Physiological, Morphological, and Histochemical Characterization of Three Classes of Interneurons in Rat Neostriatum

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Interneurons in lateral part of neostriatum were studied in isolated slices from juvenile rats (16–20 d postnatal) by whole-cell, current-clamp recording at 33–34°C, followed by intracellular staining with biocytin and double immunocytochemical or histochemical staining for parvalbumin, ChAT, and NADPH diaphorase. Medium-sized spiny neurons (MS cells) had distal dendrites with many spines and were likely projection cells, while interneurons had dendrites with fewer spines. The neostriatal interneurons could be further divided into three classes by physiological, chemical, and morphological criteria.

The first class of interneurons (fast-spiking cells, FS cells) fired very short-duration action potentials with short-duration afterhyperpolarizations at constant spike frequency during depolarizing current pulses. FS cells had more negative resting potentials and lower input resistances than the other two classes. At depolarized potentials, FS cells fired repetitive spikes in response to synaptic excitation. FS cells were immunoreactive for parvalbumin. As all parvalbumin-immunoreactive cells in the neostriatum were also immunoreactive for GABA, FS cells were considered to be GABAergic. FS cells were further divided into two morphological types: FS cells with local dendritic fields and FS cells with extended dendritic fields. The axons of both types of FS cells had their densest collateralization within or near their dendritic fields.

The other two classes of interneuron, PLTS cells (persistent and low-threshold spike cells) and LA cells (long-lasting afterhyperpolarization cells), were distinguished from FS cells by longer-duration action potentials and larger input resistances, had less negative resting potentials, and had longer-lasting afterhyperpolarizations. Afterhyperpolarizations of PLTS cells had a shorter time to peak than those of LA cells. PLTS cells fired both Na^+ -dependent, persistent depolarization spikes and Ca^{2+} -dependent, low-threshold spikes in addition to fast spikes. Low-threshold spikes in PLTS cells were induced only from hyperpolarized potentials. Both persistent depolarizations and low-threshold spikes could also be evoked by synaptic activation. PLTS cells were histochemically identified as NADPH diaphorase-positive cells. As all NADPH diaphorase-positive cells in the same tissue

were immunoreactive for nitric oxide (NO) synthase, PLTS cells were considered to release NO. PLTS cells had the largest axonal fields. Some PLTS cells appeared to have two axonal origins from the somata and dendrites.

LA cells were mostly large aspiny cells with Ca^{2+} -dependent long-lasting afterhyperpolarizations and strong time-dependent hyperpolarizing rectification. As this slowly occurring anomalous rectification was blocked by 2 mM cesium, this potential was considered to be due to activation of I_h . LA cells were ChAT immunoreactive, and thus considered to be cholinergic. All LA cells were larger than 20 μm in diameter. LA cells had more branched dendrites than PLTS cells.

Parvalbumin-, NO synthase, and ChAT-immunoreactive cells were not immunoreactive for either of the other two antigens. These findings show that neostriatal interneurons can be differentiated into at least three classes by differences in firing modes, territories of dendrites and axons, and content of neuroactive substances.

[Key words: interneuron, fast-spiking cell, low-threshold spike, parvalbumin, GABA, NADPH diaphorase, nitric oxide, ChAT, neostriatum, basal ganglia]

For functional understanding of any neuronal local circuit, it is necessary to reveal the physiological and morphological characteristics of its constituent neurons. These include membrane and synaptic potential properties, contained neurotransmitters, and dendritic and axonal arborization patterns. Within the mammalian neostriatum, the local circuitry is gradually becoming unravelled. Principal projecting cells are medium-sized cells (10–20 μm diameter) with spiny dendrites (MS cells) and have been well investigated from the above points of view (Gerfen, 1988; Wilson, 1990). Neostriatal neurons with fewer dendritic spines are considered to be interneurons. Compared to MS cells, neostriatal interneurons have not been well characterized physiologically. In particular, the physiological characteristics of neostriatal interneurons have not yet fully related to their morphological and chemical identities. Recent *in vivo* (Wilson et al., 1990) and *in vitro* (Kawaguchi, 1992) studies have shown that, compared with MS cells, large aspiny cells have different electrical properties and different dendritic and axonal arborization patterns. These studies suggest that other neostriatal interneurons may also show distinct physiological characteristics that may correlate with the morphological diversity of aspiny neuron types revealed in Golgi studies of the neostriatum.

Although morphological classifications of neostriatal cells are not completely consistent among several studies using Golgi staining (DiFiglia et al., 1976; Dimova et al., 1980; Chang et al., 1982; Bolam et al., 1984a), cumulative immunohistochem-

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ical evidence suggests that neostriatal interneurons are mainly composed of three chemically identified populations. One population of neostriatal interneurons contains the calcium-binding protein parvalbumin (Cowan et al., 1990; Kita et al., 1990). Most of these are also strongly immunoreactive for both GABA and glutamate decarboxylase (GAD), the synthetic enzyme for GABA. Another population of interneurons is stained for NADPH diaphorase, an enzyme that is identical with nitric oxide (NO) synthase (Dawson et al., 1991). These cells also colocalize somatostatin and neuropeptide Y (Vincent et al., 1983; Dawson et al., 1991). Third population of interneurons is immunostained for ChAT, the rate-limiting enzyme in the synthesis of ACh (Bolam et al., 1984b; Phelps et al., 1985). These three populations are probably separate from one another (Kita et al., 1990; Dawson et al., 1991).

The present study investigated physiological properties of neostriatal cells in a slice preparation using whole-cell current-clamp recording combined with intracellular staining and immunocytochemistry or histochemistry to clarify the following three points important for understanding the functional differentiation of neostriatal local circuit neurons. First, is there a physiological heterogeneity among neostriatal interneurons and what properties are characteristic of each class of cells? Second, are there correlations between physiological subtypes and the three chemically defined classes? Third, are there morphological differences among physiologically and chemically different groups? The results show that physiological heterogeneity among the neostriatal interneurons correlates with specific chemical and morphological characteristics, implying that neostriatal neurons fall into three classes that differ in firing modes, neurochemical identity, and spatial territory subtended.

Materials and Methods

In vitro slice preparation and solutions used. The slicing and recording procedures used have been described in detail previously (Kawaguchi, 1992). The experiments were performed on young Wistar rats (16–20 d postnatal). The animals were deeply anesthetized with ether and decapitated. The brains were quickly removed, submerged in ice-cold physiological Ringer's and hemisected. Sections of the neostriatum were cut 180–200 μ m thick on a Microslicer (Dosaka EM) in a plane oblique to the horizontal along the line of rhinal fissure. After sectioning, neocortex was trimmed off. Slices were then incubated in oxygenated Ringer's solution at 29–30°C. The standard Ringer's solution was composed of (mM) NaCl, 124.0; KCl, 3.0; CaCl_2 , 2.4; MgCl_2 , 1.3; NaHCO_3 , 26.0; NaH_2PO_4 , 1.0; glucose, 10.0; and was continuously bubbled with a mixture of 95% O_2 and 5% CO_2 . When replacing CaCl_2 by CoCl_2 , NaH_2PO_4 was omitted. After incubation for at least 2 hr, a single slice was transferred to a recording chamber placed on the stage of an upright microscope and was totally submerged in the superfusing medium at 33–34°C. The remaining slices were kept in the holding chamber containing oxygenated Ringer's solution at 26–27°C. All drugs were bath applied. Tetrodotoxin (TTX) came from Sankyo Co.

