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LOCALIZATION OF AMPA-SELECTIVE EXCITATORY AMINO ACID RECEPTOR SUBUNITS IN IDENTIFIED POPULATIONS OF STRIATAL NEURONS

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Abstract—Two-color immunofluorescence histochemistry and immunohistochemistry in combination with retrograde tract-tracing techniques were used to examine the relationship of α-amino-3-hydroxy-5-methyl-4-isoxazole proprionic acid (AMPA)-selective glutamate receptor subunits (GluR1, GluR2/3/4c and GluR4) to identified populations of striatal projection neurons and interneurons. The majority of striatonigral and striatopallidal neurons were double-labeled for GluR2/3/4c. These findings were confirmed using calbindin to label matrix projection neurons. In contrast, immunostaining of the GluR1 subunit was not observed to co-localize with any striatal projection neurons. Striatal interneurons immunostained for parvalbumin were also labeled by antibodies directed against the GluR1 subunit. Approximately 50% of parvalbumin neurons also contained GluR2/3/4c. Somatostatin immunoreactivity did not co-localize with either the GluR1 or GluR2/3/4c subunits. GluR4-immunoreactive neurons were not observed in striatum.

This study demonstrates that AMPA-selective glutamate receptors are differentially localized on subpopulations of striatal neurons and interneurons. These findings suggest that discrete striatal neuron populations may express different AMPA receptor subunit combinations which may account for their functional specificity.

The striatum is the major afferent component of the basal ganglia and receives as its main input a massive excitatory amino acidergic (EAAergic) projection from the neocortex and allied areas. Corticostriatal inputs are topographically organized based on regional and laminar origin and by site of termination.[25,43] However, the relationship between EAAergic input to specific pools of striatal projection neurons and interneurons is not completely understood. Organization of the striatum is based on segregation of input systems, segregation of output systems and compartmentalization of striatal neurons (see Ref. 25 for review). It has been postulated that changes in the specific function of subpopulations of striatal neurons may account for the variety of clinical manifestations associated with basal ganglia disease.[2] The majority (90–95%) of striatal neurons are medium spiny GABAergic output neurons, which are segregated into "patch" (striosome) and "matrix" compartments. In the rat, the smaller patch compartment is enriched in μ-opiate receptors[46] and 5′-nucleotidase.[49] Neurochemical markers distributed preferentially within the matrix include acetylcholinesterase, tyrosine hydroxylase, calbindin-D_{28k}, somatostatin and cytochrome oxidase.[25] Several populations of striatal interneurons have been classified based on morphology and the differential expression of neuractive substances, including large, aspiny cholinergic interneurons, medium aspiny interneurons expressing both parvalbumin and GABA, and interneurons co-containing somatostatin, neuropeptide Y and NADPH-diaphorase, a nitric oxide synthase. Rodent striatal output neurons are segregated into two main populations on the basis of their target structure, substantia nigra and globus pallidus. Striatonigral neurons are further characterized by their expression of substance P and dynorphin, whereas striatopallidal neurons are distinguished by expression of the peptide enkephalin.

Differential distribution of receptors among striatal projection neurons and interneurons may play a role in the regulation of striatal function. Segregation of receptors in striatum is known to occur, although the extent to which segregation is maintained, particularly with regard to dopamine receptors, is controversial.[52] Striatonigral neurons have been described as enriched in D_1 dopamine[29] and *N*-methyl-D-aspartate (NMDA) receptors,[53] whereas striatopallidal neurons have been shown to exhibit enrichment in D_2 dopamine[30] and A_{2a} adenosine receptors.[48] Autoradiographic and immunohistochemical studies have demonstrated differences in EAA receptors between striosome and matrix

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Abbreviations: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole proprionic acid; DL dorsolateral striatum; DM, dorsomedial striatum; EAA, excitatory amino acid; FG, Fluoro-Gold; GluR*x*, glutamate receptor, subunit *x*; NADPH, reduced nicotinamide adenine dinucleotide phosphate; NMDA, *N*-methyl-D-aspartate; PB, phosphate buffer; VL, ventrolateral striatum; VM, ventromedial striatum.

compartments in human[18] and monkey[41] striatum. Subpopulations of striatal projection neurons and interneurons have been shown to have a differential expression of muscarinic cholinergic receptor subtypes.[8]

Four major subtypes of functional EAA receptors have been identified; NMDA, α-amino-3-hydroxy-5-methyl-4-isoxazole proprionic acid (AMPA), metabotropic quisqualate and kainate receptors. Recently, a new "delta" class of EAA receptor has been identified, but no functional profile has been determined.[40] Molecular cloning strategies have revealed that multiple subunits of each receptor subtype appear to exist, including four major AMPA receptor subunits (GluR1–GluR4; see Ref. 23 for review). EAA receptor subtype subunits, like EAA receptor subtypes, show a differential distribution in brain, and the subunit configuration of ionotropic EAA receptor subtypes has been shown to influence their pharmacological and electrophysiological properties.[32,33,44,51] Since EAA receptor subunit configuration is likely to be an important factor in the mediation of striatal EAA neurotransmission, we examined the relationship of AMPA-selective EAA receptor subunits to identified populations of striatal projection neurons and interneurons using two-color immunofluorescence histochemistry and immunohistochemistry in combination with retrograde tract-tracing techniques.

EXPERIMENTAL PROCEDURES

Fluoro-Gold labeling

Eight male Sprague–Dawley rats (200–225 g; Spartan Labs, Hastings, MI) were anesthetized with ketamine–xylazine (10:3, 1 ml/kg, i.m.) and placed in a stereotaxic apparatus. Fluoro-Gold (FG; Fluorochrome, Inc., Englewood, CO; 0.2 μl of a 2% solution in 0.9% saline) was pressure injected into either substantia nigra or globus pallidus using coordinates taken from the atlas of Paxinos and Watson.[45] For substantia nigra, coordinates were: AP −5.4, ML +1.8, DV −8.2 from bregma. For globus pallidus, the needle was tilted 16° medially to avoid penetrating the striatum and coordinates were AP −1.0, ML +0.5, DV −7.0. Animals were allowed to survive for 12 days following FG injection.

