Synaptic endings and their postsynaptic targets in neostriatum: Synaptic specializations revealed from analysis of serial sections

(dopamine/5-hydroxydopamine/rats/basal ganglia)

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ABSTRACT Neostriatal tissue from rat brain was examined in ribbons of serial sections, using tissue from subjects in which the synaptic marker 5-hydroxydopamine had been injected into the lateral ventricle. A total of 440 synaptic terminals, identified by the presence of a population of vesicles, was studied, including those labeled by 5-hydroxydopamine and all those unlabeled in the surrounding neuropil. Three categories of synaptic profiles innervating neostriatal dendrites could be discerned. One category contained small rounded or slightly pleomorphic vesicles and consisted of a sample of 375 endings, 53 of which exhibited light to heavy label inside the synaptic vesicles. All of these, labeled and unlabeled, showed evidence of membrane specializations and other conventional criteria of synaptic contact, mostly in the form of asymmetric synaptic contacts with the heads of dendritic spines. A second category of 40 terminals, 3 of which were lightly labeled, contained large rounded or slightly pleomorphic vesicles and made predominantly symmetric contacts with the shafts of dendritic spines or spine-free portions of dendritic membrane. None of these synaptic endings appeared to lack synaptic contact with postsynaptic targets in the neostriatum. A third category of 25 terminals, 3 of which were labeled, contained small flattened vesicles, and these endings also invariably made synaptic contact, mostly onto spine-free dendritic membrane, and were characterized by symmetric membrane thickenings at the point of apposition. Our evidence supports the view that dopaminergic and other synaptic terminals in the rat caudate-putamen make synaptic contact with postsynaptic targets in the neostriatum and, at least in the adult, do not exist in appreciable numbers in the form of terminals that are not apposed to membranes of postsynaptic targets in the neostriatum.

Recently, a number of investigators have suggested that monoaminergic synaptic endings in various central nervous system sites terminate without making synaptic contact. On the basis of analogies to characterizations of similar arrangements in the innervation of the hypophysis (see ref. 1 for review) and catecholaminergic innervation of certain peripheral targets (2), a lack of synaptic membrane specializations indicative of synaptic contact has been suggested to occur for the majority of serotonergic nerve endings innervating rat locus ceruleus (3, 4), dopaminergic terminals innervating the rabbit neostriatum (5), and serotonergic and noradrenergic nerve endings in cerebral cortex (6, 7) and elsewhere. Based almost entirely on the analysis of images contained within individual thin sections, this theory is buttressed by statistical analyses in which monoaminergic endings identified by various techniques are compared to synaptic profiles in the surrounding neuropil. The latter appear to be more often engaged in synaptic contact with postsynaptic targets, leading to the conclusion that a large number of monoaminergic nerve endings may lack synaptic specializations.

Although the absence of membrane specializations in indi-

vidual sections of nerve terminals could suggest that the entire vesicle-containing structure is without synaptic contact, the more conventional interpretation of such images is that the membrane specializations characteristic of synaptic apposition are out of the plane of the section. Indeed, when 191 synaptic endings of various categories in rat locus ceruleus were examined in their entirety in adjacent serial sections, every terminal swelling was characterized by membrane specializations typical of conventional criteria for synaptic apposition (8, 9).

In the present experiments, neostriatal tissue was examined in rats pretreated with the synaptic marker 5-hydroxydopamine, which is taken up by monoaminergic neurons to act as a false transmitter. It is precipitated by glutaraldehyde and subsequently reduces osmium tetroxide, forming an electron-dense reaction product in monoaminergic synaptic vesicles and other compartments of the monoaminergic neuronal cytoplasm (10, 11). Tissue was examined in ribbons of adjacent serial sections to determine if a population of synaptic endings could be found that did not make synaptic contact and to characterize the postsynaptic targets of nerve terminals in the rat neostriatum.

METHODS

Three male Sprague-Dawley rats weighing between 390 and 420 g were anesthetized with sodium pentobarbital supplemented with chloral hydrate, and a 32-gauge stainless steel infusion cannula connected by means of a Teflon tube to a Hamilton microsyringe was placed stereotaxically into the left lateral ventricle. Then 1.5 mg of the synaptic marker 5-hydroxydopamine hydrochloride (Aldrich), dissolved in 5 μ l of Krebs-Ringer solution containing L-ascorbate at 0.2 mg/ml, was injected over a period of 10–15 min. The cannula was then removed, the wound was closed, and the animals were maintained under light sodium pentobarbital anesthesia for a survival period of 1 hr.

