neurons.

Other inputs

The striatum receives numerous other projections. While a serotonin afferent from the dorsal raphe has been well documented, many of the afferents from the raphe area are dopaminergic. Histaminergic neurons in the posterior hypothalamus project to many areas including the striatum. A pallidostriatal projection has been recently discovered as has an input from the subthalamic nucleus. A fairly large input from the amygdala has been noted. CCK immunoreactivity may be present in striatal afferents originating in the basolateral amygdala, claustrum and piriform cortex. Other minor projections from brainstem areas such as the locus ceruleus (noradrenergic) and the pedunculopontine nucleus (cholinergic) require further confirmation.

2. Striatal Outputs

Evidence from biochemical experiments following various lesions has indicated that there is a population of γ-aminobutyric acid (GABA) neurons in the striatum. These studies also indicate that striatal GABA neurons project to the pallidum and substantia nigra. The development of antibodies to the GABA synthesizing enzyme, glutamate decarboxylase (GAD), has permitted the morphological analysis of striatal GABA neurons. Bolam et al. have found that neurons which accumulate [3H]GABA or display GAD immunoreactivity are not medium spiny neurons. Rather, these cells have the morphological features of a type of medium aspiny cell. This morphology is similar to those of GAD-positive cells seen previously by Ribak et al. in the rat striatum, and by Panula et al. in cultures of rat striatum. Bolam et al. suggest that these cells might be striatal GABA interneurons, and that the striatonigral GABA projection neurons may not be labelled by the GABA uptake technique, possibly because of insufficient local axon collaterals. Similarly, the striatonigral GABA neurons may not be readily detected with GAD antisera if most of their GAD is rapidly transported out of the striatum to the pallidum and nigra. Recent studies using a different antisem have demonstrated GAD immunoreactivity in two populations of rat striatal neurons, a small population of medium to large neurons which was detected in normal animals, and a larger population of medium-sized neurons that was detected after colchicine treatment. In the cat, GAD immunoreactivity has been demonstrated in retrogradely labelled medium-sized spiny striatonigral neurons. These cells received symmetric GAD-immunoreactive axosomatic and axodendritic contacts, plus many asymmetric non-immunoreactive contacts on their soma, dendrites and spines.

Medium-spiny striatonigral neurons have also been labelled with tritiated taurine. This raises the possibility that taurine and GABA might coexist in some of these cells. In addition, many of the medium-spiny striatal projection neurons displaying GAD immunoreactivity have been found to contain leu- or met-enkephalin. Hökfelt et al. first described the presence of enkephalin-immunoreactive neurons in the rat striatum. These cells appear to project massively upon the globus pallidus. Enkephalin-immunoreactive neurons have also been observed in the cat and primate striatum.

Pickel et al. have found that medium spiny neurons in the rat striatum contain enkephalin-immunoreactivity. Kubota et al. detected axosomatic symmetrical tyrosine hydroxylase (TH)-immunoreactive contacts on enkephalin-immunoreactive medium spiny striatal neurons. Striatal enkephalin terminals form symmetric contacts with dendrites of medium spiny neurons that also receive asymmetric contacts from cortical afferents. Some investigators have reported the presence of a few asymmetric axosomatic contacts made by leu-enkephalin-immunoreactive boutons. Axosomatic contacts between cortical afferents and enkephalin terminals may also occur in the rat striatum.
In the cat, about half of the medium-sized spiny enkephalin-immunoreactive neurons contain neurotensin immunoreactivity, and half the neurotensin-immunoreactive neurons display enkephalin immunoreactivity. 63 Dynorphin-immunoreactive neurons have been detected in the rat striatum, 73 and these also appear to project to the substantia nigra. 74