Tissue processing

In all studies, the animals were deeply anesthetized with pentobarbitol (100 mg/kg, i.p.) and perfused through the ascending aorta with 100 ml of 0.1 M phosphate buffer (PB), followed by 400 ml of 2% paraformaldehyde in PB (pH 7.4). The brains were removed, blocked and placed in fresh fixative for 1–3 h. A modification of the method of Barthel and Raymond[3] was used to achieve cryoprotection adequate for cutting 4–6 μm cryostat sections. Briefly, the brains were transferred through a series of graded sucrose solutions made up in PB (5, 7.5, 12.5, 15 and 20%; rotating at 4°C for a minimum of 1 h at each step, or until the brains sank). The brains were placed in a 2:1 solution of 20% sucrose PB–Tissue-Tek OCT embedding compound (Miles, Kankakee, IL) for 45 min, transferred to embedding molds, surrounded by fresh PB:OCT, frozen over liquid nitrogen and stored at −70°C until processed for immunohistochemistry.

Immunohistochemistry

Eight FG-injected and four untreated brains were processed for one-color or two-color immunofluorescence histochemistry, respectively. All antibodies and fluorochromes were made up in 1% normal donkey serum–0.3% Triton X-100 in PB. Rinses were in PB (×3, 15 min each). For immunofluorescence staining of glutamate receptor subunits in FG-labeled tissue, 4 μm coronal sections were cut through the rostral striatum using a cryostat microtome. The tissue was rinsed and incubated in 4% normal donkey serum in 0.3% Triton X-100–PB (1 h, room temperature). Normal donkey serum was blotted off and affinity-purified antiserum was pipetted onto tissue sections (RH9, specific for GluR1, RH27, recognizing GluR2, 3 and 4c or RH25, specific for GluR4, gifts from Dr Richard Huganir, 0.5 μg/ml, overnight at 4°C). The tissue was rinsed and incubated in biotin-labeled donkey anti-rabbit immunoglobulin G (1:2000, 1 h, room temperature; Jackson ImmunoResearch, West Grove, PA). Sections were rinsed and incubated in cyanine 3.18 conjugated to streptavidin (1:300, 1 h, room temperature; Jackson ImmunoResearch). After three washes, the sections were coverslipped using a fade-retarding mounting medium.[34] For two-color immunofluorescence staining of glutamate receptor subunits and either somatostatin, calbindin or parvalbumin, 4 μm cryostat sections were processed according to the same protocol described above, except that (i) the primary antibody solution contained a mixture of rabbit anti-glutamate receptor antiserum (0.5 μg/ml) and either rat anti-somatostatin (1:50, Accurate Chemicals, Westbury, NY), mouse anti-calbindin (1:200, Sigma Chemical Co., St. Louis, MO) or mouse anti-parvalbumin (1:200, Sigma Chemical Co.), and (ii) the fluorochrome solution contained a mixture of cyanine 3.18 conjugated to streptavidin (1:300) and fluorescein-labeled donkey anti-rat or anti-mouse immunoglobulin G (1:200, Jackson ImmunoResearch).

Quantification

Sections were examined under epifluorescence illumination using a Nikon Microphot-SA microscope equipped with the following filters. FG: 330–380 nm excitor; 435 nm barrier filter. Cyanine 3.18: 510–560 nm excitor; 610 nm barrier filter. Fluorescein: 450–490 excitor; 520–560 nm barrier filter. For the purpose of analysis, the striatum was divided into four quadrants: dorsomedial (DM), dorsolateral (DL), ventromedial (VM) and ventrolateral (VL). The density of single- and double-labeled cells was determined using a grid reticle inserted in the eyepiece of the microscope and a ×20 objective. Matrix and patch compartments were distinguished by the presence or absence of calbindin immunoreactivity, respectively. Average densities for each experimental paradigm were established using measurements taken from four animals; from each rat, three sections were examined; for each area in a section, the density of single- and double-labeled cells per 0.17 mm^2 was established. In studies examining the density of AMPA receptor subtypes in patch and matrix compartments, the area of each compartment in a quadrant was often less than 0.17 mm^2 in a single section. In these instances, the density of fluorescently-labeled cells was determined by measuring the entire area of each compartment and recording the total number of labeled neurons. These numbers were then normalized to express the density as the number of single- and double-labeled neurons per 0.17 mm^2.

RESULTS

Fluoro-Gold tract tracing and glutamate receptor immunohistochemistry

Dense labeling of striatonigral neurons was observed unilaterally in all quadrants of the striatum

Table 1. Combined glutamate receptor immunohistochemistry and Fluoro-Gold labeling of striatal projection neurons

Striatal quadrant	+GluR-immunoreactive −FG	+GluR-immunoreactive +FG	−GluR-immunoreactive +FG
GluR-1 immunohistochemistry and FG labeling of striatonigral neurons			
DL	5 ± 0	0 ± 0	71 ± 6
DM	6 ± 1	0 ± 0	78 ± 8
VL	4 ± 0	0 ± 0	58 ± 15
VM	4 ± 0	0 ± 0	46 ± 16
GluR-2/3/4c immunohistochemistry and FG labeling of striatonigral neurons			
DL	37 ± 4	62 ± 2	4 ± 1
DM	39 ± 3	73 ± 5	4 ± 1
VL	53 ± 6	61 ± 6	2 ± 1
VM	43 ± 7	53 ± 5	2 ± 1
GluR-1 immunohistochemistry and FG labeling of striatopallidal neurons			
DL	4 ± 0	0 ± 0	38 ± 16
DM	5 ± 1	0 ± 0	139 ± 9
VL	4 ± 0	0 ± 0	20 ± 19
VM	4 ± 0	0 ± 0	116 ± 5
GluR-2/3/4c immunohistochemistry and FG labeling of striatopallidal neurons			
DL	85 ± 13	20 ± 7	1 ± 1
DM	10 ± 2	101 ± 4	5 ± 2
VL	99 ± 16	15 ± 14	1 ± 1
VM	11 ± 6	95 ± 0	4 ± 1

The data are expressed as the number of neurons (mean ± S.E.M.) single- or double-labeled by immunohistochemistry and/or FG, $n = 4$ rats. The counts were derived from examining areas of 0.17 mm^2 per region.

following injection of FG into the medial substantia nigra (Table 1). FG injection into the globus pallidus resulted in dense labeling of striatopallidal neurons in the unilateral DM and VM quadrants and relatively few retrogradely-labeled cells in the DL and VL quadrants (Table 1). Neurons immunostained for GluR1 or GluR2/3/4c appeared to be homogeneously distributed throughout the striatum (Table 1). No GluR4-immunoreactive neurons were observed in the striatum.