Whole-cell recording and stimulation. Individual neostriatal cells in lateral part were visualized using a 40 $\times$ water immersion objective. Neostriatal cells that appeared larger than most other cells were preferentially targeted to obtain a larger sample of types of cells other than MS cells. Electrodes (borosilicate glass pipettes) were filled with a solution containing (mM) K-methylsulfate, 120; EGTA, 0.6; MgCl_2 , 2.0; ATP, 4.0; GTP, 0.3; and HEPES, 10. The resistance of the electrodes filled with this solution was 5–6 M Ω . Whole-cell recordings with a high seal resistance (more than 2 G Ω before break-in) were obtained using an amplifier with bridge balance and capacitance compensation. Input resistances were measured from the voltage deflection (–6 to –15 mV) induced by hyperpolarizing current pulses. Time to peak and amplitude of afterhyperpolarizations following spikes were measured from spike onset by depolarizing current pulses (duration, 0.5–1.0 sec) of threshold strength for spike initiation. Spike widths were measured at half-amplitude, on action potentials induced by depolarizing pulses (duration,

50 msec) of threshold strength for spike initiation. Only one cell was recorded in each slice to facilitate comparison of physiological, immunohistochemical and morphological properties of single cells.

To identify the morphology of recorded cells, 0.5% biocytin (Sigma) was added to the pipette solution to stain cells by diffusion (Horikawa and Armstrong, 1988). Synaptic responses were evoked by electrical stimulation by bipolar electrodes at the white matter underlying neocortex (external capsule) or at sites more than 0.6–0.7 mm distant from the recorded neurons toward the external capsule. Stimuli of 100 μ sec duration and 0.2–0.5 mA intensity were delivered at 0.2–0.4 Hz.

Immunohistochemical procedure for immersion-fixed slices. Slices containing biocytin-loaded cells were fixed by immersion in 4% paraformaldehyde and 0.2% picric acid in 0.1 M phosphate buffer (PB) for 30–60 min at room temperature and incubated in PB containing 0.5% Triton X-100 (TX) for 1 hr. The tissue was frozen with dry ice and thawed in PB containing 0.5% TX at room temperature. The slices, without resectioning, were then washed with Tris-buffered saline (TBS) for 1 hr. Each slice was further treated by one of the following four procedures.

(1) The slices were incubated with a mouse monoclonal antibody against parvalbumin (Sigma; diluted 1:2000) in TBS containing 10% normal goat serum (NGS), 2% bovine serum albumin (BSA), and 0.5% TX, overnight at room temperature. After 3×10 min washing in TBS, the slices were incubated in a mixture containing the streptavidin-conjugated Texas red (Amersham; diluted 1:200) and dichlorotriazinyl-aminofluorescence-dihydrochloride (DTAF)-conjugated goat anti-mouse IgG (Chemicon; 1:200) in TBS containing 10% NGS, 2% BSA for 3 hr at room temperature.

(2) The slices were incubated with the streptavidin-conjugated Texas red (1:200) in TBS containing 10% NGS, 2% BSA for 3 hr at room temperature. After 3×10 min washing in TBS, the slices were processed for NADPH diaphorase histochemistry by incubating in a solution containing 1 mg/ml NADPH and 0.2 mg/ml nitroblue tetrazolium in 0.1 M Tris buffer (TB) at 37°C for 20–30 min.

(3) The slices were incubated with a rat monoclonal antibody against choline acetyltransferase (Boehringer; 1 μ g/ml) in TBS containing 10% NGS, 2% BSA and 0.5% TX, overnight at room temperature. After 3×10 min washing in TBS, the slices were incubated in a mixture containing the streptavidin-conjugated Texas red (1:200) and FITC-conjugated goat anti-rat IgG (Jackson; 1:200) in TBS containing 10% NGS, 2% BSA for 3 hr at room temperature.

(4) In some slices, no histochemical procedures were applied.

In the cases treated with histochemical procedures 1–3, after 3×10 min washing in TBS, the slices were observed and photographed in a fluorescence microscope (Nikon Microphot-FXA) using epifluorescence the G-2A (barrier filter > 590 nm) dichroic mirror system for Texas red to identify biocytin-labeled cells, epifluorescence B-2E (barrier filter, 520–560 nm) for DTAF and FITC to examine immunoreactive cells, or bright-field optics for NADPH diaphorase-positive cells. The immunoreactive cells were seen in red or green fluorescence with the respective filter systems, and only one color was visualized by each filter combination in epifluorescence.

In all cases, slices were next incubated to make staining for biocytin more permanent. They were incubated in TBS containing 0.5% H_2O_2 for 30 min to suppress endogenous peroxidase activity. After washing in TBS for 3×20 min, the slices were incubated with avidin–biotin–peroxidase complex (Vector; 1:100) in TBS for 3 hr at room temperature. After washing in TBS for 20 min, the slices were again incubated with avidin–peroxidase (Vector; 1:100) in TBS for 3 hr at room temperature. After washing in PB, the slices were reacted with 3,3'-diaminobenzidine tetrahydrochloride (DAB) (0.05%) and H_2O_2 (0.003%) in PB. The sections were postfixed in 0.1% osmium tetroxide in PB for 10 min and mounted on gelatin-coated glass slides. Mounted slices were dehydrated and embedded in Epon 812 (Fluka). The dendrites, axonal processes, and somata of the biocytin-labeled neurons were drawn at 600 $\times$ using a 60 $\times$ 1.4 NA objective and a camera lucida. Data are given as means $\pm$ SD. For statistical analysis in comparing cell classes, the Mann–Whitney *U* test was used.