Substance P-immunoreactive neurons are also found in the striatum. 75-78 In fact, substance P coexists in many GAD- and enkephalin-immunoreactive neurons in the rat and cat striatum. 60 In an ultrastructural study of the rat striatum, Bolam et al. 48 identified two types of substance P-immunoreactive neurons. One appears to correspond to the medium spiny neuron, while the other appears to be an aspiny medium-sized neuron, probably distinct from the aspiny GABA or somatostatin neurons. 48 Substance P-immunoreactive boutons appear to form symmetric synaptic contacts usually on dendrites or spines of what are perhaps medium spiny neurons. 48 The morphology of these contacts is similar to that of substance P terminals in the substantia nigra, and may thus derive from collaterals of striatonigral substance P neurons. 71,79

Although substance P, enkephalin and dynorphin are found in medium spiny neurons together with GAD, the extent to which these substances coexist in the striatal efferents is not known. Dense terminal staining for enkephalin is present in the external segment of the globus pallidus, while for substance P the densest terminal fields are present in the internal pallidal segment and substantia nigra. 78,80,81 These observations suggest a chemical coding of the striatal GABA efferents, with those projecting to the globus pallidus containing predominantly enkephalin, and those projecting to the entopeduncular nucleus and substantia nigra containing mainly substance P.

3. STRIATAL INTERNEURONS

It is clear from numerous immunohistochemical studies in various species that the cholinergic neurons of the striatum correspond to the large aspiny neurons described in Golgi studies. This was originally proposed by Lehmann et al. 82 on the basis of pharmacohistochemical studies of acetylcholinesterase (AChE). Kimura et al. 83 subsequently demonstrated that the large striatal cells in the rat and guinea pig did in fact display choline acetyltransferase (ChAT) immunoreactivity. This has since been confirmed in various species including rat, cat, 84-86 and primate. 87 The ultrastructure of these neurons has been examined in the rat using both AChE histochemistry, 88,89 and ChAT immunohistochemistry. 89,90 Although often referred to as the large or giant aspiny neuron, the soma and dendrites of this cell type are often sparsely spiny. These neurons receive rare symmetric axosomatic and axodendritic contacts, 85,86,90 plus some asymmetric contacts. 89,90 In addition, the axonal initial segments of these neurons appear to receive symmetric synapses. 90 Bolam et al. 91 have recently demonstrated that the cell bodies and proximal dendrites of striatal cholinergic neurons in the rat receive symmetrical contacts from substance P-immunoreactive boutons. The cholinergic terminals make symmetric contacts with somata, dendrites and axon initial segments of what appear to be medium spiny neurons. 90,92

In addition to the giant aspiny cholinergic neurons, other smaller aspiny interneurons are present in the striatum. Light and electron microscopic immunohistochemical studies have indicated that somatostatin is contained in one such population. 93-95 Another peptide, neuropeptide Y (NPY) is present in the striatal somatostatin neurons, 95,96 which are also characterized by the presence of NADPH-diaphorase activity. 97,98 These striatal neurons receive only a few symmetric and asymmetric inputs to their soma and proximal dendrites, 99,101 while the distal dendrites usually have asymmetric contacts. 99,100 Somatostatin-immunoreactive boutons form symmetrical contacts with dendrites and spines. 99,100 The spines receiving somatostatin-immunoreactive input also receive other asymmetric contacts. 100

Although other neuropeptides have been noted in striatal neurons, these have not been analysed in detail. CCK-immunoreactivity is found in a small population of medium aspiny neurons. 102 A few neurons containing VIP 103 and galanin 104 may also be present.

Although important questions regarding the organization of the striatum remain, it may be helpful to summarize our current knowledge of the basic striatal circuit as follows: the major functional unit of the striatum appears to be the medium spiny neuron. These cells receive the major inputs from cortex, thalamus and substantia nigra, and supply the major output to the pallidum and substantia nigra. The cholinergic, GABAergic, and somatostatin/NPY-containing interneurons could thus act to modulate the activity of the medium spiny neurons. Major questions that are still unanswered include: 1) Do the striatal interneurons receive cortical, thalamic or nigral input to their distal dendrites? 2) Do all medium spiny neurons receive similar inputs? 3) What are the connections of the aspiny striatonigral neurons, and the aspiny GABA interneurons? 4) How is this striatal circuitry accommodated in the plan of the regional heterogeneity that is now becoming apparent in the striatum (see below)?