In all quadrants, the majority (≥94%) of FG-labeled striatonigral (Fig. 1B) or striatopallidal (Fig. 2B) neurons were double-labeled for GluR2/3/4c (Table 1). In contrast, immunostaining of the GluR1 subunit was not observed to co-localize with any FG-labeled striatonigral (Fig. 1A) or striatopallidal (Fig. 2A) neurons (Table 1).

Immunohistochemical double-labeling

GluR1 and GluR2/3/4c AMPA receptor subunits appeared to be evenly distributed throughout the striatum. We did not observe any differences in the density of either GluR1- or GluR2/3/4c-immunoreactive neurons between patch and matrix compartments (data not shown). Calbindin immunoreactivity was observed throughout the matrix in all but the DL striatal quadrant (Table 2). In DM, VM and VL striatum, the majority (92, 88 and 93%, respectively) of GluR2/3/4c-immunoreactive neurons were double-labeled for calbindin (Table 2, Fig. 3B). Virtually no co-existence of GluR1 and calbindin was observed (Table 2, Fig. 3A).

Striatal neurons immunostained for parvalbumin were also labeled by antibodies directed against the GluR1 subunit (Table 3, Fig. 4A). Approximately 50% of parvalbumin neurons also contained GluR2/3/4c (Table 3, Fig. 4B).

Somatostatin immunoreactivity did not co-localize with either the GluR1 or GluR2/3/4c subunits (Table 4, Fig. 5).

DISCUSSION

We have shown previously that EAA receptor subtypes are expressed on striatonigral projection neurons.[53] In the present study, we have extended these findings demonstrating that subunits of AMPA-selective EAA receptors are differentially localized on subpopulations of striatal interneurons and projection neurons. We used anti-peptide antibodies to examine the relationship of AMPA-selective EAA receptor subunits to specific striatal neuron populations identified by (i) retrograde labeling with FG as striatonigral or striatopallidal projection neurons, (ii) calbindin immunostaining as matrix projection neurons, (iii) parvalbumin immunostaining as parvalbumin/GABAergic interneurons, or (iv) somatostatin immunostaining as somatostatin/neuropeptide Y/NADPH-diaphorase (nitric oxide synthase)-containing medium aspiny interneurons. The characterization and specificity of the GluR antisera have been described previously.[9,10,42] The antisera to EAA receptor subunits are both directed against C-terminal peptides common to both "flip and flop" splice