Immunohistochemistry in perfusion-fixed brains. Four male Wistar rats (16 and 20 d postnatal) were used. The animals were given an overdose of Nembutal and perfused through the heart with phosphate-buffered saline, followed by 150 ml of fixative containing 2% paraformaldehyde, 0.2% picric acid, and 0.1% glutaraldehyde in PB. The brains were removed and postfixed in the same fixative for 3 hr at 4°C. After incubation in PB containing 30% sucrose for 3 d at 4°C, the sections

Table 1. Properties of four types of neostriatal cells

	MS cells (<i>n</i> = 17)	FS cells (<i>n</i> = 13)	PLTS cells (<i>n</i> = 11)	LA cells (<i>n</i> = 26)
Soma diameter (μm)	13.0 ± 2.1 ($< \text{FS}, < \text{PLTS}, < \text{LA}$)	17.4 ± 3.5 ($> \text{MS}, < \text{LA}$)	18.7 ± 3.2 ($> \text{MS}, < \text{LA}$)	26.7 ± 4.3 ($> \text{MS}, > \text{FS}, > \text{PLTS}$)
Resting potential (mV)	-81.1 ± 4.4 ($< \text{PLTS}, < \text{LA}$)	-79.9 ± 3.2 ($< \text{PLTS}, < \text{LA}$)	-56.4 ± 15.7 ($> \text{MS}, > \text{FS}$)	-57.3 ± 5.0 ($> \text{MS}, > \text{FS}$)
Input resistance ^a (M Ω)	193 ± 77 ($> \text{FS}, < \text{PLTS}, < \text{LA}$)	86 ± 38 ($< \text{MS}, < \text{PLTS}, < \text{LA}$)	638 ± 245 ($> \text{MS}, > \text{FS}$)	433 ± 231 ($> \text{MS}, > \text{FS}$)
Spike width at half amplitude (msec)	1.05 ± 0.20 ($> \text{FS}$)	0.29 ± 0.04 ($< \text{MS}, < \text{PLTS}, < \text{LA}$)	1.00 ± 0.41 ($> \text{FS}$)	0.82 ± 0.19 ($> \text{FS}$)
Time to peak of AHP ^b (msec)	22.9 ± 6.6 ($> \text{FS}, < \text{LA}$)	1.3 ± 0.27 ($< \text{MS}, < \text{PLTS}, < \text{LA}$)	13.6 ± 8.2 ($> \text{FS}, < \text{LA}$)	53.2 ± 17.1 ($> \text{MS}, > \text{FS}, > \text{LA}$)
Amplitude of AHP ^b (mV)	-13.7 ± 2.4 ($> \text{FS}, > \text{LA}$)	-23.3 ± 3.8 ($< \text{MS}$)	-19.9 ± 6.5 ($< \text{MS}$)	-21.6 ± 5.4 ($< \text{MS}$)

Data are mean $\pm$ SD. MS cells, medium-sized spiny neurons; FS cells, fast-spiking cells; PLTS cells, persistent and low-threshold spike cells; LA cells, long-lasting afterhyperpolarization cells. Data in parentheses indicate that the given value is significantly larger or smaller than that of indicated cells ($p \leq 0.005$).

^a Input resistance and time constant were determined by hyperpolarizing current pulses inducing voltage shift of 6–15 mV negative to rest.

^b Time to peak and amplitude of afterhyperpolarization (AHP) were measured from the spike onset.

were cut along the line of the rhinal fissure in a cryostat at 10 μm thickness and mounted on gelatin-coated slides. The sections were washed several times in TBS and were further treated by one of the following three procedures.

(1) In preparations to be triple stained for parvalbumin, NO synthase, and choline acetyltransferase, the sections were incubated with the same monoclonal mouse antibody against parvalbumin as above (diluted 1:2000), with a rabbit antiserum against NO synthase that had been purified from rat cerebellum and was an enzyme of constitutive type (from Drs. Hattori and Yui, Kyoto University; diluted 1:2000) and with the rat monoclonal antibody against choline acetyltransferase (1 $\mu\text{g}/\text{ml}$) in TBS containing 10% NGS, 2% BSA, and 0.5% TX for overnight at room temperature. After 3×10 min washing in TBS, the sections were incubated with TRITC-conjugated goat anti-mouse IgG (Chemicon; 1:200), biotinylated goat anti-rabbit IgG (Vector; 1:200) and DTAF-conjugated goat anti-rat IgG (Chemicon; 1:200) in TBS containing 10% NGS, 2% BSA for 3 hr at room temperature. After 3×10 min washing in TBS, the sections were incubated with AMCA-streptavidin (Vector; 1:100) in TBS for 3 hr at room temperature.

(2) In preparations to be double stained for NADPH diaphorase and NO synthase, the sections were incubated with the rabbit serum against NO synthase in TBS containing 10% NGS, 2% BSA, and 0.5% TX for overnight at room temperature. After 3×10 min washing in TBS, the sections were incubated with biotinylated goat anti-rabbit IgG (1:200) in TBS containing 10% NGS, 2% BSA for 3 hr at room temperature. After 3×10 min washing in TBS, they were incubated with streptavidin Texas red (1:200) for 3 hr at room temperature. After 3×10 min washing in TBS, they were washed with TB and were processed for NADPH diaphorase histochemistry by incubation in a solution containing 1 mg/ml NADPH and 0.2 mg/ml nitroblue tetrazolium in TB at 37°C for 20–30 min.

(3) In preparations to be double stained for parvalbumin and GABA, the sections were incubated with the monoclonal mouse antibody against parvalbumin (diluted 1:2000) and a rabbit antiserum against GABA (Sigma; diluted 1:4000) in TBS containing 10% NGS, 2% BSA, and 0.5% TX overnight at room temperature. After 3×10 min washing in TBS, the sections were incubated in DTAF-conjugated goat anti-mouse IgG (1:200) and TRITC-conjugated donkey anti-rabbit IgG (Chemicon; 1:200) in TBS containing 10% NGS, 2% BSA for 3 hr at room temperature.

After each of the above three procedures, the sections were washed in TBS several times and mounted on slides with 50% glycerin in TBS. The sections were observed and photographed in a fluorescence microscope (Nikon Microphot-FXA) using the G-2A (barrier filter > 590 nm) dichroic mirror system for TRITC and Texas red, B-2E (barrier filter, 520–560 nm) for DTAF, UV-2A (barrier filter > 420 nm) for AMCA, or bright-field optics for NADPH diaphorase-positive cells. In addition, a band-pass filter (400–500 nm; BPB-45 FUJI) was used for AMCA. The cells were seen in red, green, or blue fluorescence with the respective filter systems, and only one color was visualized by each filter combination.

Cross-reactivity of the secondary antibodies. Selected sections were incubated with a single primary antibody and then incubated with the secondary antibody appropriate to the other primary; for example, one set of sections was incubated in mouse monoclonal antibody against parvalbumin and then in TRITC-conjugated goat anti-rabbit IgG. In none of the possible combinations was specific staining observed.

