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DIFFERENTIAL CHANGES OF SYNAPTIC TRANSMISSION IN SPINY NEURONS OF RAT NEOSTRIATUM FOLLOWING TRANSIENT FOREBRAIN ISCHEMIA

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Abstract—Spiny neurons in neostriatum are vulnerable to cerebral ischemia. To reveal the mechanisms underlying the postischemic neuronal damage, the spontaneous activities, evoked postsynaptic potentials and membrane properties of spiny neurons in rat neostriatum were compared before and after transient forebrain ischemia using intracellular recording and staining techniques *in vivo*. In control animals the membrane properties of spiny neurons were about the same between the left and right neostriatum but the inhibitory synaptic transmission was stronger in the left striatum. After severe ischemia, the spontaneous firing and membrane potential fluctuation of spiny neurons dramatically reduced. The cortically evoked initial excitatory postsynaptic potentials were suppressed after ischemia indicated by the increase of stimulus threshold and the rise time of these components. The paired-pulse facilitation test indicated that such suppression might involve presynaptic mechanisms. The inhibitory postsynaptic potentials in spiny neurons were completely abolished after ischemia and never returned to the control levels. A late depolarizing postsynaptic potential that was elicited from ~5% of the control neurons by cortical stimulation could be evoked from ~30% of the neurons in the left striatum and ~50% in the right striatum after ischemia. The late depolarizing postsynaptic potential could not be induced after acute thalamic transection. The intrinsic excitability of spiny neurons was suppressed after ischemia evidenced by the significant increase of spike threshold and rheobase as well as the decrease of repetitive firing rate following ischemia. The membrane input resistance and time constant increased within 6 h following ischemia and the amplitude of fast afterhyperpolarization significantly increased after ischemia.

These results indicate the depression of excitatory monosynaptic transmission, inhibitory synaptic transmission and excitability of spiny neurons after transient forebrain ischemia whereas the excitatory polysynaptic transmission in neostriatum was potentiated. The facilitation of excitatory polysynaptic transmission is stronger in the right neostriatum than in the left neostriatum after ischemia. The suppression of inhibitory component and the facilitation of excitatory polysynaptic transmission may contribute to the pathogenesis of neuronal injury in neostriatum after transient cerebral ischemia.

Key words: excitation, inhibition, cell death, lateralization, intracellular recording, *in vivo*.

The neostriatum is one of the discrete brain regions highly vulnerable to transient forebrain ischemia (Pulsinelli et al., 1982; Ren et al., 1997). It has been demonstrated that the spiny neurons in the striatum are vulnerable whereas cholinergic, somatostatin and neuropeptide Y-containing interneurons are resistant to schemic insult (Francis and Pulsinelli, 1982; Pulsinelli, 1985; Zoli et al., 1997). Spiny neurons are innervated by cortical and thalamic glutamatergic pathways (Fonnum et al., 1981; Kemp and Powell, 1971; Parent and Hazrati, 1995) and by nigrostriatal dopaminergic projections (Bjorklund and Lindvall, 1983; Hokfelt et al., 1976, 1977). Extracellular glutamate concentration dramatically increased in the striatum during cerebral ischemia (Globus et al., 1988; Obrenovitch et al., 1990) and glutamate excitotoxicity has been hypothesized as the major cause of neuronal injury following cerebral ischemia (Rothman and Olney, 1986; Choi and Rothman, 1990). The changes in cortically evoked synaptic transmission following ischemia, therefore, may provide important information regarding the excitotoxic effects on striatal neurons after transient forebrain ischemia.

A massive increase of extracellular dopamine has also been reported in the striatum during cerebral ischemia (Globus et al., 1988; Obrenovitch et al., 1990; Akiyama et al., 1991; Chang et al., 1993; Richards et al., 1993). Detrimental effects of excessive dopamine release during ischemia on postischemic cell death has been suggested by the finding that depletion of dopamine input to the striatum attenuates the degree of neuronal damage following ischemia (Weinberger et al., 1985; Globus et al.,

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Abbreviations: EPSP, excitatory postsynaptic potential; fAHP, fast afterhyperpolarization; IPSP, inhibitory postsynaptic potential; ISI, inter-stimulus inter-spike interval; KPBS, potassium phosphate-buffered saline; L-PSP, late depolarizing postsynaptic potential; MCAO, middle cerebral artery occlusion; PPF, paired-pulse facilitation.

1987a,b; Clemens and Phebus, 1988). However, other investigators fail to reproduce the protective effects on striatal neurons after dopamine depletion (Pulsinelli and Block, 1987; Wieloch et al., 1990). Such controversy has been resolved by recent studies demonstrating the asymmetrical effects of dopamine depletion on striatal neurons following ischemia (Ren et al., 1997; Xu et al., 1999). Injection of 6-hydroxydopamine into the right substantia nigra (SN), which destroys $>90\%$ of dopamine neurons, significantly reduces the cell death in the right striatum following ischemia while the same treatment on the left SN has no protection on neurons in the left striatum (Ren et al., 1997). Lateralization of the dopamine system in the nigrostriatal pathway has been under active investigation (Schneider et al., 1982; Sullivan and Szechtman, 1994) but little is known about the lateralization of the electrophysiology of spiny neurons before and after transient cerebral ischemia.

Many studies have investigated the electrophysiological changes associated with the pathogenesis of spiny neurons following cerebral ischemia. It has been shown that the membrane potential of spiny neurons depolarized during *in vitro* hypoxia and hypoglycemia while interneurons showed hyperpolarization (Calabresi et al., 1995, 1997a,b). Brief periods of aglycemia (5–10 min) caused depression of excitatory postsynaptic potentials (EPSPs) without any change in membrane potentials whereas longer periods of aglycemia (15–20 min) and brief periods of hypoxia (1–2 min) induced depression of EPSPs with membrane depolarization (Calabresi et al., 2000a,b). However, the changes in hypoxia/hypoglycemia in an *in vitro* preparation probably differ from the pathophysiological changes in ischemia *in vivo*. In addition, the electrophysiological changes during ischemia/hypoxia may not be the same as those after ischemia. In an attempt to address these issues, an *in vivo* intracellular recording study has been conducted to compare the neurophysiological changes in striatal neurons during and after transient forebrain ischemia (Xu, 1995). The spontaneous activity and cortically evoked EPSPs of spiny neurons are transiently suppressed following 5–8 min forebrain ischemia and the inhibitory postsynaptic potentials (IPSPs) also depress shortly following ischemia (Xu, 1995). The excitability of spiny neurons transiently decreases after ischemia. Because 5–8 min ischemia is insufficient to induce neuronal damage in neostriatum, the above observation may be the neuronal response to ischemic stress rather than the events associated with postischemic cell death. Further investigation is needed to study the neurophysiological changes following severe ischemia that will cause neuronal damage in neostriatum.

To reveal the electrophysiological changes associated with postischemic cell death in neostriatum, the present study examined the synaptic transmission and membrane properties of spiny neurons before and after severe transient forebrain ischemia using intracellular recording and staining techniques *in vivo*. Special attention was also paid to examine the cellular bases of lateralization by comparing the neuronal responses between the left and right striatum.

EXPERIMENTAL PROCEDURES

Transient forebrain ischemia

NIH guidelines for the care and use of laboratory animals were strictly followed. All efforts were made to minimize both the suffering and the number of animals used. The procedures were approved by the Institutional Animal Care and Use Committee of Indiana University School of Medicine. Male adult Wistar rats (220–350 g, Charles River, MA, USA) were used in the present study. Transient forebrain ischemia was induced using the four-vessel occlusion method (Pulsinelli and Brierley, 1979) with modification (Ren et al., 1997). The animals were fasted overnight to provide uniform blood glucose levels. For surgical preparation the animals were anesthetized with halothane. A silicon-tube loop was placed loosely around each common carotid artery to allow subsequent occlusion of these vessels. The animals were then placed on a stereotaxic frame and the core body temperature was maintained with a heating pad through a temperature control unit (TC-120, Medical System, NY, USA). Anesthesia was maintained with a mixture of 1–2% halothane, 33% O_2 and 66% N_2 via a gas mask placed around the nose of the animal. The vertebral arteries were electrocauterized. A very small temperature probe (0.025 inches in diameter, Physitemp) was inserted beneath the skull in the extradural space and the brain temperature was maintained at 37°C with a heating lamp using a temperature control system (BAT-10, Physitemp). Severe forebrain ischemia was induced by occluding both common carotid arteries to induce ischemic depolarization for $\sim$21 min (Ren et al., 1997). Cerebral blood flow resumed immediately upon release of the carotid artery clasps.