4. HETEROGENEITY IN THE STRIATUM

Studies over the past decade have revealed that the striatum displays considerable heterogeneity with respect to cytoarchitecture 105-107 and, in particular, the distribution of various neurotransmitter-related markers, and afferent and efferent connections. In addition to regional differences, there is a mosaic pattern in which the presence or absence of a given anatomical marker is localized to “patches” against the background or “matrix”. Moreover, the “patches” revealed by different markers do not appear to be independent from each other, but display varying degrees of correspondence. These observations have led to the suggestion that this mosaic pattern may represent the basic organizational plan of compartmentalization in the mammalian striatum.

As intriguing as the concept is, there are limitations in the current data that suggest a striatal patch-matrix organization. The concept is at present based entirely on qualitative observations. The patch and matrix compartments have been commonly defined by either opiate receptor binding, neuropeptide immunoreactivity, or AChE staining, and increasingly these are being assumed to demarcate identical regions. Until these matches are firmly established in quantitative terms, the results of the studies using different markers to define the patch and matrix compartments may not be directly comparable. The observations reviewed below should be considered with these caveats in mind.
Neurochemistry

Early reports on macroscopic heterogeneity in the striatum were made with AChE staining in cat, monkey, and human, and opiate receptor binding with \(^{1}H\)-diprenorphine in the rat. AChE histochemistry reveals occasional zones, about 0.5 mm in width, of pale staining. These AChE-poor zones, termed "striosomes", were subsequently reported to match the "islands" of opiate receptors revealed by \(^{1}H\)-naloxone binding in the rat striatum, as well as the compartments of high met-enkephalin-like and dynorphin-B-like immunoreactivity in the dorsal striatum of cats and kittens.

Neurotensin-positive neuropil also appears to be in register with enkephalin-like immunoreactive neuropil and AChE-poor zones in the cat. This is consistent with the coexistence of these two peptides. However, unlike opiates and opiate receptors, the distributions of neurotensin and its receptors do not appear to be coincident; high densities of neurotensin receptors are found in the opiate-poor, AChE-rich matrix.

Substance P immunoreactive neuropil is largely confined to AChE-poor striosomes in rat and cat. Substance P-positive perikarya are seen more frequently in the patches, defined by dense substance P neuropil, than in the matrix in rat, baboon, and human striatum.

Somatostatin-immunoreactive fibers have been reported to be dense in the matrix defined by the absence of opiate receptor binding, substance P, or enkephalin immunostaining in rat and cat. The distribution of NPY is heterogeneous, with patchy zones of weak immunoreactivity in the cat, whereas it is homogeneous in the monkey. Dense NADPH-diaphorase staining appears to be in register with the AChE-rich matrix in the cat. Somatostatin-immunoreactive cell bodies are found in both patches and matrix in the rat, although their processes are seen mostly in the matrix.

The distribution of cholinergic neurons has been reported to be homogeneous. More recently, using an antiserum to ChAT combined with AChE staining in cat and monkey, Graybiel et al. have reported that ChAT-positive neuropil is confined to AChE-rich matrix zones. Concentrations of muscarinic receptors revealed by \(^{1}H\)-propylbenzilylcholine mustard in the cat appear to correspond to AChE-poor patches in the dorsal striatum. However, a homogeneous density was seen with \(^{1}H\)-quinuclidinyl benzilate.

Neither dopamine fluorescence nor TH immunoreactivity displays obvious heterogeneity in adult animals, although they form "islands" in immature animals (see below). A heterogeneity has been reported to be detected in adult animals by quantitative analyses of TH immunohistochemistry. The patch pattern of TH immunoreactivity can also be "unmasked" in adult rats by pretreatment with a TH inhibitor. In the human striatum, the density of D2 receptors is high in AChE-rich matrix regions.