Results

All neurons sampled by whole-cell recording from the lateral part of the neostriatum had resting potentials more negative than -40 mV and overshooting spikes. All electrophysiological data used for the analysis came from 17 MS cells and 50 cells of other types, which were all morphologically identified and satisfied the above electrophysiological criteria (Table 1). Staining intracellularly with biocytin clearly distinguished the morphologies of MS cells and other classes of cells. In no cases were two or more cells stained by a single whole-cell recording. Diameter of MS cells ranged from 9 to 17 μm . MS cells had distal dendrites with many spines (Fig. 1*A,B*). The axons of MS cells emitted local collaterals that remained close to the dendritic field. Main axons of MS cells sometimes could be traced into fiber bundles in the neostriatum. Other types of cells had dendrites either lacking spines or with fewer spines than MS cells;

Figure 1. Photomicrographs of four classes of rat neostriatal cells intracellularly stained by biocytin. *B, D, F, and H* are higher magnifications of *A, C, E, and G*, respectively. *A and B*, An MS (medium spiny) cell. Note the dendrites with many spines. *C and D*, An FS (fast-spiking) cell. *E and F*, A PLTS (persistent and low-threshold spike) cell. Note the dendrites with fewer spines in FS and PLTS cell than MS cell. *G and H*, An LA (longer-duration afterhyperpolarization) cell. Note a larger soma than other cells. Scale bars: *G*, 100 μm for *A, C, E, and G*; *H*, 50 μm for *B, D, F, and H*.

Figure 2. Plot of spike widths at half-amplitude (*spike-width*) versus input resistances (*Ri*) of three classes of interneurons in rat neostriatum, FS cell (open circles), PLTS cell (solid triangles), and LA cell (open triangles). FS cells had spike widths shorter than 0.4 msec and input resistances lower than 140 MΩ. PLTS and LA cells had spike widths longer than 0.5 msec and input resistances higher than 180 MΩ.

Figure 4. Distributions of time to peak of afterhyperpolarization (AHP) following spikes of PLTS cells and LA cells. Time to peak of afterhyperpolarization was measured from onsets of spikes that were elicited by threshold depolarizing current stimuli. Note times to peak of PLTS cells and LA cells are smaller and larger than 30 msec, respectively.

Figure 3. Physiological characteristics of an FS cell. *A1*, A spike elicited by a depolarizing pulse. Note short-duration spike and short afterhyperpolarization following a spike. *A2*, Voltage responses induced by current pulses and repetitive spike discharges induced by current pulse close to the threshold. Note the slowly depolarized phase before spike initiation (arrowhead), abrupt cessation of firing, and late resumed firing. Spikes are truncated due to sampling frequency. *A3*, A stronger pulse induced early single firing and late repetitive firing. Note abrupt cessation of repetitive firing and weak adaptation of firing rate during repetitive discharges. *A1–A3* are from the same FS cell and have the same calibration. Resting membrane potential, -73 mV. *B*, The slowly depolarized phase in FS cells became clear by elimination of spike discharges by $1 \mu\text{M}$ TTX (arrowhead). Spontaneous synaptic potentials could be observed. *C1*, Repetitive firing by synaptic stimulation (arrowhead) of FS cell in combination with depolarization (-38 mV) by depolarizing currents. *C2*, Synaptic excitation of FS cell with a spike elicited by synaptic stimulation (arrowhead) at resting potential (-73 mV). *C1* and *C2* have the same calibration.

Figure 5. Physiological characteristics of PLTS cells. *A1*, A single spike was elicited by a depolarizing pulse. Note longer duration of spike compared to FS cells (Fig. 3*A1*). *A2*, An all-or-none low-threshold spike with a fast spike was induced by synaptic excitation (arrowhead) at hyperpolarized potential. *A3*, A long-lasting persistent depolarization followed by low-threshold spikes was elicited by cessation of the hyperpolarizing pulse from depolarized potentials. This long-lasting persistent depolarization was terminated by the afterhyperpolarization following a fast spike. *A4*, Low-threshold spikes were also elicited by depolarizing pulses from hyperpolarized potentials. *B1–B5*, Persistent depolarizations following low-threshold spikes could be induced by both a current step and synaptic excitation. *B1* and *B2*, Low-threshold spike and following persistent depolarization by cessation of hyperpolarizing pulses at depolarized potentials (*B1*) and by depolarizing pulses at hyperpolarized potentials (*B2*) in all-or-none fashion. *B3–B5*, Persistent depolarizations could be also induced by sufficient synaptic excitation. At hyperpolarized potentials, no spikes were induced by synaptic excitation (arrowhead) (*B5*). Only low-threshold spikes could be elicited synaptically at -73 mV (*B4*), but at -66 mV persistent depolarizations could be also elicited following low-threshold spikes (*B3*). Membrane potentials are shown on each record. In slower sweeps, spikes are truncated.

their main axons did not run in the neostriatal fiber bundles (Fig. 1*C–H*) and they were considered interneurons.

The electrophysiological characteristics of MS cells were essentially the same as previously described (Kita et al., 1984; Kawaguchi et al., 1989; Calabresi et al., 1990; Jiang and North, 1991; Kawaguchi, 1992). Morphological types of cells other than MS cells that were considered to be interneurons could be further divided into three physiological classes. Interneurons of the first class fired very short-duration action potentials at constant spike frequency and are called fast-spiking cells (FS cells). Interneurons of the second class fired both Na^+ -dependent persistent depolarization spikes and Ca^{2+} -dependent low-threshold spikes, and are called persistent and low-threshold spike cells (PLTS cells). The third class of interneurons were mostly large aspiny cells with long-lasting afterhyperpolarizations and strong time-dependent hyperpolarizing rectification, and are called long-lasting afterhyperpolarization cells (LA cells). They were also different in chemical and morphological characteristics. First, the electrophysiological properties of the three classes of interneurons will be described.

Physiological properties of three classes of neostriatal interneurons

Fast-spiking cells (FS cells). Among 50 identified neostriatal interneurons, 13 cells belonged to the FS class. A readily distinguishable feature of FS cells was short-duration action potentials with afterhyperpolarizations that had shorter times to peak than other classes of cells (Table 1; Figs. 2, 3*A1*). Another characteristic feature was their lower input resistances compared with other classes of cells. FS cells had spike widths at half-amplitude shorter than 0.4 msec and input resistances lower than $140 \text{ M}\Omega$, while PLTS cells and LA cells had spike widths at half-amplitude longer than 0.5 msec and input resistances higher than $180 \text{ M}\Omega$ (Fig. 2). Resting membrane potentials of FS cells were more negative than those of PLTS cells and LA cells. In FS cells, depolarizing current pulses with strength just below threshold produced membrane depolarization having two distinct phases, an initial rapidly rising component followed by slow depolarization with a duration of about 100 msec (Fig. 3*A2*). Eliminating Na^+ -dependent fast spikes by TTX made this

Figure 6. Properties of low-threshold spikes and persistent depolarizations in PLTS cells. *A1* and *A2*, Low-threshold spikes induced by depolarizing pulses (*A1*) and cessation of hyperpolarizing pulses (*A2*). *A3* and *A4*, Low-threshold spikes could not be abolished by 1 μ M TTX application. *A5* and *A6*, Low-threshold spikes were abolished by replacement of Ca^{2+} by 2 mM Co^{2+} . *B1*, All-or-none induction of low-threshold spike followed by persistent depolarization. *B2*, Replacement of Ca^{2+} by 2 mM Co^{2+} blocked initial low-threshold spike, but not following all-or-none persistent depolarizations with fast spikes. *B3*, In addition to Co^{2+} , TTX (1 μ M) abolished persistent depolarizations. Membrane potentials are written on each record. Voltage and current calibrations in *A1* are applicable to all traces. Time calibrations in *A1*, *A2*, and *B1* are applicable to *A3,5*, *A4,6*, and *B2,3*, respectively.

slow phase clear (Fig. 3*B*). At threshold, spike firing was delayed because of the slow phase of the depolarizing potential (Fig. 3*A2*). Just above threshold, FS cells fired several spikes with a constant interspike interval. Firing abruptly ceased (Fig. 3*A2,3*) and sometimes resumed again after a quiescent period within the period of the same depolarizing pulse (Fig. 3*A2*).