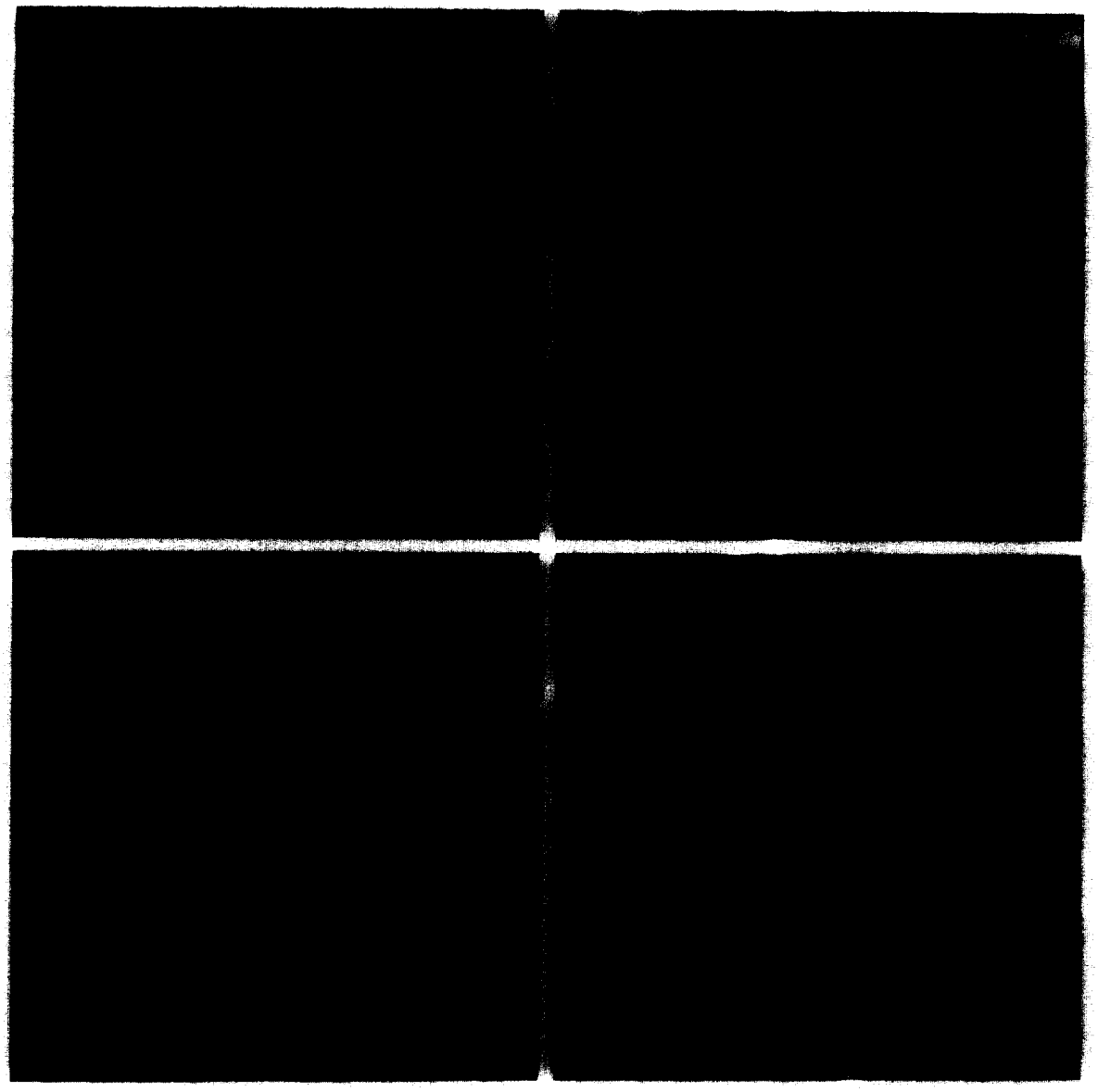

Fig. 1. (A) Fluorescence photomicrographs of the same tissue section demonstrating that striatal neurons immunoreactive for GluR1 (left panel) are distinct from striatonigral neurons labeled with FG (right panel). Arrowheads indicate GluR1-immunoreactive neurons that are not labeled with FG. (B) Paired fluorescence photomicrographs demonstrating that striatonigral neurons express GluR2/3/4c subunits. Arrows point to FG-labeled striatonigral neurons (right panel) double-labeled for GluR2/3/4c (left panel).

variants. The GluR1 antibody is specific for the GluR1 subunit, while the GluR2/3/4c antibody recognizes an epitope conserved on GluR2, GluR3 and GluR4c subunits. In the present study, GluR2/3/4c results are specific for GluR2/3 as *in situ* studies have shown that the GluR4c subtype is expressed only in the cerebellum.[36] Our results indicate that the GluR1 subunit is selectively expressed by interneurons containing parvalbumin. A portion ($\approx 50\%$) of parvalbumin interneurons also stain for GluR2/3/4c. The notion that striatal parvalbumin interneurons are not a homogeneous cell population is further supported by the work of Kawaguchi,[35] who has described two types of striatal parvalbumin interneuron based on dendritic field size and axon arborization pattern. Most ($\geqslant 88\%$) striatal projection neurons, identified by either retrograde labeling with FG or calbindin immunostaining, were double-labeled with GluR2/3/4c. These findings raise the possibility that subpopulations of striatal neurons may be differentially regulated by EAA receptors displaying unique subunit configurations.

Multi-color fluorescence microscope techniques were employed in these studies because they allow the visualization of multiple fluorochromes in a single tissue section and, with the appropriate filters, it is