In vivo intracellular recording and staining

The animals were divided into two groups, one for control and one for ischemia. In the ischemia group, most of the recordings were performed immediately following ischemia to study the electrophysiological changes within 12 h after ischemia while some animals were returned to the cage after recovery from ischemia and anesthetized again to study the changes 12–24 h following ischemia. *In vivo* intracellular recording was performed as described in previous studies (Xu, 1995). In brief, the animals were anesthetized with a mixture of 1–2% halothane, 33% O_2 and 66% N_2 and the skull was opened to expose the cortex above the recording site and for placement of stimulus electrodes. One pair of bipolar stimulating electrodes was made from 000 stainless steel insect pins, insulated except for within 1.0 mm of the tips. The electrodes were separated by 1.0 mm and placed into the ipsilateral medial agranular cortical field at an angle of 30° to the vertical, 2.2 mm from the dural surface. Stimuli were constant current pulses of 0.1-ms duration ranging in amplitude from 0.1 to 3 mA. Recording electrodes were pulled from glass capillaries with a glass filament (A-M systems, WA, USA) using a vertical electrode puller (Kopf 750). The impedance of the electrodes was 40–70 MΩ when filled with a solution of 3–5% neurobiotin (Vector Laboratories, CA, USA) in 2 M potassium acetate. Cerebrospinal fluid was drained via a cisternal puncture at the posterior atlanto-occipital membrane to reduce brain pulsation and the animals were suspended by a clamp applied at the base of the tail. After placement of a microelectrode in the cortex above the neostriatum for recording, the exposed surface of the brain was covered with soft paraffin wax to reduce the brain pulsation. In order to record neurons in the dorsal striatum, the electrodes were advanced no more than 4.0 mm from the surface. After impalement, only neurons with stable membrane potentials of -50 mV or greater were selected for the study. In some animals, the neuronal activities in cortex above the striatum were recorded before and after ischemia. Recordings were digitized with data acquisition program Axodata (Axon Instruments, CA, USA) and stored on Macintosh computers for off-line analysis.

After each successful recording neurobiotin was iontophoresed into the cell by passing depolarizing current pulses (2 Hz,

300 ms, 0.5–1.3 nA) for 10–30 min. At the end of the experiment, the animal was deeply anesthetized and perfused transcardially with 0.01 M phosphate-buffered saline (pH 7.4) followed by 4% paraformaldehyde. The brain was removed and stored in fixative overnight. The brains were trimmed and parasagittal sections were cut at 50-μm thickness using a vibratome (Ted Pella Inc., CA, USA). The sections were incubated in 0.1% horseradish peroxidase-conjugated avidin-D (Vector) in 0.01 M potassium phosphate-buffered saline (KPBS, pH 7.4) with 0.5% Triton X-100 for 6–8 h at room temperature. After detection of peroxidase activity with 3′,3′-diaminobenzidine as the chromogen, the sections were examined in KPBS. Those sections containing labeled neurons and stimulation sites were mounted on gelatin-coated slides and counterstained with Cresyl Violet for light microscopy.

Acute thalamic lesions

In 10 animals an acute knife cut was made through the thalamus after the forebrain ischemia. The knife was a thin sheet of 3 mm wide plastic and cut to conform to the ventral contour of the thalamus. It was oriented mediolaterally and lowered stereotaxically into the thalamus at the rostral border of the parafascicular nucleus (anterior–posterior: 5.0–6.0; mediolateral: 0.5–1.5; dorsoventral: 7.0). It transected the thalamus except for 0.3–0.5 mm at the medial border and extended ventrally to cut the medial lemniscus and penetrated slightly into zona incerta (Wilson et al., 1983a).

Data analysis

The spontaneous membrane potential distribution was normalized and the weighted sum of three Gaussian distributions was used to fit the data (Reynolds and Wickens, 1999) by using Axograph 4.0 (Axon Instruments):

$$(a\exp((-1(x-b)(x-b))/(2cc))/(\mathrm{sqrt}(2\pi)c))+$$

$$(d\exp((-1(x-e)(x-e))/(2ff))/(\mathrm{sqrt}(2\pi)f))+$$

$$((1-(a+d))\exp((-1(x-g)(x-g))/(2hh))/(\mathrm{sqrt}(2\pi)h))$$

where:

Down state:

a = weighting factor for down state.
b = average potential.
c = variance.

Intermediate state:

d = weighting factor for intermediate state.
e = average potential.
f = variance.

Up state:

$(1-(a+d))$ = weighting factor for up state.
g = average potential.
h = variance.

Data was presented as mean ± S.E.M. Analysis of variance was used for statistical analysis using Statview 4.1 (Abacus Concepts).

RESULTS

The experiments were performed on 98 rats, of which 30 served as control animals and 68 were subject to forebrain ischemia that resulted in ischemic depolarization of 21.5 ± 0.8 min. Ischemia of such degree and duration consistently produced >90% cell death in dorsal striatum under halothane anesthesia (Ren et al., 1997).

Intracellular recordings were performed on 151 neurons in either left or right striatum, of which two were excluded from the present study because they were morphologically identified as aspiny neurons. A total of 149 neurons were selected, of which 60 were successfully stained and identified as spiny neurons (Fig. 1A). All histologically recovered neurons were located in the dorsal half of the neostriatum. They had round or oval somata with dendrites radiating in all directions. The dendrites were heavily loaded with spines except in their most proximal parts. Eighty-nine unidentified cells were considered as spiny neurons based on the electrode track, stereotaxic coordinates and characteristic spontaneous fluctuation of membrane potential between −60 and −90 mV. In some neurons recorded during 9–12 h after ischemia, signs of degeneration such as swollen stomata and beaded dendrites were observed (Fig. 1B). Intracellular recording became extremely difficult 12 h after ischemia. Recordings were attempted in five ani-

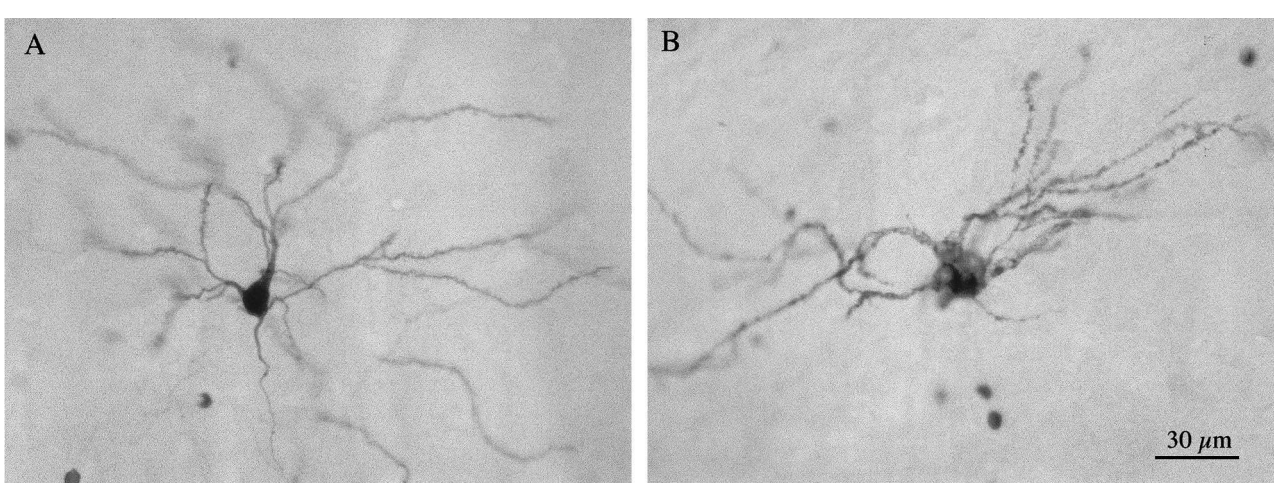

Fig. 1. Photomicrographs of intracellularly stained spiny neurons after recording. (A) A spiny neuron recorded from a control animal. The dendrites are loaded with dendritic spines. (B) A spiny neuron recorded ~10 h after ischemia. The cell body of this neuron is swollen and many dendrites show degenerating signs such as fragmentation and beaded appearance.