After deep sodium pentobarbital anesthesia the animals were perfused intracardially, first briefly with oxygenated Krebs-Ringer solution, then for 5 min with a solution of 0.25% glutaraldehyde and 1% paraformaldehyde in 0.16 M sodium cacodylate buffer (pH 7.2 with CaCl_2 at 0.25 g/liter), and finally for 15 min with a solution of 1% glutaraldehyde and 4% (wt/vol) paraformaldehyde in the same buffer. The brain was removed and stored overnight in the latter fixative at 4°C. Tissue blocks from caudate-putamen were washed briefly in buffer and postfixed for 60 min in 1% OsO_4 in 0.16 M cacodylate buffer. They were then stained *en bloc* by immersion for 16 hr in 0.5% aqueous uranyl acetate, dehydrated with a graded acetone series, and embedded in Epon/Araldite.

Ribbons of up to 75 adjacent serial selections of caudate-

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putamen were cut from selected, trimmed blocks by using a Sorvall MT-2 Porter-Blum ultramicrotome. These were mounted on Formvar-coated slotted grids, stained with lead citrate, and examined with a JEOL 100C electron microscope at 80 kV. Three-dimensional reconstructions of selected endings were performed by a CDC 6400 computer, displayed on a Tektronix 4006-1 graphics terminal, and drawn by using a Tektronix digital plotter. The placement of the infusion cannula was verified in every case from 60- μ m frozen sections stained with cresyl violet.

RESULTS

Synaptic endings, defined simply by the presence of a population of vesicles, contained entirely within ribbons of serial sections were characterized according to the sizes and shapes of their vesicle populations, the presence or absence of membrane specializations characteristic of synaptic apposition, and other features. The postsynaptic targets of these endings were also classified according to whether they were composed of the heads or shafts of dendritic spines, or spine-free regions of dendrites. Axosomatic synapses were not analyzed. The results of this analysis are depicted in Table 1.

Three categories of synaptic profiles could be discerned on the basis of the sizes and shapes of their vesicle populations and their associated postsynaptic targets in neostriatum. The first and most numerous of these categories displayed small rounded or slightly pleomorphic vesicles engaged predominantly in synaptic contacts with the heads of dendritic spines having asymmetrical membrane specializations. A total sample of 375 synaptic endings of this category was analyzed in serial sections. The presence of light to heavy label, identified by small or large, sometimes eccentrically placed, dense cores inside the synaptic vesicles was noted in 53 of these endings. When these labeled and unlabeled synaptic endings were examined in serial sections, every terminal showed evidence of membrane specializations characteristic of synaptic contact, and in nearly all cases the postsynaptic site could also be identified unambiguously, as revealed in the summary in Table 1.

A perspective drawing of one of these endings, containing small, rounded synaptic vesicles with dense cores indicative of the presence of the synaptic marker, is shown in Fig. 1, derived from a computer-assisted, three-dimensional reconstruction. Individual micrographs show clearly the asymmetric synaptic contact between the terminal swelling and the head of a dendritic spine. The synaptic terminal originates from a fine preterminal axon and expands into the interstices of the neuropil. As shown in the two rotations of the three-dimensional reconstruction, the terminal makes synaptic contact only over a relatively small portion of its surface and a substantial portion of the ending is not directly apposed to the postsynaptic target.

A second category of 40 synaptic endings, 3 of which were lightly labeled, contained large rounded or slightly pleomorphic vesicles and made symmetric synaptic contacts with spine shafts and spine-free dendritic membrane. When these endings were examined in serial sections, all of them showed clear evidence of synaptic contact.

A third category of 25 terminal swellings, of which 3 were labeled, contained small flattened vesicles, and symmetrical membrane specializations characterized their synaptic contacts with spine shafts or spine-free dendritic membrane. All of these, too, showed evidence of synaptic contact characterized by membrane specializations and other conventional criteria.