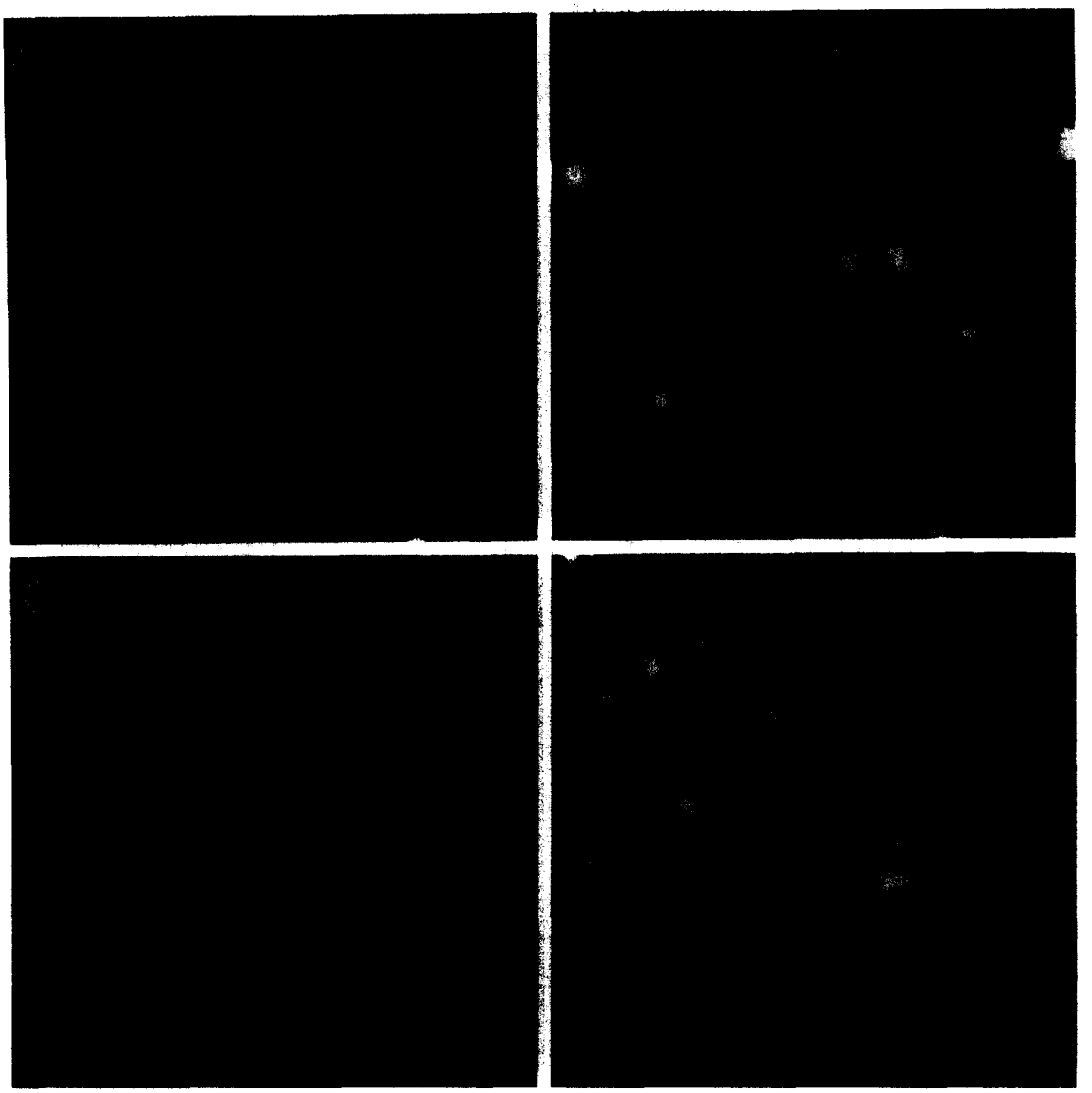

Fig. 2. (A) Fluorescence photomicrographs illustrating that striatopallidal neurons labeled with FG (right panel) are separate from striatal neurons immunoreactive for GluR1 (left panel). Arrowheads indicate single-labeled structures. (B) Expression of GluR2/3/4c subunits on striatopallidal neurons demonstrated by striatal neurons immunoreactive for GluR2/3/4c and retrogradely labeled with FG following an injection of tracer in the ipsilateral globus pallidus (arrows).

Table 2. Immunohistochemical staining of AMPA-selective glutamate receptor subunits and calbindin in striatal matrix

Striatal quadrant	+GluR-immunoreactive −Calbindin-immunoreactive	+GluR-immunoreactive +Calbindin-immunoreactive	−GluR-immunoreactive +Calbindin-immunoreactive
GluR-1 subunit			
DM	2 ± 0	0 ± 0	102 ± 8
VL	2 ± 0	1 ± 0	118 ± 15
VM	3 ± 0	0 ± 0	97 ± 12
GluR-2/3/4c subunit			
DM	8 ± 1	98 ± 14	2 ± 1
VL	15 ± 4	108 ± 15	6 ± 4
VM	8 ± 3	101 ± 12	3 ± 2

The data are expressed as the number of neurons (mean ± S.E.M.) single- or double-labeled by immmunohistochemistry, $n = 4$ rats. The counts were derived from examining areas of 0.17 mm^2 per region.

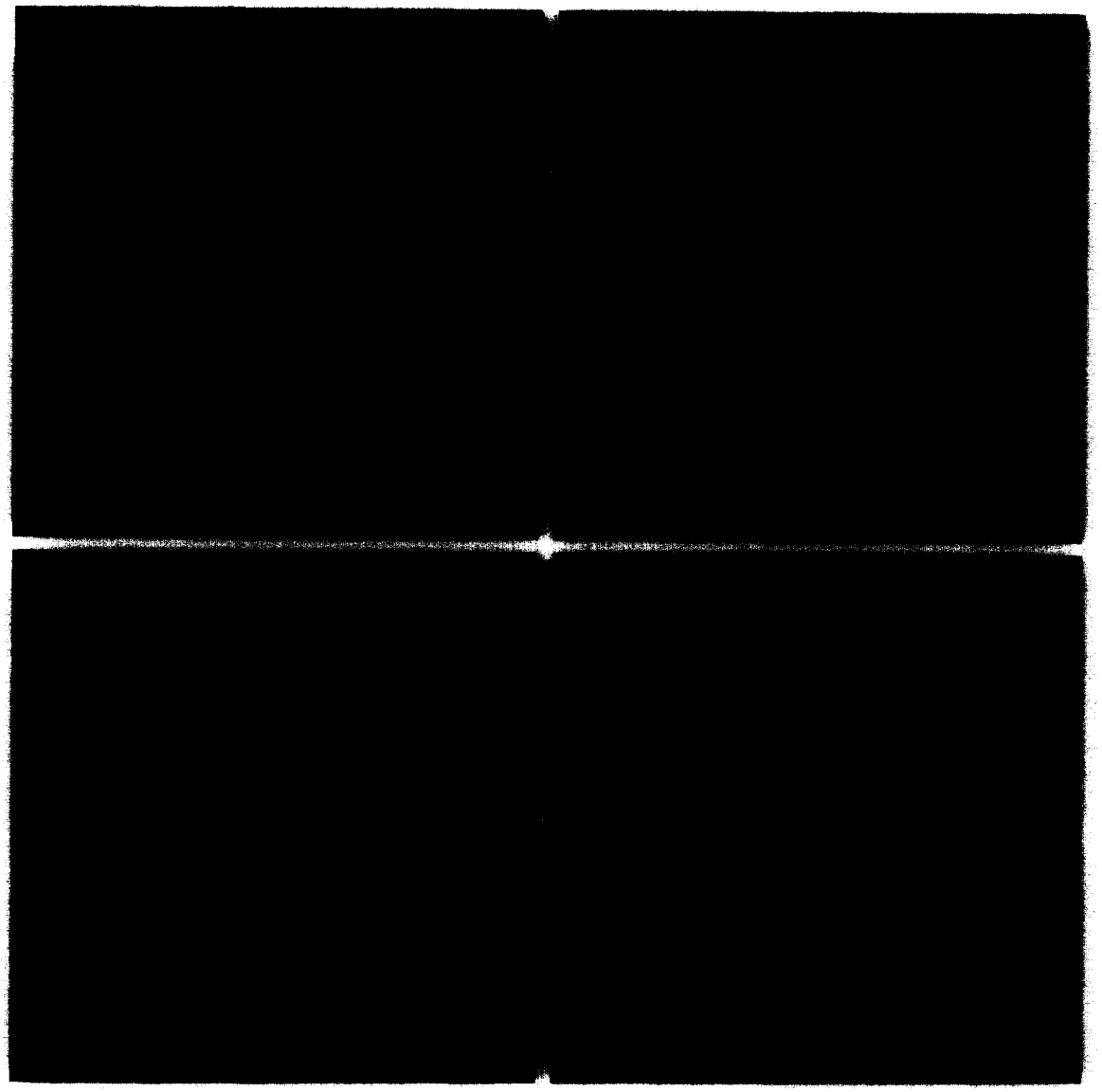

Fig. 3. Dark-field photomicrographs of striatal sections showing simultaneous two-color immunofluorescence staining for GluR subunits (left panels) and calbindin (right panels). (A) Arrowheads point to GluR1-immmunoreactive neurons (left panel) that do not stain for calbindin (right panel). The section shown in B demonstrates that co-existence of GluR2/3/4c and calbindin immunoreactivity is common (arrows).