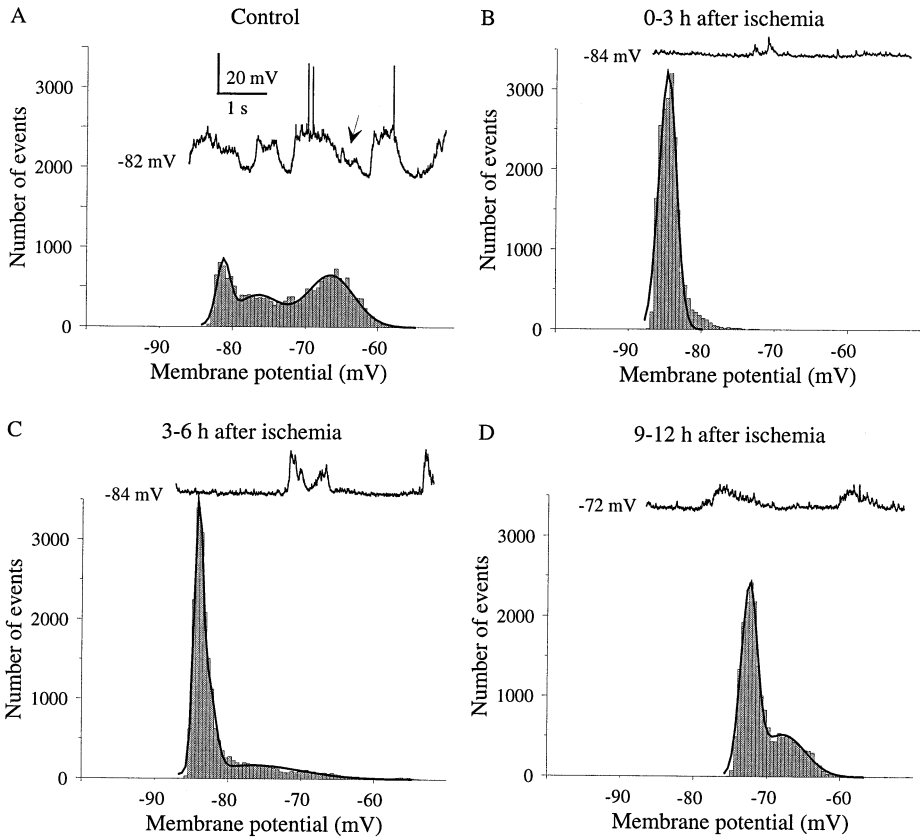

Fig. 2. The changes of spontaneous membrane potential fluctuation in spiny neurons before and after ischemia. Recordings of spontaneous activities were digitized at 1 kHz. Histograms represent the time spent at various membrane potentials. The insets are the original recording traces. Each histogram is 20 s of the membrane potential recordings in gray bars. The black lines are the best fitting Gaussians. (A) In control neurons, the membrane potential fluctuates between the hyperpolarizing down state (~−80 mV) and depolarizing up state (~−60 mV). Sometimes the membrane potential stays at the intermediate state (arrow). The three peaks of the histogram represent the down state, intermediate state and up state of the membrane potential of control neurons. (B) At 0–3 h after transient forebrain ischemia the spontaneous activity of spiny neurons was depressed with occasional membrane potential depolarization at the intermediate state or up state. (C) The spontaneous activity of spiny neurons slightly recovered at 3–6 h after ischemia. (D) The spontaneous activity of spiny neurons at 9–12 h after ischemia. The membrane potential became more depolarized and the histogram only showed two peaks.

mals 12–24 h after ischemia and extensive cell death was found in dorsolateral striatum with hematoxylin and eosin staining. We therefore focused on the electrophysiological changes in spiny neurons during the first 12 h following ischemia. Neurons were divided into five groups based on the time of recording (control, 0–3, 3–6, 6–9 and 9–12 h after ischemia). In three postischemia neurons, the continuous intracellular recording lasted for 4–6 h and the temporal changes of these neurons coincided with the changes across the groups.

Changes of spontaneous membrane potential fluctuation following ischemia

In control animals the spontaneous membrane potential of spiny neurons fluctuates mainly between two subthreshold states. The quiescent hyperpolarized down state and the noisier depolarized up state are separated by 15–30 mV (Wilson, 1993). The action potentials are generated from the up state. Sometimes the membrane potential rests at a level between these two states and is

Table 1. Properties of up and down states of spiny neurons before and after ischemia

	V_m down (mV)	V_m intermediate (mV)	V_m up (mV)	Percentage (%) of events at		
				down	intermediate	up
Control	−81 ± 8.09 (46)	−76 ± 8.05 (46)	−66 ± 7.66 (46)	16 ± 0.09 (46)	29 ± 0.14 (46)	54 ± 0.19 (46)
0–3 h	−80 ± 6.00 (22)	−76 ± 2.63 (8)	−66 ± 3.66 (9)	92 ± 0.09* (22)	5 ± 0.08* (22)	3 ± 0.06* (22)
3–6 h	−79 ± 6.16 (52)	−76 ± 5.85 (27)	−69 ± 6.45 (19)	85 ± 0.19* (52)	9 ± 0.11* (52)	6 ± 0.11* (52)
9–12 h	−72 ± 1.23* (11)	–	−67 ± 1.33 (8)	81 ± 0.48* (11)	–	19 ± 0.48* (11)

Values are mean ± S.E.M.; with number of neurons in parentheses. V_m, membrane potential; intermediate, intermediate state; down, hyperpolarized down state; up, depolarized up state. *$P < 0.01$.

called the intermediate state (Reynolds and Wickens, 1999). To quantitatively analyze the spontaneous membrane potential fluctuations, the histograms were constructed by sampling the recordings of spontaneous activity at 1 kHz and fitted by Gaussian's distribution (Reynolds and Wickens, 1999) (Fig. 2A). This gives the proportion of the time spent by the neuron at each membrane potential. The spontaneous membrane potential fluctuation ceased immediately following ischemia leaving the neurons at the hyperpolarized state. Approximately 50% of the neurons slowly recovered the spontaneous membrane potential fluctuation showing short periods of depolarization at the intermediate state or up state following ischemia. The time of membrane potential staying at the up state increased from 3% at 0–3 h after ischemia to 6 and 14% at 3–9 and 9–12 h after ischemia, respectively (Fig. 2, Table 1). The membrane potential at down state gradually depolarized from −81 mV of control level to −72 mV at 9–12 h after ischemia ($P < 0.01$) and the intermediate state virtually disappeared at this time (Table 1).

Studies have demonstrated that the continuous membrane potential fluctuation of spiny neurons between the up state and down state is influenced by activities of corticostriatal projection neurons (Wilson, 1994). To determine whether the postischemia change in membrane potential fluctuation in spiny neurons is due to the changes in cortex or in striatum, the spontaneous activity of cortical neurons was compared before and after ischemia. The depolarized up state of cortical neurons was totally suppressed after ischemia and the resting membrane potential became more hyperpolarized ($n = 5$, Fig. 3).