Connections

The mosaic patterns seen with various neurochemical markers described above have been reported to be superimposed, to a considerable extent, on the terminal patterns of various afferents and the distributions of projection neurons. Heterogeneous patterns of terminations described as "patchy" were noted in autoradiographic studies of corticostriatal and thalamostriatal projections. Recently, Donoghue and Herkenham have shown in adult rats that prelimbic frontal cortical afferents tend to terminate in opiate receptor dense patches, and the afferents from the somatosensory, visual, motor, and cingulate cortices terminate in the matrix. The termination of the afferents from the medial prefrontal (or prelimbic) cortex in the patches defined by the absence of somatostatin neuropil has also been reported in the rat. In adult cat and monkey, Ragsdale and Graybiel have reported that in the dorsal half of the caudate nucleus, the presence of afferent terminals from the frontal cortex matches with AChE-poor striosomes, whereas in the ventral half, the absence of afferent terminals tends to be in register with the striosomes. Variance terminals of afferents from the parafascicular thalamic nucleus are found to distribute heterogeneously in the cat, outside of opiate receptor dense islands, and within the AChE-rich matrix in the rat. Amygdalostrial projections have been reported to terminate in patchy patterns in the monkey.

The nigral afferents also appear to have a heterogeneous distribution of terminal labelling in the striatum of the rat and cat. The projection from the ventral tegmental area has been reported to terminate predominantly in the matrix of the ventral striatum, including the nucleus accumbens, in the rat. More recently in a systematic study of the projection to the striatum from the ventral tegmental area (A10), substantia nigra (A9), and retrorubral area (A8), Gerfen et al. have reported that dopaminergic fibers from the ventral part of the substantia nigra pars compacta and the ventral tier of the pars reticulata (displaced A9 cells) terminate in the opiate receptor-dense patches, whereas both dopaminergic and non-dopaminergic afferents from all the other areas terminate outside of the patches. In addition, those dopaminergic neurons giving rise to the afferents to the matrix, but not those innervating the patches, contain a calcium binding protein, and appear to develop later than those without this protein.

A mosaic pattern is also seen in efferent projections of the striatum. Graybiel et al. have reported that projection neurons (mostly medium-sized) retrogradely labelled following HRP injections into the globus pallidus and substantia nigra are found largely in the AChE-rich matrix compartment in the cat. In the rat, Gerfen has reported that striatal neurons projecting to the substantia nigra pars compacta are located in somatostatin-poor patches, whereas those projecting to the pars reticulata are located in the matrix.

The above findings suggest that the striatum may be segregated in a mosaic manner into two compartments which represent two separate input-output channels. The patch compartment receives a major input from the prefrontal cortex and its output is directed to the substantia nigra pars compacta. The matrix compartment receives major afferents from the sensory and motor cortices and the centromedian-parafascicular complex of the thalamus, and directs its output to the substantia nigra pars reticulata.

Development

The heterogeneity of some striatal neurochemical markers develops during embryonic development and is already present at birth. These include enkephalin neuropil in the cat, and opiate receptor binding, muncarinic receptors, neurotensin, and neurotensin receptors in the rat. However, there are also markers whose distributions change during development. For example, AChE staining reveals dense patches, rather than pale patches as seen in adults, in the striatum of...
neonatal rats, fetal and neonatal cats, and human fetus and young infants. Dopamine fluorescence, which is homogeneous in adult rats, begins to display heterogeneity on embryonic day 19; this develops into conspicuous “islands” by the time of birth, but then gradually fades by postnatal day 16. Lança et al. have shown that the ratio of the opiate receptor-dense patches to the total striatal area peaks on postnatal day 7.

In neonatal and fetal cats, the AChE-rich patches correspond to dopamine islands, as well as to the patches of enkephalin- and, to some extent, substance P-positive neuropil. Matching of dopamine islands and dense AChE staining is not surprising because most of the AChE seen early during development is probably contained within dopaminergic fibers from the substantia nigra. It is, however, not clear how the reversal of the AChE staining pattern occurs during development.