Table 3. Immunohistochemical staining of AMPA-selective glutamate receptor subunits and paravalbimin striatum

Striatal quadrant	+GluR-immunoreactive −Parvalbumin-immunoreactive	+GluR-immunoreactive +Parvalbumin-immunoreactive	−GluR-immunoreactive +Parvalbumin-immunoreactive
GluR-1 subunit			
DL	1 ± 0	7 ± 1	0 ± 0
DM	2 ± 0	5 ± 1	0 ± 0
VL	2 ± 0	5 ± 1	0 ± 0
VM	1 ± 0	6 ± 1	0 ± 0
GluR-2/3/4c subunit			
DL	106 ± 13	2 ± 1	3 ± 1
DM	112 ± 17	1 ± 0	3 ± 1
VL	114 ± 5	2 ± 1	2 ± 1
VM	115 ± 16	1 ± 0	2 ± 1

The data are expressed as the number of neurons (mean ± S.E.M.) single- or double-labeled by immunohistochemistry, $n = 4$ rats. The counts were derived from examining areas of 0.17 mm^2 per region.

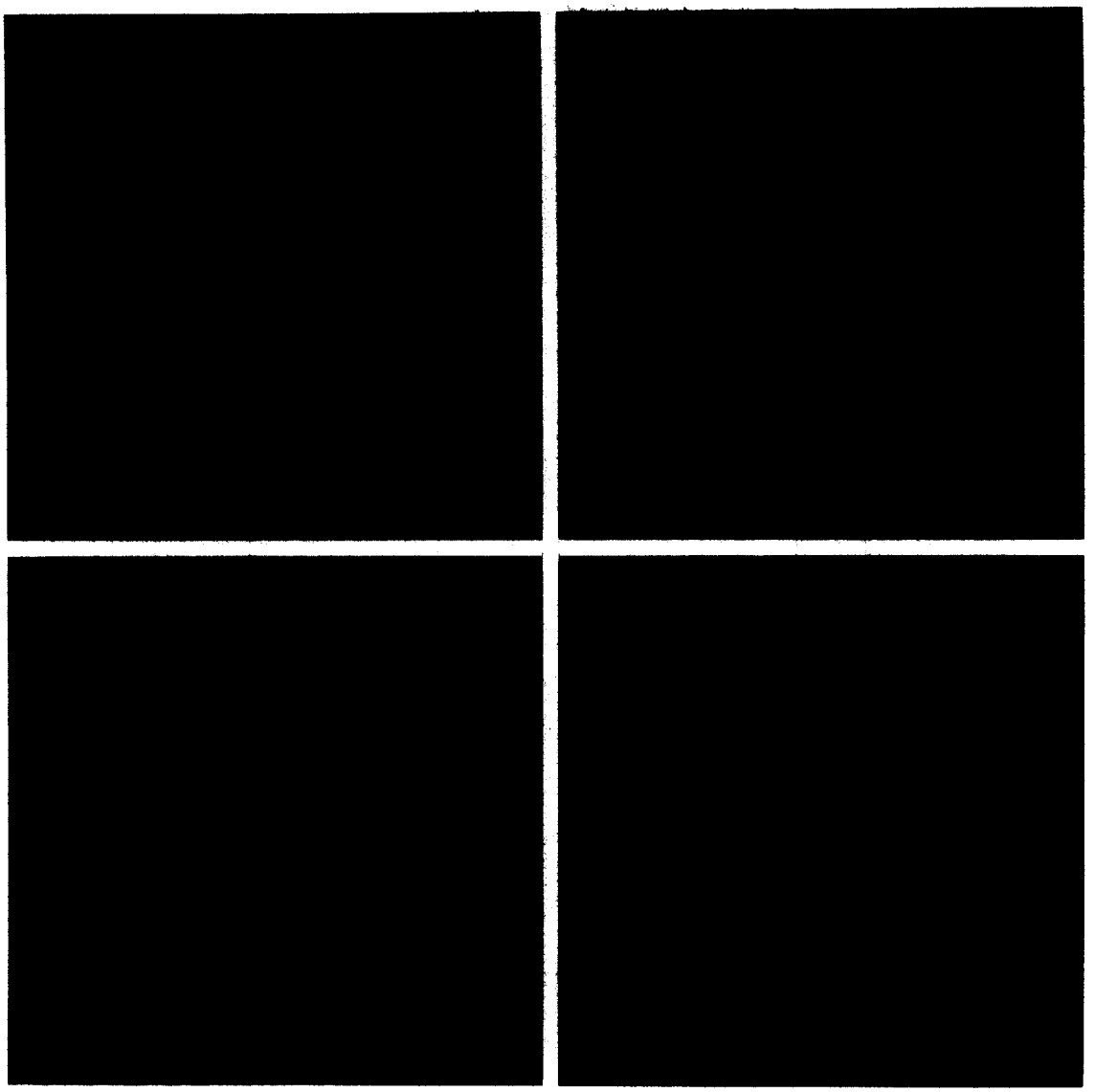

Fig. 4. Fluorescence photomicrographs illustrating co-existence of parvalbumin- (A and B, right panels) and GluR1- (left panel) or GluR2/3/4c-immunoreactivity (B, left panel) in striatum. Double-labeled neurons are indicated by arrows. Arrowheads point to single-labeled neurons.