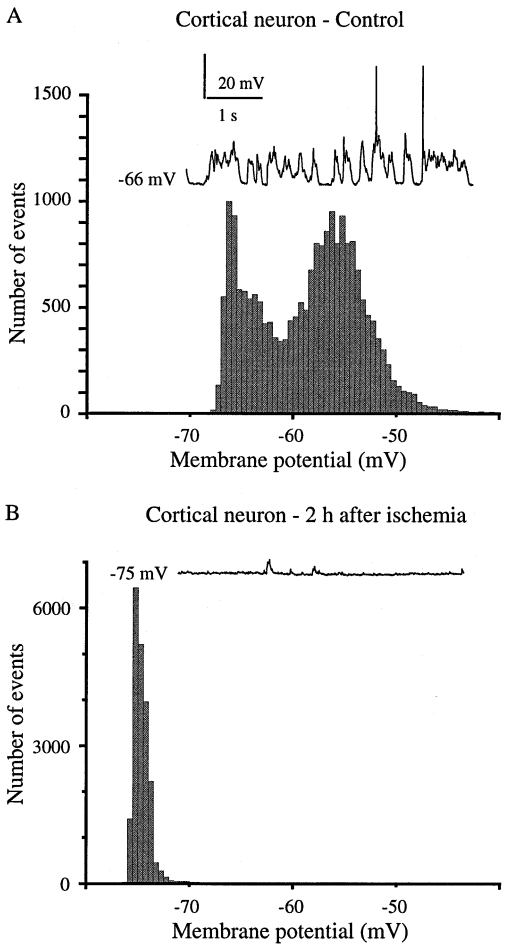

Fig. 3. Spontaneous membrane potential fluctuation of cortical neurons. Recordings of spontaneous activities were digitized at 1 kHz. Histograms represent the time spent at various membrane potentials. The inserts are the original recording traces. Each histogram is 20 s of the membrane potential recordings in gray bars. (A) Recordings from a control neuron showing the spontaneous membrane potential fluctuation with action potentials generated at depolarizing state. The histogram shows two peaks indicating the depolarizing up state and hyperpolarizing down state. (B) Recording from a cortical neuron 2 h after ischemia showing the dramatic depression of spontaneous activities. The histogram shows only one peak at the hyperpolarizing down state.

Changes of EPSPs following ischemia

EPSPs in spiny neurons were evoked by stimulation of ipsilateral medial agranular cortex. In control neurons the initial EPSPs were followed by a long-lasting hyperpolarization and a subsequent rebound excitation (Fig. 4Aa). The synaptic transmission of spiny neurons was compared between the left and right striatum in control animals. No significant difference in spontaneous firing rate was found between neurons in the left and right striatum. The EPSPs were elicited with a stimulus intensity 2.5 times as strong as the threshold stimulus intensity (2.5 T). The stimulus threshold, the amplitude and duration of initial EPSPs were about the same between the neurons in the left and right striatum (Table 2). The same comparison was made in neurons after ischemia and no significant difference in these parameters was found between left and right striatum either. Therefore, the neurons in both left and right striatum were pooled together for statistical analysis unless otherwise noted.

In addition to the initial EPSPs, a late depolarizing

Table 2. Synaptic transmission of spiny neurons in the left and right neostriatum

	Spontaneous firing rate (Hz)	T_{EPSP} (mA)	Initial EPSP			
			latency (ms)	amplitude (mV)	rise time (ms)	duration (ms)
Left	0.56 ± 0.18 (22)	0.45 ± 0.02 (16)	4.00 ± 0.22 (20)	9.87 ± 1.25 (22)	6.64 ± 0.53 (22)	20.32 ± 1.41 (22)
Right	0.47 ± 0.11 (31)	0.37 ± 0.03 (31)	3.66 ± 0.23 (30)	8.20 ± 0.67 (31)	6.38 ± 0.42 (31)	22.07 ± 1.53 (31)

Values are mean ± S.E.M.; with number of neurons in parentheses. T_{EPSP} is the threshold stimulus intensity of EPSP. The initial EPSPs are elicited with 2.5T stimulus intensity. Rise time is measured from 10 to 90% of the peak amplitude. Duration is measured at half of its peak amplitude.