Firing patterns of FS cells induced by depolarizing pulses were also reflected in those induced by synaptic excitation. Because resting potentials of FS cells were about -80 mV, a single shock of synaptic stimulation at rest induced only an EPSP or an EPSP with a single spike (Fig. 3*C2*). When FS cells were depolarized to -40 to -30 mV by constant current injection, they responded to synaptic stimulation with a short period of repetitive firing at a relatively constant firing frequency (Fig. 3*C1*).

Persistent and low-threshold spike cells (PLTS cells). The other two classes of interneurons were distinguished from FS cells by longer-duration action potentials and larger input resistances (Figs. 2, 5*A1*), less negative resting potentials, and longer time to peak of afterhyperpolarizations (Table 1). They also had less negative resting potentials and larger input resistances than MS cells (Table 1). These two classes were discriminated by duration of afterhyperpolarizations following spikes. PLTS cells and LA cells had time to peak of afterhyperpolarization following spikes shorter and longer than 30 msec, respectively (Table 1, Fig. 4).

PLTS cells were easily distinguished from other classes of cells by two prominent types of spikes in addition to fast spikes, low-threshold spikes, and persistent depolarizing spikes (Fig. 5). Low-threshold spikes could be induced by depolarizing pulses only at potentials more negative than about -60 mV (Fig. 5*A4*). In some cases, low-threshold spikes from hyperpolarized potentials were followed by persistent depolarizing spikes (Fig. 5*B2*). Low-threshold spikes were also elicited by cessation of hyperpolarizing pulses at depolarized potentials and were continuously followed by persistent depolarizations (Fig. 5*A3, B1*). Once activated, persistent depolarizations were noninactivated during depolarized state and were terminated by afterhyperpolarizations following fast spikes (Fig. 5*A3, B1,2*) or by cessation of depolarizing current pulses (Fig. 6*B1*).

Both persistent depolarizations and low-threshold spikes in PLTS cells could be evoked by synaptic excitation. At hyperpolarized potentials, all-or-none low-threshold spikes could be induced on evoked EPSPs (Fig. 5*A2, B4*). If sufficient depolarization resulted from EPSPs and/or by low-threshold spikes on evoked EPSPs, persistent depolarizations could also be induced by synaptic excitation (Fig. 5*B3*).

Electrophysiological characteristics of persistent depolarizations and low-threshold spikes in PLTS cells were investigated by blockers of Na^+ and Ca^{2+} channels (Llinás, 1988). Low-

Figure 7. Physiological properties of LA cells. *A1*, Voltage responses and a spike induced by current pulses. Note large afterhyperpolarization following spike and prominent sag during hyperpolarizing pulses. *A2* and *A3*, A spike was induced by synaptic excitation (arrowheads) at depolarized potentials, but repetitive discharges could not be induced and a large afterhyperpolarization followed the spike. *B1* and *B2*, Large afterhyperpolarizations following a spike (*B1*) were much reduced by replacement of Ca^{2+} by 2 mM Co^{2+} (*B2*). *C1* and *C2*, Prominent sag during hyperpolarizing current pulses (*C1*) were suppressed by addition of 2 mM Cs^+ (*C2*). Membrane potentials are written on each record. Voltage calibration in *A1* is applicable to *A2*. Calibrations in *B1* and *C1* are applicable to *B2* and *C2*, respectively.

threshold spikes induced by depolarization from hyperpolarized potentials (Fig. 6*A1,2*) could not be blocked by 1 μM TTX, a Na^+ channel blocker ($n = 4$) (Fig. 6*A3,4*). The remaining low-threshold spikes in TTX-containing solutions were blocked by replacement of Ca^{2+} by 2 mM Co^{2+} ($n = 2$) (Fig. 6*A5,6*). On the other hand, persistent depolarizations were resistant to replacement of Ca^{2+} by Co^{2+} ($n = 4$) (Fig. 6*B1,2*). However, these persistent depolarizations as well as fast spikes were diminished by addition of TTX to the solution ($n = 3$) (Fig. 6*B3*). These results showed that low-threshold spikes were induced by influx of Ca^{2+} , while persistent depolarizations were due to influx of Na^+ .

Long-lasting afterhyperpolarization cells (LA cells). LA cells were separated from FS cells by less negative resting potentials, larger input resistances, larger spike width, and longer-duration afterhyperpolarizations. LA cells had longer times to peak of afterhyperpolarizations following spikes (Table 1). LA cells were also discriminated from MS cells by less negative resting potentials, larger input resistances, and afterhyperpolarizations of longer duration and larger amplitude than MS cells.

LA cells displayed neither persistent depolarization spikes nor low-threshold spikes. Instead, they showed strong time-depen-

dent hyperpolarizing rectification, which was observed as a clear return to the starting potential during hyperpolarizing pulses (Fig. 7*A1*). Although a time-dependent voltage sag was also observed in PLTS cells during hyperpolarizing pulses (Figs. 5*B1,6A2*), that of LA cells was much more prominent. Synaptic excitation could evoke only single spikes even by strong depolarization (Fig. 7*A2*), in contrast to FS cells or PLTS cells. This was apparently due to the large long-lasting hyperpolarization following the spike, which suppressed consecutive spike firing.

The ionic basis for long-lasting afterhyperpolarizations and slowly occurring hyperpolarizing rectifications characteristic of LA cells was studied by ionic blockers. Replacing Ca^{2+} in the perfusate with 2 mM Co^{2+} made afterhyperpolarizations much smaller without affecting hyperpolarizing rectifications and tail potentials after cessation of current pulses ($n = 3$) (Fig. 7*B1,2*). Slowly occurring hyperpolarizing rectifications were blocked by adding 2 mM cesium in the external solution ($n = 4$) (Fig. 7*C1,2*). Depolarizing tail potentials after cessation of hyperpolarizing pulses were also suppressed. These results suggested that long-duration afterhyperpolarizations followed by spikes of LA cells were caused by activation of Ca^{2+} -induced K^+ channels, while

Figure 8. Histochemical identification of FS cells, PLTS cells and LA cells. *A*, A biocytin-injected FS cell visualized by Texas red-conjugated avidin. *B*, Parvalbumin (PV) immunoreactivity visualized by DTAF-conjugated secondary antibody in the same section as *A*. An identified FS cell was parvalbumin immunoreactive (arrowhead). *C*, A biocytin-injected PLTS cell visualized by Texas red-conjugated avidin. *D*, NADPH diaphorase (NADPH-D) histochemistry in the same section as *C*. An identified PLTS cell was stained by NADPH diaphorase histochemistry (arrowhead). *E*, A biocytin-injected LA cell visualized by Texas red-conjugated avidin. *F*, ChAT immunoreactivity visualized by FITC-conjugated secondary antibody in the same section as *E*. An identified LA cell was ChAT immunoreactive (arrowhead). Scale bar, 50 μ m.

slow hyperpolarizing rectifications were induced by activation of I_h (Halliwell and Adams, 1982; McCormick and Pape, 1990; Jiang and North, 1991).