In the cat, using [3H]-thymidine autoradiography, Graybiel and Hickey have shown that neurons which became postmitotic around embryonic day 24 to 30 (the gestation period of the cat is 65-68 days) tend to form clusters which were superimposed on AChE-poor striosomes and enkephalin-rich compartments. On the basis of [3H]-thymidine-dense patches as a marker, Nastuk and Graybiel suggested that the AChE-dense patches seen in neonatal cats are the precursor of the AChE-poor striosomes seen in adults. In the rat, van der Kooy and Fishell have reported that neurons which become postmitotic earliest (embryonic day 13-15) are located in the patches defined by opiate receptor binding, whereas those cells leaving the mitotic cycle later (embryonic day 18-20) are found in the matrix. Similar observations have been made by Marchand and Lajoie.

Although the mechanisms of the formation of patch-matrix compartments are unknown, Lança et al. suggested that the striatal connections with the brainstem are important in the formation and/or maintenance of the matrix-patch compartments (see also).

**Conclusions**

The mammalian striatum, particularly its dorsal part, appears to be segregated into two neurochemically and hodologically separate compartments: patches and matrix. The two compartments are organized in a mosaic pattern in which the patch compartment forms a labyrinth through the matrix compartment, giving the appearance of Swiss cheese. Although the functions of the two compartments need to be examined by physiological techniques, this mosaic structure raises the possibility of parallel information processing through two anatomically segregated input-output channels. Similar compartmentalization of functionally related neurons has been seen in ocular dominance columns and vibrissal barrels in the cerebral cortex.

Historically, the concept of the patch-matrix organization began with a few early independent observations of macroscopic heterogeneity in histochemical staining and connections. These initial observations have been extended, with the aid of more recently developed anatomical tools, and integrated into a novel concept of striatal organization. The underlying hypothesis which has stimulated all these studies has been that the striatal heterogeneities reflect anatomically and functionally segregated compartments. As previously stated, the hypothesis has so far been based entirely on qualitative observations, and there is a need to determine the degree of matching in quantitative terms. Quantitative tests should include an examination of the extent of matching among different neurochemical/hodological labelling conducted on the same or alternate sections. Such examinations would provide a foundation on which studies using different markers to define the patch and matrix compartments become mutually comparable, and also may indicate the “best” marker to use in future studies.

Attention also should be paid to the fact that the mosaic pattern may not be evident in all regions of the striatum. Heimer and Wilson have proposed that the striatum consists of two subregions: the dorsal, non-limbic part, and the ventral, limbic part. At present it appears that the most conspicuous patches as well as the most consistent matching between different markers occur in the dorsal striatum. In the ventral striatum, patches are less obvious and the matching is either less convincing, absent, or sometimes, reversed. One explanation for such regional differences is that some peptides are contained in afferents which terminate heterogeneously in the striatum.

Although the segregation of patches and matrix in the dorsal striatum seems relatively convincing at the macroscopic level, the information at the cellular level remains limited to some preliminary data with projection neurons and somatostatin-containing interneurons. Gerfen has reported that striatal neurons retrogradely labelled with fluorescent tracers have dendrites mostly confined to the somatostatin-dense compartment containing their cell bodies. Consistent results have been reported for medium spiny neurons in an abstract by Penny et al. using the intracellular HRP technique, which can reveal more extensive dendritic fields than retrograde labelling.

The issue of somatostatin-containing neurons as link neurons which connect the patch and the matrix compartments requires further clarification. Gerfen has reported that, although somatostatin-immunoreactive cell bodies are found in both matrix and patches, axons of these neurons in patches extend into surrounding matrix in rat. This has led Gerfen to suggest that somatostatin-containing neurons may play a role as a link from the patches to the matrix. Similar observations have been made in the cat. However, these authors considered it unlikely that a major function of somatostatin neurons is linking, because cross-compartmental somatostatin fibers do not occur frequently. The question of possible link neurons, as well as the dendritic morphology of striatal neurons, in general, in relation to the patch-matrix organization might be better addressed by combining intracellular injection techniques with immunohistochemistry.

**Acknowledgements**

Supported by grants from the Medical Research Council of Canada, and the British Columbia Health Care Research Foundation.

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