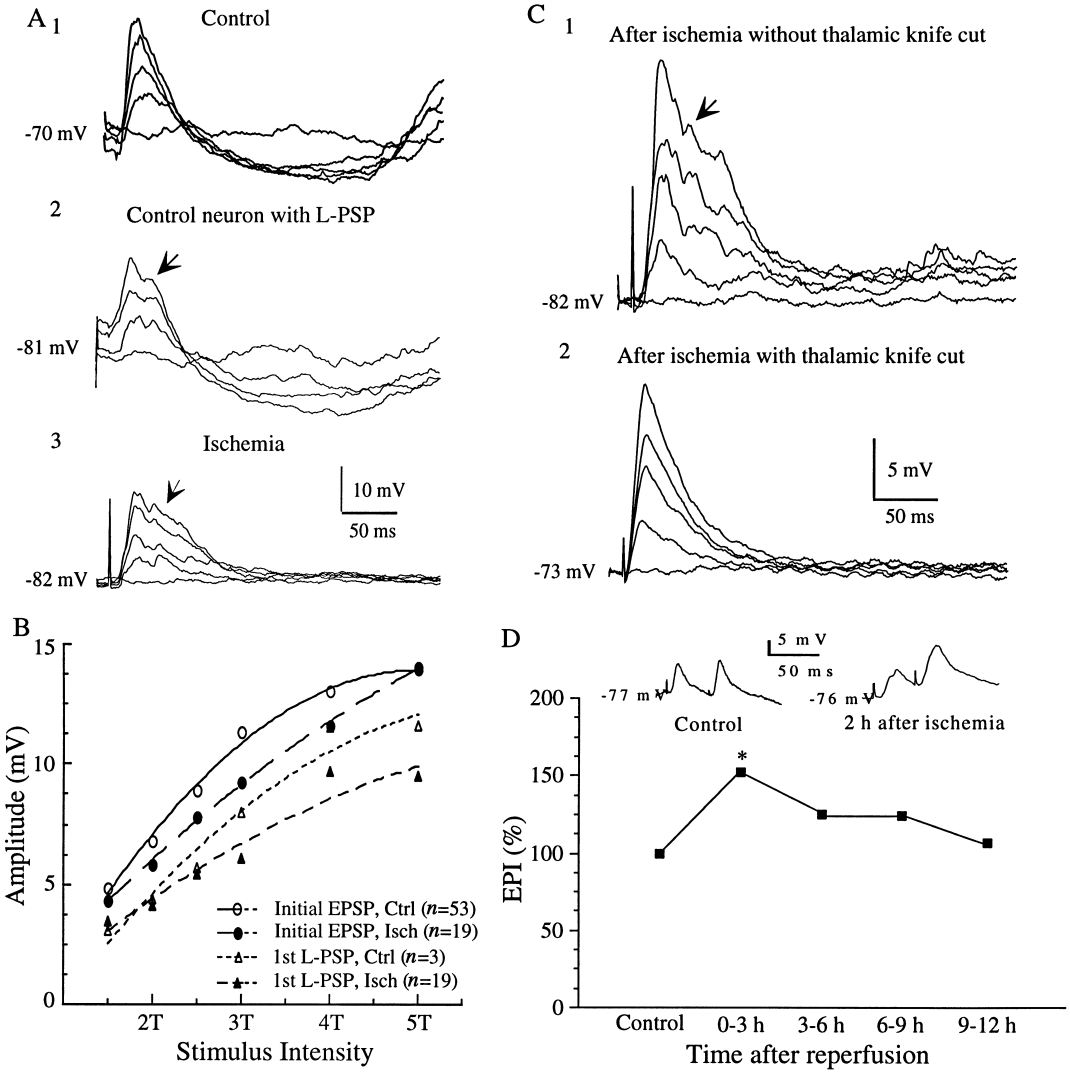

Fig. 4. The changes of evoked postsynaptic potential in striatum after cerebral ischemia. (A) Cortically evoked postsynaptic potentials of spiny neurons before and after transient forebrain ischemia. (1) Recordings from a control neuron in which stimulation of ipsilateral medial agranular cortex elicited an initial EPSP followed by a hyperpolarization and rebound excitation. (2) In a small percentage of control neurons L-PSP was evoked in addition to initial EPSP upon cortical stimulation. (3) An example of an L-PSP neuron recorded after ischemia. The number of L-PSP neurons significantly increased in striatum after transient cerebral ischemia. (B) The plots shows the relationship between stimulus intensity and the amplitude of initial EPSPs and L-PSPs. The values in the plotting are means. The stimulus intensity is the magnitude of the threshold (T) for inducing the initial EPSPs. The amplitude of initial EPSPs and L-PSPs decreased after ischemia. The numbers of recorded neurons are in the parentheses. (C) Cortically evoked postsynaptic potentials recorded after transient ischemia. (a) After ischemia, cortical stimuli elicited L-PSPs following the initial EPSPs. (b) After acute thalamic transection, no L-PSP was elicited from spiny neurons upon cortical stimulation. (D) The PPF test of spiny neurons before and after ischemia. The inset shows the representative responses recorded in neurons before and 2 h after ischemia. Paired-pulse stimulation was delivered with an inter-stimulus interval of 40 ms. The plotting shows the temporal changes of paired-pulse EPSP facilitation index (EPI) following ischemia. EPI is defined as the ratio of testing EPSP amplitude to the conditioning EPSP amplitude. Each point is the mean value of EPI. The EPI was significantly higher at 0–3 h after ischemia and gradually returned to the control levels. All representative traces in this figure are the average of four individual recordings.

postsynaptic potential (L-PSP) was evoked from a small portion of spiny neurons in control animals (3/53, Fig. 4A2). The L-PSPs had more than one component with the latency to peak of 30–50 and 55–75 ms for the first and second component, respectively. The latency of L-PSPs decreased with increasing stimulus intensity indicating that they were polysynaptic events. The amplitude of L-PSPs increased with increasing stimulus intensities (Fig. 4B). The amplitude of L-PSP was potentiated by paired-pulse with inter-stimulus interval (ISI) between 100 and 140 ms (Fig. 5A). After severe forebrain ischemia, the number of neurons with L-PSP significantly increased in the striatum. The proportion of L-PSP neurons increased to ~30% (14/52) in the left striatum and ~50% (12/25) in the right striatum, respectively. The percentage of neurons exhibiting L-PSP was different at different intervals following ischemia (0–3 h: 25%; 3–6 h: 50%; 6–9 h: 25%; 9–12 h: 0%). Such tem-

Table 3. Synaptic transmission of spiny neurons before and after ischemia

	Spontaneous firing rate (Hz)	T_{EPSP} (mA)	Initial EPSP			
			latency (ms)	amplitude (mV)	rise time (ms)	duration (ms)
Control	0.51 ± 0.09 (53)	0.44 ± 0.02 (52)	3.80 ± 0.16 (50)	8.90 ± 0.65 (53)	6.49 ± 0.32 (53)	21.35 ± 1.07 (53)
0–3 h	0.00 ± 0.00** (26)	0.60 ± 0.04* (25)	3.76 ± 0.36 (25)	7.20 ± 0.75 (26)	13.48 ± 1.99** (26)	43.22 ± 3.96** (16)
3–6 h	0.01 ± 0.00** (41)	0.51 ± 0.04 (41)	3.53 ± 0.24 (39)	8.02 ± 0.64 (41)	10.17 ± 0.83** (41)	34.89 ± 2.33** (29)
6–9 h	0.01 ± 0.00** (18)	0.44 ± 0.06 (17)	3.35 ± 0.32 (17)	9.82 ± 1.06 (18)	8.33 ± 0.98 (18)	28.17 ± 5.04 (10)
9–12 h	0.00 ± 0.00** (11)	0.58 ± 0.10* (11)	3.10 ± 0.10 (10)	7.06 ± 1.25 (10)	10.98 ± 1.50* (10)	24.04 ± 3.41 (9)

Values are mean ± S.E.M.; with number of neurons in parentheses. T_{EPSP} is the threshold stimulus intensity of EPSP. The initial EPSPs are elicited with 2.5T stimulus intensity. Rise time is measured from 10 to 90% of the peak amplitude. Duration is measured at half of its peak amplitude. *$P < 0.05$; **$P < 0.01$.

poral distribution of L-PSP neurons was about the same in both left and right striatum. The amplitude of first L-PSPs was decreased after ischemia (Fig. 4B).

The appearance of L-PSPs in spiny neurons after ischemia resembled the polysynaptic EPSPs evoked by thalamic stimulation in decorticated animals (Wilson et al., 1983a). To reveal the origin of L-PSPs in postischemia neurons, a transection was made in the thalamus after ischemia in 10 animals. Intracellular recording was performed on 12 spiny neurons at various intervals after ischemia. No L-PSP was elicited from these neurons suggesting that the postischemic L-PSPs were mediated by cortico–thalamo–striatal circuits (Fig. 4C).

The spontaneous firing rate and cortically evoked initial EPSPs of control neurons were compared with those recorded at different intervals after ischemia (Table 3). The spontaneous firing was completely abolished following ischemia and never returned to control levels. The stimulus threshold for inducing initial EPSPs significantly increased following ischemia (0.44 ± 0.02 mA for control versus 0.60 ± 0.04 mA at 0–3 h reperfusion, $P < 0.01$) with a transient return to control level at 6–9 h after ischemia. The amplitude of initial EPSPs was decreased ~20% at 0–3 and 9–12 h after ischemia and the rise time of initial EPSPs significantly increased at all time points after ischemia. The duration of initial EPSPs was measured in postischemia neurons without L-PSP and compared with that of control ones. The result indicated a significant increase in EPSP duration within 6 h after ischemia. All the above data indicated the depression of excitatory monosynaptic transmission after transient forebrain ischemia.

To determine whether the depression of initial EPSPs after transient ischemia is mediated through presynaptic or postsynaptic mechanism, paired-pulse facilitation (PPF) test was conducted to reveal the temporal changes of PPF of these neurons following ischemia. As shown in Fig. 4D, the PPF index increased by 50% ($P < 0.01$) shortly after ischemia and gradually returned to control levels at 12 h after ischemia suggesting the involvement of presynaptic mechanisms.

Changes of IPSPs following ischemia

The IPSPs in spiny neurons are masked by initial EPSPs. The paired-pulse test with different ISI showed a significant decrease in amplitude of testing EPSPs at 20 ms ISI in control neurons (~35% as the amplitude of conditioning EPSPs) indicating a short duration IPSP (Fig. 5A). In control animals this short duration IPSP was stronger in the left striatum (20 ± 6.30% as the conditioning one, Fig. 5D) than in the right striatum (46 ± 6.38% as the conditioning one, $P < 0.05$). Shortly after ischemia, the amplitude of testing EPSPs at 20 ms ISI dramatically increased to ~170% as the conditioned ones suggesting not only the disappearance of IPSPs but also a facilitation of synaptic transmission at this time (Fig. 5B, C, E). Then the amplitude of testing EPSPs at 20 ms ISI returned to the level of conditioning EPSPs before being potentiated again at 9–12 h after ischemia. At no time after ischemia the amplitude of testing EPSPs at 20 ms ISI returned to the control levels suggesting the complete abolishment of inhibitory components in neostriatum by ischemic insult.

Changes of membrane properties following ischemia

No difference in membrane properties of spiny neurons was found between the left and right neostriatum before (Table 4) and after transient cerebral ischemia.