Table 4. Immunohistochemical staining of AMPA-selective glutamate receptor subunits and somatostatin in striatum

Striatal quadrant	+GluR-immunoreactive −Somatostatin-immunoreactive	+GluR-immunoreactive +Somatostatin-immunoreactive	−GluR-immunoreactive +Somatostatin-immunoreactive
GluR-1 subunit			
DL	6 ± 1	0 ± 0	3 ± 0
DM	9 ± 1	0 ± 0	3 ± 0
VL	4 ± 0	0 ± 0	3 ± 0
VM	7 ± 1	0 ± 0	3 ± 0
GluR-2/3/4c subunit			
DL	124 ± 15	0 ± 0	3 ± 0
DM	128 ± 14	0 ± 0	2 ± 0
VL	132 ± 14	0 ± 0	3 ± 0
VM	145 ± 20	0 ± 0	3 ± 0

The data are expressed as the number of neurons (mean ± S.E.M.) single- or double-labeled by immunohistochemistry, $n = 4$ rats. The counts were derived from examining areas of 0.17 mm^2 per region.

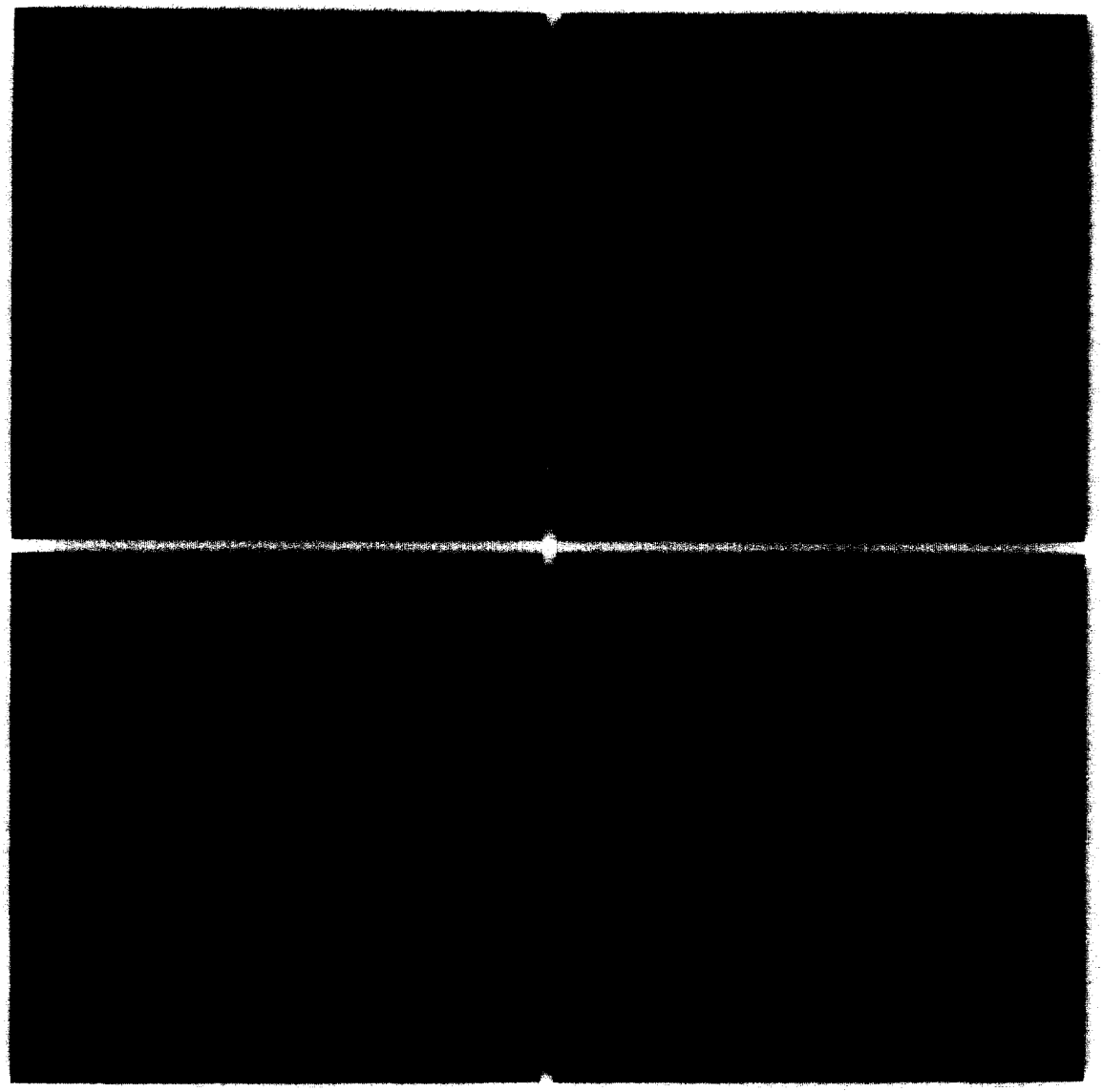

Fig. 5. Paired fluorescence photomicrographs of sections through the striatum demonstrating segregation of neurons stained for somatostatin (right panels) and either GluR1 (A, left panel) or GluR2/3/4c (B, left panel) subunits. Arrowheads point to single-labeled neurons.

easy to distinquish between different colored labels. In striatum, AMPA receptor subtypes are expressed abundantly in the neuropil and in neuronal perikarya.[42] In these studies, we used thin (4 μm) cryostat sections to maximize visualization of perikaryal staining. However, we cannot exclude the possibility that some neurons might express receptor without having significant perikaryal epitope.