Correlation of three chemically identified cell groups with three classes of interneurons in the neostriatum

Neostriatal interneurons are considered to be chemically divided into at least three groups, parvalbumin-immunoreactive cells,

NADPH diaphorase-positive cells, and ChAT-immunoreactive cells. Parvalbumin-positive cells are considered to be more active cells that could induce higher firing rates in the same brain areas (Celio, 1990); in the hippocampus and neocortex a type of cell similar to neostriatal FS cells shows parvalbumin immunoreactivity (Kawaguchi et al., 1987; Kawaguchi and Kubota, 1993). ChAT-positive cells have the largest cell body among neostriatal cells (Bolam et al., 1984b), while parvalbumin- and

Figure 9. Parvalbumin-, NO synthase (NADPH diaphorase)-, ChAT-positive cells belonged to immunohistochemically separate populations and parvalbumin-immunoreactive cells were also positive for GABA in neostriatum in 20-d-old rats. *A–C*, Triple photomicrographs from the same microscopic field, showing relationships of neostriatal cells stained immunohistochemically for parvalbumin (*PV*), NO synthase (*NOS*), and ChAT. Each type of immunoreactive cells showed no immunoreactivity for the other antigens. *D* and *E*, Paired photomicrographs from the same microscopic field, showing relationships of cells stained histochemically for NADPH diaphorase (*NADPH-D*) and immunohistochemically for NO synthase (*NOS*). NADPH diaphorase-positive cells overlapped with NO synthase-immunoreactive cells. *F* and *G*, Paired photomicrographs from the same microscopic field, showing relationships of cells stained immunohistochemically for parvalbumin (*PV*) and GABA. Parvalbumin-immunoreactive cells show GABA immunoreactivity. Scale bar, 50 μ m.

FS cell (local dend.)

Axon

FS cell (extended dend.)

Dend.

Axon

200 μ m

Figure 10. Reconstruction of an FS cell with local dendritic field (*local dend.*) and an FS cell with extended dendritic field (*extended dend.*). Dendrites (*Dend.*) and axons (*Axon*) are separately drawn. For comparison of morphological differences among three classes of neostriatal interneurons, Figures 10, 12, and 13 have the same calibration. Axonal origins are indicated by arrows. An FS cell with local dendritic field had smallest dendritic fields. Local axon collaterals were ramified extensively close to the dendritic field. Axon collaterals of an FS cell with extended dendritic field also ramified extensively in similar spatial extent to dendrites.

NADPH diaphorase-positive cells have medium-sized perikarya (Vincent et al., 1983; Cowan et al., 1990; Kita et al., 1990). Taken together with the morphological and physiological characteristics of FS cells, PLTS cells, and LA cells described above, these data suggest that FS cells may contain parvalbumin, LA cells may express ChAT, and the remaining PLTS cells may correspond to NADPH diaphorase-positive cells.

To test the hypothesis that each physiologically different class of interneurons corresponds to one of the three separate histochemical classes, histochemical staining was combined with intracellular staining. Physiologically identified neostriatal cells injected with biocytin were visualized by Texas red-conjugated avidin (red fluorescence) (Fig. 8*A,C,E*). FS cells and LA cells were stained immunohistochemically for parvalbumin and ChAT, respectively. Parvalbumin and ChAT immunoreactivities in recorded cells were visualized by fluorescein-conjugated secondary antibodies (green fluorescence) (Fig. 8*B,F*). PLTS cells were stained histochemically for NADPH diaphorase. NADPH diaphorase activity could be seen as a blue reaction product in bright field.

Among 11 FS cells tested, 10 cells showed parvalbumin immunoreactivity (Fig. 8*A,B*). Among nine PLTS cells tested, seven cells showed NADPH diaphorase activity (Fig. 8*C,D*). Among 15 LA cells tested, 14 cells showed ChAT immunoreactivity (Fig. 8*E,F*). As a control, seven biocytin-loaded MS cells were histochemically stained for parvalbumin, seven for NADPH diaphorase, and five for ChAT. No tested MS cells were stained for any of these substances. Five parvalbumin-immunoreactive FS cells were also further stained histochemically for NADPH diaphorase. All five FS cells tested were negative for NADPH diaphorase histochemistry.

Triple immunofluorescence methods were used to determine whether these three groups belong to separate populations (Fig. 9*A,B,C*). Because cells stained by NADPH diaphorase histochemistry were also stained by NO synthase immunohistochemistry (Fig. 9*D,E*), the relationship of parvalbumin-, NO synthase-, and ChAT-immunoreactive cells could be studied by triple immunofluorescent staining. Parvalbumin-positive cells ($n = 244$), NO synthase-positive cells ($n = 144$), and ChAT-positive cells ($n = 139$) were always singly stained and were never immunoreactive for the other two antigens (Fig. 9*A–C*). It was also confirmed by double immunohistochemical staining that all parvalbumin-positive cells were immunoreactive for GABA (Fig. 9*F,G*).

These findings showed that three physiological classes of neostriatal interneurons were also unique chemically (FS cells are parvalbumin positive; PLTS cells are NADPH diaphorase positive; LA cells are ChAT positive).

Morphological characteristics of three classes of neostriatal interneurons

To compare the morphological differences among the three classes of neostriatal interneurons, reconstructions of cells are shown at the same magnification in Figures 10, 12, and 13. Dendrites and axons are separately drawn. Although morphological data, particularly the full extent of dendrites and axons, were limited by slice thickness, each class of interneurons showed a characteristic soma size and arborization of dendrites and axons.

FS cells. The somatic diameters of FS cells sampled in this study ranged from 13 to 28 μ m (mean = 17 μ m; Table 1). Two of 13 cells had diameters larger than 20 μ m. FS cells had round

or oval somata and three to eight smooth primary dendrites. FS cells seemed to be further divided into two classes depending on the pattern of dendrite extension. Most FS cells (FS cells with local dendritic field, $n = 10$) had very restricted dendritic fields compared to PLTS cells and LA cells (Fig. 10). Their dendritic fields were 200–300 μm . In this type of FS cell, the dendrites branched frequently, forming a compact and elaborate field. Local axon collaterals were much more dense than those of PLTS cells and LA cells and were very dense near the dendritic fields. Local axon fields extended to 400–600 μm in diameter of field. Some FS cells (FS cells with extended dendritic field, $n = 3$) had more extended dendrites (Fig. 10). Their dendritic fields were 500–600 μm . However, they also had frequently branched dendrites and dense axonal branches close to the dendritic field.