Table 4. Membrane properties of spiny neurons in left and right neostriatum

	RMP (mV)	H_{spk} (mV)	W_{spk} (ms)	T_{spk} (mV)	R_{in} (MΩ)	T_{con} (ms)	Rheobase (nA)	fAHP (mV)
Left	−74 ± 1.56 (27)	76 ± 1.94 (27)	1.29 ± 0.07 (26)	−50 ± 0.89 (27)	30 ± 1.19 (25)	6.14 ± 0.60 (24)	0.36 ± 0.04 (27)	7.98 ± 0.60 (27)
Right	−75 ± 0.87 (28)	77 ± 2.54 (28)	1.16 ± 0.06 (26)	−51 ± 0.99 (28)	29 ± 1.83 (25)	6.59 ± 0.62 (25)	0.36 ± 0.04 (28)	6.30 ± 0.59 (25)

Values are mean ± S.E.M.; with number of neurons in parentheses. RMP, resting membrane potential. H_{spk}, spike height is measured from the resting membrane potential. W_{spk}, spike width is measured at the base of the action potential. T_{spk}, spike threshold is measured at the beginning of the upstroke of the action potential. R_{in}, input resistance is derived from the linear portion of the I–V curve (0–0.5 nA). T_{con}, time constant is derived from transients of hyperpolarizing pulses (−0.3 nA, 200 ms). fAHP, fast afterhyperpolarization is measured as deviation from the beginning of the upstroke of an action potential within 5 ms after the peak of a single spike.

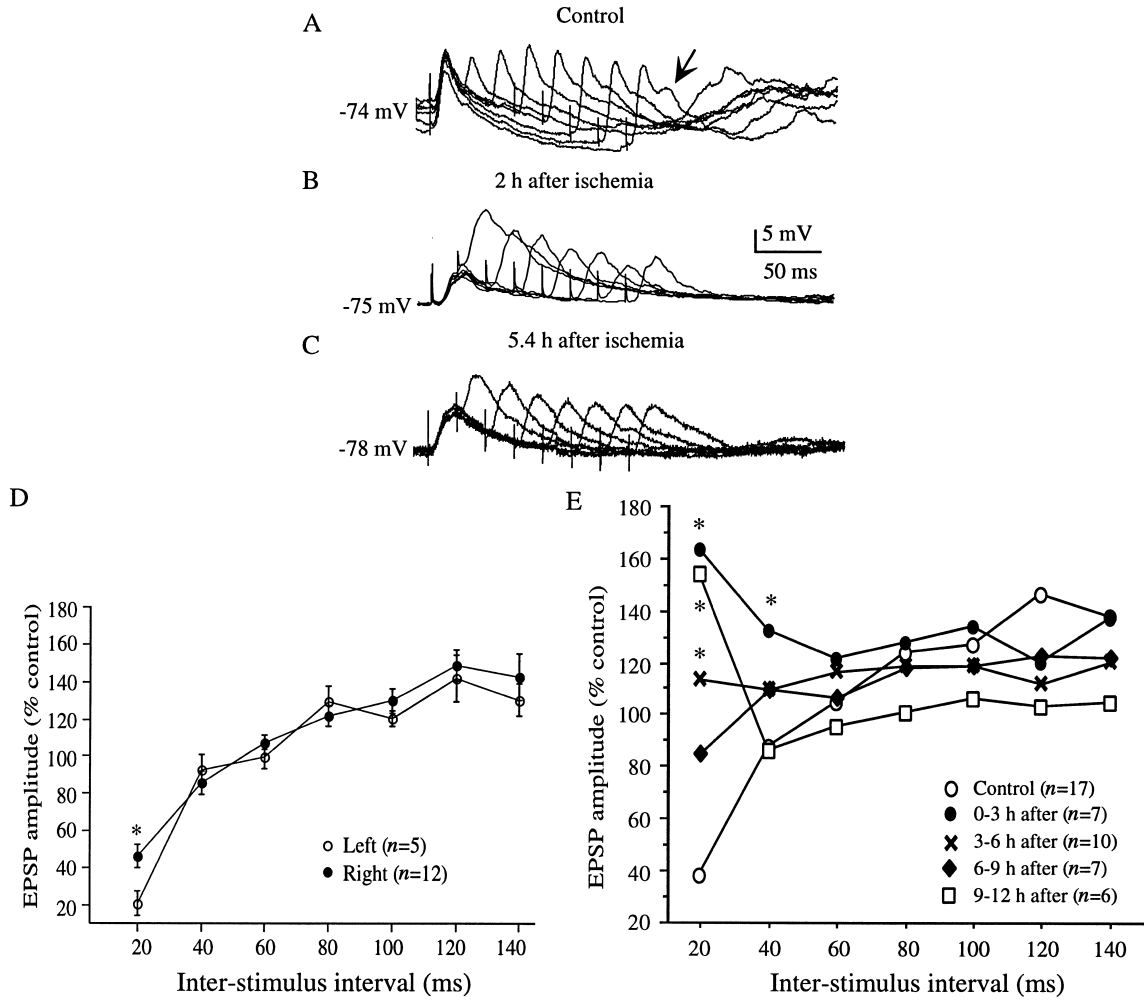

Fig. 5. Responses of spiny neurons to cortically evoked paired-pulse stimulation with different ISIs before and after ischemia. (A) Superimposed recordings from a control neuron showing the responses to paired-pulse stimulation. The amplitude of initial EPSPs elicited by testing stimulation at 20 ms ISI was much smaller than that of conditioning one. The L-PSPs were potentiated at an ISI of 100–140 ms (arrow). (B) Responses to paired-pulse stimulation from a neuron recorded 2 h after ischemia. The amplitude of initial EPSPs elicited by testing stimulation at 20 ms ISI was much larger than that of the conditioning one. (C) Recordings from a neuron 5.4 h after ischemia. The amplitude of testing initial EPSPs was smaller than that in B but still showed a significant facilitation. (D) Plots showing the difference in amplitude of testing EPSPs between the left and right striatum. The EPSP amplitude of 20 ms ISI in the left striatum was significantly smaller than that of the right striatum ($P<0.05$) suggesting the stronger inhibitory components in the left side. (E) Plots showing the relationship between ISIs and the percentage of testing EPSP amplitude to the conditioning EPSP amplitude. The values in the plotting are means. The amplitude of EPSPs elicited by the testing stimulation of 20 ms ISI within 6 h after ischemia was significantly larger than that of control ($P<0.01$). No significant difference in EPSP amplitude was found when the ISI was longer than 40 ms.

Therefore, neurons from both left and right striatum were grouped together in Table 5. The resting membrane potential of spiny neurons hyperpolarized immediately after ischemia and then slowly depolarized. The spike threshold significantly increased from -51 ± 0.66 mV before ischemia to -47 ± 1.01 mV shortly after ischemia ($P<0.01$) and remained at this level up to 12 h after ischemia. The rheobase also significantly increased up to 9 h after ischemia before returning to control levels.

The current–voltage relationship of spiny neurons was compared before and after ischemia (Fig. 6). As shown in Fig. 6C, the outward rectification at the depolarizing extreme was reduced immediately after ischemia and slowly returned to the preischemic level. The inward rectification in the hyperpolarizing direction also reduced after ischemia. The membrane input resistance of spiny neurons was 30 ± 1.08 MΩ in control animals and increased to 34 ± 1.57 and 36 ± 1.31 MΩ within 0–3 and 3–6 h after ischemia, respectively ($P<0.05$; Table 5). The input resistance returned to preischemic level 9–12 h after ischemia. The time constant increased from 6.37 ± 0.43 ms at control level to 7.52 ± 0.39 and 7.41 ± 0.23 ms at 0–3 and 3–6 h after ischemia, respectively, and gradually returned to control levels thereafter.