The predominant EAAergic input to the striatum is from the cerebral cortex, with other probable EAA inputs from the amygdala, hippocampus and intralaminar thalamic nuclei.[43] Electrophysiological studies indicate that cortically evoked excitatory postsynaptic potentials in striatum are mediated largely by non-NMDA receptors,[11,13,15] although NMDA receptors may be recruited under depolarizing conditions.[15] Several striatal cell types have been shown to receive direct synaptic input from the cerebral cortex, including parvalbumin interneurons (in primate)[39] and neuropeptide Y interneurons (in rodent).[54]

Autoradiographic,[20,28,53] immunohistochemical[41,42] and *in situ* hybridization[24] studies have shown that NMDA, AMPA, kainate and metabotropic glutamate receptors subtypes are inhomogeneously distributed in striatum. The majority of striatal EAA binding sites, including AMPA binding sites, are thought to be localized postsynaptically on striatal neurons.[20,28,53] AMPA binding sites have been reported on striatal projection neurons.[28,53]

Recently, the production of anti-peptide antibodies to EAA receptor subunits has improved the cellular

resolution of receptor localization. Several studies have identified EAA receptor subunits co-localized with neurochemical markers used to identify subsets of neurons. In rat,[42] but not in monkey.[41] GluR1 subunits have been reported to co-localize extensively with cholinergic interneurons. GluR1 has also been reported to co-localize with a small population of NADPH-diaphorase-positive neurons in rat striatum.[42] NADPH-diaphorase has been shown to be co-contained with neuropeptide Y and somatostatin in medium aspiny striatal interneurons. However, using somatostatin as a marker, we did not observe any co-localization of GluR1 with somatostatin/neuropeptide Y/NADPH-diaphorase (nitric oxide synthase) interneurons. This discrepancy may be methodological, since the two studies used separate immunohistochemical procedures and different means of visualizing histochemical staining (fluorescence vs chromagens). Alternatively, GluR1 may co-localize with a population of NADPH-diaphorase neurons that do not contain somatostatin, although previous studies suggest that the co-existence of NADPH-diaphorase and somatostatin in striatal interneurons may be complete. GluR1 immunoreactivity has been described previously in a sparse population of medium spiny neurons.[42] Using calbindin as a marker, we observed very few medium spiny neurons immunostained for GluR1 (Table 1). Martin *et al.*[42] have reported that medium-sized spiny neurons are the only striatal cell type to express GluR2/3/4c. However, in addition to extensive GluR2/3/4c-immunostaining of striatal projection neurons, we observed co-localization of GluR2/3/4c with some parvalbumin neurons, which have been classified as medium aspiny interneurons.[26] These findings are similar to what has been described in the monkey.[41] We observed a small population of calbindin-stained neurons that did not express GluR2/3/4c. These cells may be matrix projection neurons[25] or may belong to a much smaller population of calbindin neurons that have recently been characterized as medium aspiny neurons with NADPH-diaphorase activity.[7]

Excessive stimulation of EAA receptors is known to play a role in certain acute neurodegenerative changes seen following brain trauma, such as hypoxia/ischemia and hypoglycemia.[16] EAA-induced "excitotoxicity" has also been proposed as a causative factor in chronic neurological diseases, such as Huntington's disease, Parkinson's disease and Alzheimer's disease, where the susceptibility of discrete subpopulations of neurons could be determined by the subunit composition of EAA receptors expressed. Neurotoxicity studies suggest that receptor distribution may only partially explain neuronal vulnerability to EAAs. For example, somatostatin/neuropeptide Y/NADPH-diaphorase (nitric oxide synthase) interneurons have been shown to be selectively vulnerable to AMPA toxicity,[5,6] although the present study and others[42] indicate that AMPA receptor subtypes are not expressed on the majority of these interneurons.

In Huntington's disease, the prominent striatal atrophy characteristic of the disease is believed to be due to the sequential degeneration of specific subpopulations of striatal projection neurons.[1,17,21,22,31,47] Subtype-specific EAA agonists acting at NMDA, kainate and AMPA receptors all induce loss of striatal projection neurons. However, NMDA agonists appear to most closely replicate the pattern of cell loss and interneuron sparing observed in Huntington's disease.[4,6] These findings support the theory that NMDA-mediated neurotoxicity is involved in the pathogenesis of Huntington's disease, although Dure *et al.*[19] have recently reported a uniform reduction in binding to all three ionotropic EAA receptor subtypes (NMDA, AMPA and kainate) in the striatum of Huntington's disease patients compared to control subjects.

In Parkinson's disease, the principal neurochemical defect is the loss of striatal dopamine, although recent evidence suggests that an increase in glutamatergic activity in the subthalamic nucleus may play an important, if not essential, role in the development of parkinsonian symptoms.[12,27] Interactions between glutamate and dopamine systems are known to occur in striatum. Anatomically, there is a "triadic" arrangement between dopaminergic terminals synapsing on the neck of dendritic spines of striatal neurons in close proximity to corticostriate terminals synapsing on the tips of spines.[50] Several groups have suggested that EAA antagonists could be used to manipulate basal ganglia function of Parkinson's disease patients (for review, see Ref. 27). Suppression of glutamatergic neurotransmission has been shown to produce antiparkinsonian effects. NMDA antagonists have been shown to produce an increase in motor behavior similar to that induced by dopamine agonists[14,37] and to potentiate the effects of L-DOPA in monoamine-depleted rats[37]. Similarly, AMPA antagonists have been shown to stimulate locomotor activity and decrease muscular rigidity in monoamine-depleted rats and to reduce tremor and improve akinesia and posture in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-treated monkeys.[38] Development of subtype-specific drugs may prove to be useful in the treatment of Parkinson's disease.

CONCLUSIONS

The results of the present study confirm and extend the findings of previous reports demonstrating that AMPA-selective EAA receptors are differentially localized on subpopulations of striatal neurons and interneurons. The differential distribution of EAA receptor subtypes may prove to be an important factor in the selective vulnerability of striatal neurons in basal ganglia disease. Knowledge of the cellular localization of striatal EAA receptor subtypes is

important to our understanding of how EAA neurotransmission is mediated in specific striatal neuron populations. Such information may provide insights into the etiology and/or pathophysiology of basal ganglia disease and lead to the development of subtype-specific therapeutic agents useful in slowing the progression of or ameliorating disease symptoms. Further studies will be needed to determine the full complement of EAA receptor subunits expressed by specific striatal neuron subpopulations.

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