DISCUSSION

The synaptic endings having small rounded or slightly pleomorphic vesicles appear to be those described by numerous previous authors as neostriatal afferents derived predominantly from cerebral cortex, thalamus, and substantia nigra (12-16). Endings of this category have also been suggested to include dopaminergic nerve endings from the pars compacta of the substantia nigra in the brainstem, with supporting evidence coming from a variety of methods, including anterograde degeneration techniques, autoradiography, 5-hydroxydopamine labeling, and others. Our results, showing that a substantial proportion of these endings exhibited the presence of the synaptic marker, are consistent with this view. The proportion of labeled endings is similar to that reported by others, using several techniques for identification, although our evidence does not exclude the possibility that the population of labeled endings of this category includes those from nondopaminergic afferents. However, when Tennyson *et al.* (5) examined 5-hydroxydopamine-labeled synaptic endings of this type in rabbit neostriatum, the vast majority were thought to end without making synaptic contact. Our evidence shows that this is not the case in rat neostriatum, and that all 375 of the synaptic endings of this category that were examined in their entirety in adjacent serial sections, labeled or unlabeled, made clear synaptic contact with postsynaptic targets in the neostriatum. Our results support the view that these endings engage in asymmetric synaptic contact predominantly with the heads of dendritic spines, which may be presumed to reside on the common medium-sized spiny cell of the neostriatum (17). The larger endings with large rounded or slightly pleomorphic vesicles have also recently been identified with axons of the common medium spiny cell (18).

When a total of 440 synapses was examined in serial sections, every synaptic ending showed evidence of membrane specializations and synaptic contact with postsynaptic targets in the neostriatum. A similar result was obtained when 191 synapses were examined in nucleus locus ceruleus, including a sample of endings labeled with 5-hydroxydopamine (8, 9). The

Table 1. Characteristics of axon terminals in rat neostriatum

Synapse type		Synaptic ending				Postsynaptic target			
Vesicles	Label	Number	Symmetrical	Asymmetrical	Oblique	Spine head	Spine shaft	Dendrite	Unclear
Small round	Unlabeled	322	5	288	29	285	11	17	9
	Labeled	53	3	45	5	46	4	2	1
Large round	Unlabeled	37	27	2	8	1	11	21	4
	Labeled	3	2	—	1	—	1	2	—
Small flattened	Unlabeled	22	20	1	1	1	3	18	—
	Labeled	3	1	1	1	1	—	1	1

Vesicle-containing axon terminals examined in serial sections are grouped into three categories, further categorized as those with and without 5-hydroxydopamine label. The total number in each category is indicated, whether the membrane specializations appeared to be symmetrical or asymmetrical or if they were sectioned obliquely. None of the terminals examined lacked membrane specializations characteristic of synaptic contact. The postsynaptic targets for each group are shown as the number engaged in contact with spine heads, spine shafts (necks), spine-free dendritic membrane, or as unclear, in which case the postsynaptic target could not be identified with certainty.

FIG. 1. Three sections from a series of 34 sections through a bouton containing small rounded vesicles heavily labeled by 5-hydroxydopamine are shown, indicating the presence of an asymmetrical synaptic contact with the head of a dendritic spine. Their approximate placements within the perspective drawing of a computer-assisted three-dimensional reconstruction of the entire synapse, including the postsynaptic target, are indicated. The reconstruction is shown to the lower right and a different rotation of approximately 180° around the horizontal axis is shown at the upper left.

lack of synaptic contact revealed in individual sections may simply indicate that the region of synaptic apposition is out of the plane of that section. Previous statistical comparisons between experimentally identified categories of monoaminergic synaptic endings and those in the surrounding neuropil may have been affected not only by the quality of preservation of the fine structure but also by many other factors, including the proportion of presynaptic membrane that is apposed to the postsynaptic site, the relative sizes of various categories of endings and their synaptic membrane specializations, and others. It appears from our evidence that, at least in locus ceruleus and neostriatum, where a nonsynaptic arrangement of monoaminergic endings has been proposed, such an arrange-

ment is only an appearance in the absence of analysis of serial sections through the entirety of the vesicle-containing structure.

Our results do not disprove theories suggesting release of neurotransmitters and other neurotropic factors from nonsynaptic regions of the nerve cell terminal, although we consider it more likely that monoamines may be released from sites of synaptic apposition and diffuse beyond the boundary of the region of membrane apposition at the synapse to act on receptors on nonjunctional neuronal membrane.

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