The repetitive firing pattern of spiny neurons was investigated by application of depolarizing current pulses to generate a spike train (400 ms, $0.1\sim2.0$ nA). Representative recordings at different time points are shown in Fig. 7A–C. The action potentials were followed by fast afterhyperpolarizations (fAHPs). The spike frequency

Table 5. Membrane properties of spiny neurons before and after ischemia

	RMP (mV)	H_{spk} (mV)	W_{spk} (ms)	T_{spk} (mV)	R_{in} (MΩ)	T_{con} (ms)	Rheobase (nA)	fAHP (mV)
Control	−74 ± 0.89 (55)	76 ± 1.59 (55)	1.22 ± 0.05 (54)	−51 ± 0.66 (55)	30 ± 1.08 (50)	6.37 ± 0.43 (49)	0.36 ± 0.26 (55)	7.18 ± 0.43 (52)
0–3 h	−82 ± 1.32** (31)	84 ± 1.65* (31)	1.40 ± 0.06** (30)	−47 ± 1.01** (31)	34 ± 1.57* (30)	7.52 ± 0.39* (30)	0.74 ± 0.06** (31)	12.25 ± 0.79** (31)
3–6 h	−76 ± 1.11 (53)	77 ± 3.69 (49)	1.36 ± 0.04* (49)	−45 ± 0.94** (50)	36 ± 1.31** (52)	7.41 ± 0.23* (52)	0.63 ± 0.04** (50)	11.38 ± 0.49** (50)
6–9 h	−71 ± 2.15 (20)	78 ± 1.83 (20)	1.32 ± 0.08 (20)	−43 ± 1.52** (20)	33 ± 1.74 (18)	7.21 ± 0.59 (18)	0.71 ± 0.08** (20)	9.96 ± 0.73** (20)
9–12 h	−67 ± 2.39** (12)	69 ± 3.06 (12)	1.31 ± 0.07 (12)	−47 ± 1.80 (12)	33 ± 2.76 (11)	5.57 ± 0.73 (11)	0.41 ± 0.07 (12)	8.91 ± 0.89 (12)

Values are mean ± S.E.M.; with number of neurons in parentheses. RMP, resting membrane potential. H_{spk}, spike height is measured from the resting membrane potential. W_{spk}, spike width is measured at the base of the action potential. T_{spk}, spike threshold is measured at the beginning of the upstroke of the action potential. R_{in}, input resistance is derived from the linear portion of *I–V* curve (0–0.5 nA). T_{con}, time constant is derived from transients of hyperpolarizing pulses (−0.3 nA, 200 ms). fAHP, fast afterhyperpolarization is measured as deviation from the beginning of the upstroke of an action potential within 5 ms after the peak of a single spike. *$P < 0.05$; **$P < 0.01$.

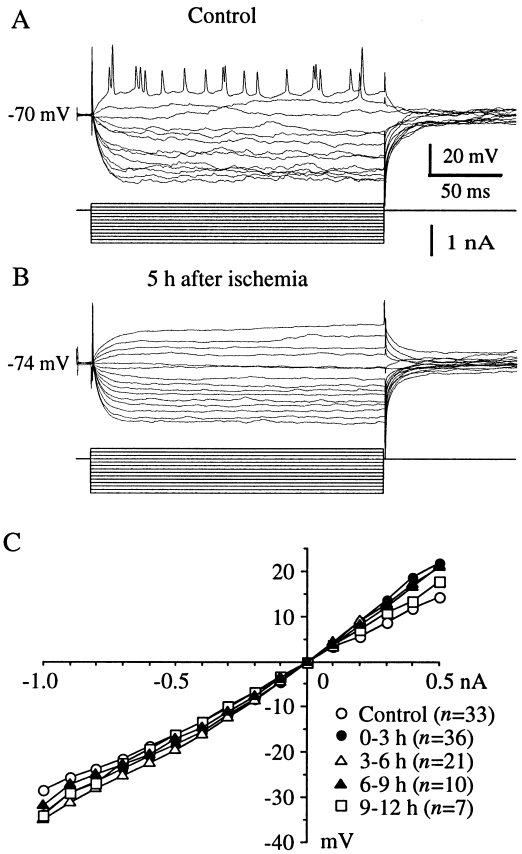

Fig. 6. Current–voltage (*I–V*) relationship of spiny neurons before and after ischemia. (A) Representative recording of a control neuron. Each trace is the average of four recordings. Top panels are membrane potential deflections caused by the current pulses. Bottom panels are intracellularly applied current pulses. The traces showed a noisy voltage deflection due to the spontaneous membrane potential fluctuations. Scales in A apply to B. (B) A recording of the *I–V* relationship from a neuron 5 h after ischemia. The spontaneous membrane potential fluctuation was significantly reduced after ischemia and the traces were smooth. (C) A plot of the *I–V* curve showing the changes at different intervals after ischemia. The outward rectification at the depolarizing extreme in control neurons was reduced following ischemia.

increased linearly with the increased current intensity. The spike frequency was significantly depressed within 6 h after ischemia and gradually returned to control level 9–12 h after ischemia (Fig. 7D, E). The amplitude of fAHP was measured as the deviation from the beginning of the upstroke of an action potential within 5 ms after the peak of the action potential. The amplitude of the fAHP significantly increased within 9 h after ischemia ($P < 0.01$) and returned to the control level 9–12 h after ischemia (Fig. 8).

DISCUSSION

Using *in vivo* preparation, the present study characterizes the electrophysiological changes in spiny neurons following severe forebrain ischemia and compares the differences between the left and right striatum. In control animals, no significant difference was found in most of the electrophysiological properties in spiny neurons