PLTS cells. The somatic diameters of PLTS cells sampled in this study ranged from 12 to 25 μm (mean = 19 μm ; Table 1). Four of 11 PLTS cells had diameters larger than 20 μm . PLTS cells had fusiform or polygonal somata and two to five primary dendrites. The dendrites tended to radiate in straight lines a long way, and were slender and infrequently branched (Figs. 11, 12). The dendrites, to form a field, extended up to 600 μm in diameter. Two origins of axon-like processes from the somata and dendrites could be observed in some cases (Figs. 11, 12) (Takagi et al., 1984b). The axons also ran in straight lines, emitting collateral branches and extending to form a field more than 1000 μm in diameter (Fig. 12), which was the widest innervation among the three classes of interneurons.

LA cells. The somatic diameters of LA cells sampled in this study ranged from 20 to 40 μm (mean = 27 μm ; Table 1). The cells had polygonal or fusiform cell bodies with two to five primary dendrites. Compared to PLTS cells, the dendrites had more branching points and ran in more tortuous trajectories (Fig. 13). The dendrites extended to 600 μm , similar to those of FS cells with extended dendritic fields and to PLTS cells. The axons changed direction frequently and also took tortuous courses (Fig. 13). The axons extended to 500–700 μm , less than PLTS cells.

These findings showed that three classes of neostriatal interneurons with specific physiological and histochemical characteristics had different morphologies as regarding somatic sizes, branching patterns and field sizes of dendrites, and arborization patterns of axons.

Discussion

This study reveals that neostriatal interneurons can be differentiated into three classes differing in firing modes, chemical content, and territories of dendrites and axons (Fig. 14). FS cells fired very short-duration action potentials with short-duration afterhyperpolarizations at constant spike frequency during depolarizing current steps. FS cells had more negative resting potentials and lower input resistances than the other two classes. At depolarized potentials, FS cells fired repetitive spikes by synaptic excitation. FS cells were identified as parvalbumin-immunoreactive cells, which were also GABA immunoreactive. FS cells had densest innervation within or near the dendritic fields. PLTS cells and LA cells were distinguished from FS cells by longer-duration action potentials, larger input resistances, less negative resting potentials, and longer-lasting afterhyperpolarizations. PLTS cells had smaller time to peak of afterhyperpolarizations than LA cells. PLTS cells fired both Na^+ -dependent persistent depolarization spikes and Ca^{2+} -dependent

Figure 11. Photomicrograph of a PLTS cell with two axon-like processes. Two origins of axon-like processes indicated by arrowheads and axon-like processes are indicated by arrows. Scale bar, 50 μm .

low-threshold spikes in addition to fast spikes. Both persistent depolarizations and low-threshold spikes could also be evoked by synaptic activation. PLTS cells were histochemically identified as NADPH diaphorase-positive cells. As NADPH diaphorase-positive cells were also immunoreactive for NO synthase,

PLTS cell

Figure 12. Reconstruction of a PLTS cell with two axon-like processes. Dendrites (*Dend.*) and two axon-like processes (*Axon-1*, *Axon-2*) are separately drawn. Axonal origins are indicated by arrows. Axons of PLTS cells were most widely distributed. Axons tended to run straight.

LA cell

Figure 13. Reconstruction of an LA cell. Dendrites (*Dend.*) and axons (*Axon*) are separately drawn. Axonal origin is indicated by arrow. LA cells were largest cells. Compared to PLTS cells, dendritic and axonal courses were more tortuous than PLTS cells.

Figure 14. Schematic summary of morphologies, synaptic firing patterns, and neurochemical substances and putative transmitters that the three classes of neostriatal interneurons contain. Dendrites are drawn by solid lines. Axonal drawings are omitted. FS cells were divided into two morphological classes with different dendritic fields. FS cells with local dendritic field (*top*) and FS cells with extended dendritic field (*bottom*). Both types of FS cells innervated more densely within or close to the dendritic field than PLTS cells and LA cells. FS cells were parvalbumin (PV)-immunoreactive cells that were GABAergic. FS cells showed repetitive firing induced by synaptic activation (arrowhead) at depolarized potentials, which ceased abruptly after a short time. PLTS cells innervated wider areas, in some cells with two axon-like processes with different origins. PLTS cells were NADPH diaphorase (NADPH-D)-positive cells, which were considered to release NO. PLTS cells showed Na^+ -dependent persistent depolarization. Ca^{2+} -dependent low-threshold spikes were also induced by synaptic activation (arrowhead) at hyperpolarized potentials. LA cells were large aspiny cells with diameter larger than 20 μm . Because LA cells were immunostained for ChAT, they were considered to release ACh. LA cells showed long-lasting afterhyperpolarization following spikes, which presumably prevented repetitive firing by synaptic excitation (arrowhead).

PLTS cells were considered to release NO. PLTS cells had the largest axonal fields. Some PLTS cells appeared to have two axonal origins from the somata and/or dendrites. LA cells were large aspiny cells with long-lasting afterhyperpolarizations and strong time-dependent hyperpolarizing rectification. LA cells were identified as ChAT-immunoreactive cells, and are therefore considered to be cholinergic. All LA cells were larger than 20 μm in diameter. LA cells had more branched dendrites than PLTS cells. It was also immunohistochemically confirmed that parvalbumin-, NADPH diaphorase-, and ChAT-positive cells belonged to separate neuronal populations.

It is important to note that this study was carried out on tissue from juvenile rats (16–20 d old). It has been shown that membrane potentials and synaptic potentials of neostriatal MS cells mature gradually after birth (Misgeld et al., 1986). Input resistances of MS cells are higher, and fast anomalous rectification of MS cells is less prominent, in rats of postnatal days 7–20. These findings suggest that physiological parameters obtained here in other types may also vary somewhat from adult rats.

To sample a greater number of aspiny interneurons, larger cells were preferentially recorded. This might lead to a lack of recording from additional populations of smaller interneurons. Although parvalbumin-immunoreactive cells and NADPH diaphorase-positive cells contain populations with small somatic diameters similar to those of MS cells (Vincent et al., 1983; Kita et al., 1990), these may have physiological characteristics similar to those with larger diameters. However, there may be aspiny cells with diameters similar to or smaller than those of MS cells

and with physiological properties qualitatively different from those of the three types of aspiny cells described here. For example, neostriatal cells immunoreactive for cholecystikinin (Takagi et al., 1984a), vasoactive intestinal polypeptide (Theriault and Landis, 1987), or calretinin (Bennett and Bolam, 1993) are aspiny in type and have diameters similar to MS cells. Therefore, in addition to the three types of interneurons mentioned above, other physiological subclasses of aspiny cells may be found.

FS cells

From electrophysiological and parvalbumin-immunohistochemical data in this report, FS cells are considered to be GABAergic interneurons with phasic repetitive firing induced by synaptic activation at depolarized potentials. MS cells in the striatum receive disynaptic IPSPs from the cortex by a feed-forward pathway via striatal intrinsic neurons (Wilson et al., 1989; Pennartz and Kitai, 1991) and these IPSPs are blocked by bicuculline, a GABA_A receptor antagonist (Pennartz and Kitai, 1991). The cortical afferents end directly on parvalbumin-immunoreactive cells in asymmetric synapses, while the parvalbumin-immunoreactive terminals have symmetrical synapses on somata and dendrites of the neostriatal cells, probably including MS cells (Kita et al., 1990; Lapper et al., 1992). Therefore, FS cells may be excited by cortical afferents, mediate feed-forward GABA_A inhibition on neostriatal projection neurons, and thus regulate excitability or threshold of the output cells. As parvalbumin-immunoreactive fibers make synapses also on

spine-free dendrites (Kita et al., 1990), FS cells may also mediate inhibition on interneurons.

FS cells had very dense axon collaterals near their somata. The neostriatum has patch and matrix compartments that differ in histochemical characteristics and in the origin of their afferents (Graybiel, 1990; Gerfen, 1992). Because axonal branches were dense near the somata and not particularly widespread, each FS cell may inhibit neostriatal cells belonging to only the compartment in which it lies, patch or matrix. The other intra-striatal inhibitory interactions are lateral and recurrent connections among MS cells that are GABAergic (Park et al., 1980; Wilson and Groves, 1980; Somogyi et al., 1981). This inhibition is only activated when projection MS cells fire, which corresponds to feedback inhibition. It is very important to clarify different functional roles between feedforward inhibition by FS cells and feedback inhibition by MS cells.

Because FS cells could fire repetitively by synaptic activation at depolarized potentials, they should be able to inhibit neostriatal cells strongly. Neostriatal MS cells exhibit characteristic shifts of membrane potential between depolarized and hyperpolarized states *in vivo* (Wilson and Groves, 1981; Wilson, 1990). By their phasic repetitive firing, FS cells may suppress depolarization in the dendrites where prolonged depolarization may be caused by afferent inputs (Wilson, 1990) and change prolonged depolarizations to a long-lasting hyperpolarized state in MS cells, or may reduce spike firing in a prolonged episode of depolarization.

PLTS cells

PLTS cells exhibited unique firing properties due to Ca^{2+} -dependent low-threshold spikes and Na^{+} -dependent persistent depolarized spikes, in addition to Na^{+} -dependent fast spikes. PLTS cells were histochemically active for NADPH diaphorase. Because NADPH diaphorase is identical to NO synthase (Dawson et al., 1991), PLTS cells are considered to release NO within the neostriatum. These cells are also considered to contain neuropeptide Y (NPY) and somatostatin (Vincent et al., 1983; Dawson et al., 1991). However, a "classical" neurotransmitter in this group of cells has not been identified yet. There is one report that NPY-immunoreactive cells are also stained for GAD, the synthetic enzyme for GABA (Vuillet et al., 1990). If PLTS cells are also GABAergic, neostriatal GABAergic interneurons would be functionally divided into two classes by differences in firing patterns, innervation areas and co-released substances. As NPY-immunoreactive cells directly receive cortical inputs (Vuillet et al., 1989a) and NPY-immunoreactive terminals, like parvalbumin-immunoreactive ones, make synapses on perikarya and dendrites of neostriatal cells including MS cells (Vuillet et al., 1989b), PLTS cells are considered to regulate MS cells in a feedforward manner from the cortex, like FS cells. Thus, cortical cells may affect MS cells through two types of interneurons of medium size within the neostriatum.

Some PLTS cells had two origins of axon-like processes, which innervated different areas. In the neostriatum, a medium aspiny cell with two axons forming synapses has been reported by Golgi-impregnated material (Takagi et al., 1984b), which also suggests that some neostriatal interneurons can have two axons. In the visual cortex of the cat, nonpyramidal cells with two axon-like processes are also described (Mayer, 1982). In the neostriatum and the neocortex, a subpopulation of interneurons may have multiple axons.

NO may be released when PLTS cells are activated. NO is a

messenger molecule that is suggested to be involved in the regulations of blood flow, synaptic efficacy, and glutamate toxicity (Bredt and Snyder, 1992). Excitation in the corticostriatal pathway is mediated by excitatory amino acids released from cortical afferents onto both AMPA/kainate and NMDA receptor subtypes (Cherubini et al., 1988). Recently it has been shown that NO inhibits NMDA receptor function in cultured neostriatal cells (Manzoni et al., 1992). During prolonged depolarizations PLTS cells may produce NO, and modulate excitatory NMDA receptor-mediated transmission in MS cells over a wide area.

LA cells

Physiological properties of these large aspiny cells were qualitatively similar to those investigated previously (Jiang and North, 1991; Kawaguchi, 1992). In this article, these cells were identified as the cells immunoreactive for ChAT. Large afterhyperpolarizations following spikes and slowly occurring hyperpolarizing rectification were revealed here to be due to Ca^{2+} influx by spikes and I_h current, respectively. LA cells fire single spikes due to large afterhyperpolarizations that suppress repetitive firing. The morphological and electrophysiological data suggest that tonically active large aspiny cells described *in vivo* (Kimura et al., 1984; Wilson et al., 1990) are identical to LA cells, and may thus be cholinergic.

ChAT-immunoreactive cells receive thalamic inputs from the parafascicular nucleus of the thalamus, at least on their proximal dendrites (Lapper and Bolam, 1992). Compared with FS cells and PLTS cells, activities of LA cells may be influenced more by thalamic afferents. However, afferent fibers from the parafascicular nucleus almost exclusively innervate the matrix compartment (Herkenham and Pert, 1981; Sadikot et al., 1992) and distal dendrites of LA cells extend also into the patch compartment (Penny et al., 1988; Kawaguchi, 1992). LA cells innervate preferentially the matrix compartment (Kawaguchi, 1992). These data suggest that cholinergic LA cells may integrate input from both the thalamic axons at the matrix and the unidentified fibers in the patch, and affect projection cells in the matrix.

ChAT-immunoreactive terminals end on dendritic shafts and perikarya of MS cells in symmetrical synapses (Izzo and Bolam, 1988). It has been proposed that ACh stabilizes the state of MS cells, whether it be a depolarized or a hyperpolarized state (Akins et al., 1990). Firing of LA cells may thus stabilize membrane potential of nearby MS cells either at excitable states in which MS cells can fire or at a hyperpolarized state in which MS cells cannot fire. This suggests that membrane potentials and synaptic potentials in MS cells may be modulated differentially by three classes of interneurons, GABAergic FS cells, PLTS cells producing NO, and cholinergic LA cells.

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