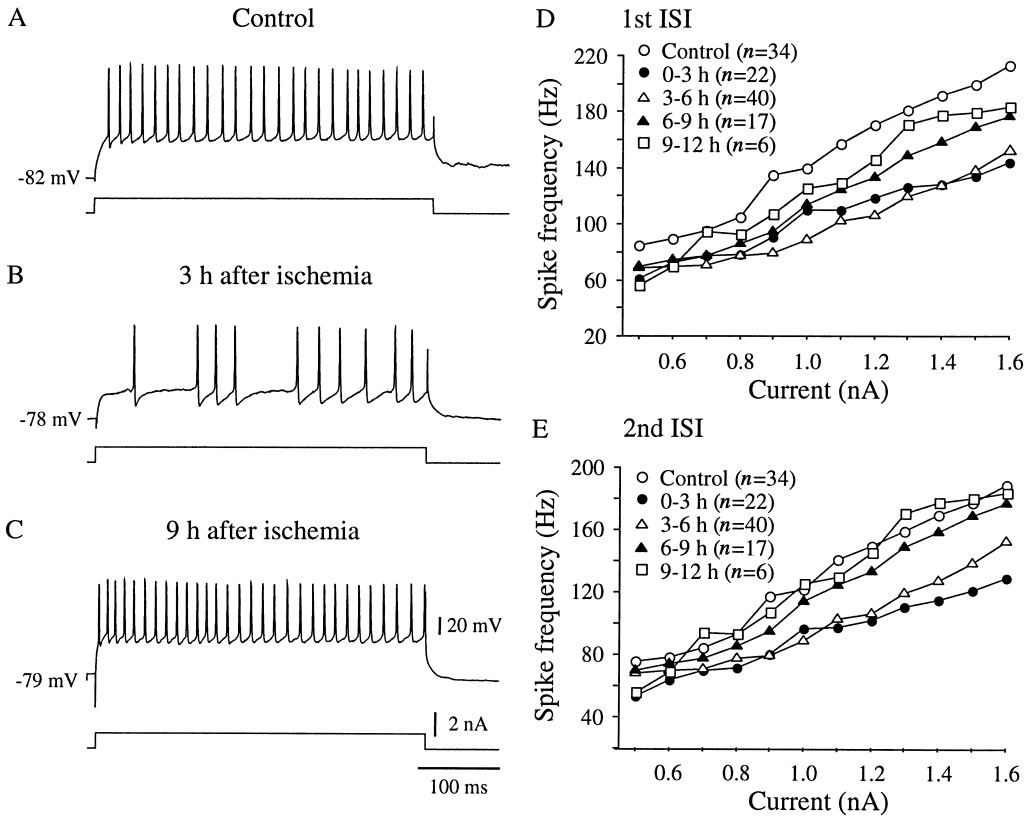

Fig. 7. The changes of repetitive firing pattern in spiny neurons after ischemia. (A–C) Representative recordings of repetitive firing induced by depolarizing current pulses in spiny neurons before ischemia and at different intervals after ischemia. The upper panels are the recordings of repetitive firing pattern. Bottom panels are intracellularly injected current pulses. At 3 h after ischemia (B), the spike frequency of spike train induced by 1 nA depolarizing current pulse was lower than the control one (A). The amplitude of fAHP significantly increased in B. At 9 h after ischemia (C), the spike frequency returned to the control levels. Scales in C apply to A and B. (D, E) Plots showing the relationship between spike frequency and depolarizing currents of first inter-spike interval (ISI, D) and second ISI (E) of spiny neurons before and after ischemia. Value of each individual plot is the mean. The spike frequency increased linearly with increasing current intensities. In comparison with control, the spike frequency was dramatically decreased within 6 h after ischemia. The spike frequency returned to the pre-ischemic level 9–12 h after ischemia.

between the left and right striatum. However, the inhibitory synaptic transmission was stronger in the left striatum. Despite the decrease of excitability and suppression of initial EPSPs, the excitatory polysynaptic transmission, such as L-PSPs, is facilitated in spiny neurons after ischemia. The facilitation of excitatory polysynaptic transmission after ischemia is more prevalent in the right striatum than in the left striatum. The IPSPs are completely abolished following ischemia. The suppression of inhibitory component and facilitation of excitatory polysynaptic transmission may be associated with the neuronal damage in neostriatum following transient forebrain ischemia.

Lateralization of electrophysiological responses in neostriatum

Neuroanatomical, neurochemical, and behavioral asymmetries have been reported in rat brain (Glick et al., 1977; Sherman and Galaburda, 1984). Asymmetrical differences in neurotransmitters such as GABA and dopamine have been demonstrated in the neostriatum (Glick et al., 1974; Guarneri et al., 1985, 1988). To evaluate the electrophysiological asymmetry at the cellular level, the present study compared the membrane properties and cortically evoked postsynaptic potentials of spiny neurons between the left and right striatum. The results have indicated that the membrane properties and initial EPSPs of spiny neurons in the left side are similar to those in the right side. However, the short-lasting IPSPs are significantly stronger in the left striatum than those in the right striatum. The inhibition in striatum comes from GABAergic spiny projection neurons and aspiny interneurons (Groves, 1983; Kita et al., 1990; Wilson, 1990). Previous studies have suggested that the IPSPs in neostriatum may result from collateral inhibition of neighboring spiny neurons and from the GABAergic inhibitory interneurons (Park et al., 1980; Lighthall and Kitai, 1983; Flores-Hernandez et al., 1994). Recent reports, however, indicated that most of the IPSPs in striatum are generated by the interneurons (Jaeger et al., 1994; Koos and Tepper, 1999). The possible mechanisms underlying the stronger inhibition in the left striatum are either to have more GABAergic synapses or to have higher GABAergic synaptic efficacy. It has been shown that the number of ^{3}H-GABA binding

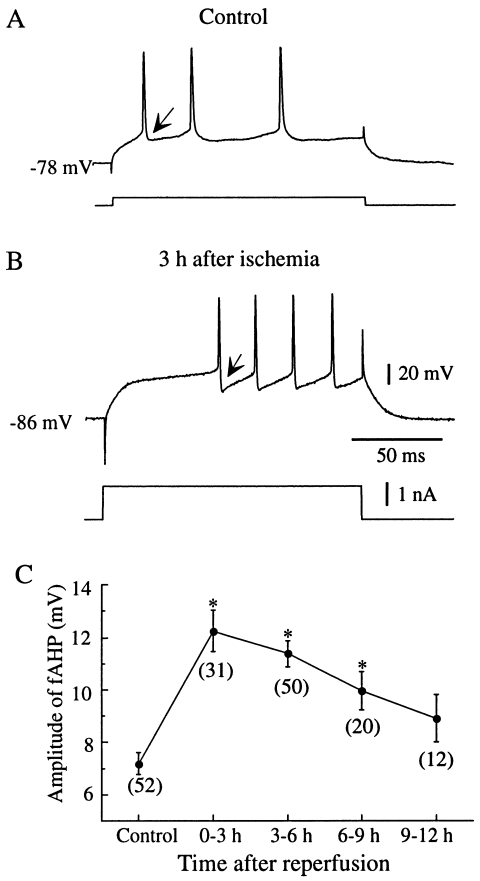

Fig. 8. fAHP of spiny neurons before ischemia and after ischemia. (A, B) Representative recordings of response of spiny neurons to depolarizing current pulse. The upper panels are the action potentials evoked by the current pulse. Bottom panels are intracellularly injected depolarizing current pulses. The arrow indicates the fAHP. The amplitude of fAHP of a neuron 3 h after ischemia (B) is larger than that of control one (A). Scales in B apply to A. (C) Plots showing the temporal changes in amplitude of fAHP at different intervals after ischemia. Each point is the mean value. The amplitude of the fAHP significantly increased within 9 h after ischemia ($P < 0.01$).

sites in the left striatum is higher than those in the right striatum and the presynaptic activity of glutamic acid decarboxylase is also higher in the left striatum (Guarneri et al., 1988) suggesting more GABA receptors and higher GABAergic activities in the left striatum. It is not clear, however, whether these GABA receptors and GABAergic activities are associated with the collaterals of spiny projection neurons or the terminals of aspiny interneurons.

The asymmetry of inhibitory tone between the left and right striatum may be responsible for the difference in excitatory polysynaptic response after ischemia. As shown in the present study, the number of neurons exhibited L-PSPs increase from ~5% of control level to ~30% in the left striatum and ~50% in the right striatum after severe ischemia. It is conceivable that the more prevalent facilitation of excitatory synaptic transmission in the right striatum after ischemia is at least partially due to the weaker inhibition as compared with that of the left side. Coinciding with our observation, it has been reported that animals with right hemispheric infarction following ipsilateral middle cerebral artery occlusion (MCAO) showed hyperactivity while those with left MCAO showed no difference as compared with the control ones (Robinson, 1979). The same study also showed a significant reduction of dopamine concentration in the ipsilateral SN after MCAO in the right side. Inhibitory influence of dopamine on corticostriatal excitatory neurotransmission in the neostriatum has been well documented (Calabresi et al., 2000a,b; Nicola et al., 2000). The hyperactivity of animals following MCAO may be due to the decrease of dopamine inhibitory influence over corticostriatal excitatory neurotransmission. Further studies are needed to elucidate the mechanisms of dopamine effects on spiny neurons following cerebral ischemia.

Differential changes of synaptic transmission after ischemia

The present study has shown differential changes of cortically evoked postsynaptic potentials in spiny neurons after transient cerebral ischemia. The spontaneous neuronal activities and the cortically evoked initial EPSPs were suppressed whereas the cortically evoked L-PSPs was potentiated following ischemia. The characteristic spontaneous membrane potential fluctuation between the hyperpolarizing down state and depolarizing up state in spiny neurons was dramatically depressed after ischemia leaving the membrane potential at the down state most of the time (Table 1). The membrane potential depolarization maintaining the up state in spiny neurons is driven by the activity of cortical neurons (Wilson, 1994). Decortication *in vivo* or brain slice preparation that removes most of the cortical afferent fibers abolish or dramatically reduce the up state (Wilson et al., 1983a,b; Calabresi et al., 1990). Cerebral ischemia completely depresses the activity of cortical neurons and acts as another way of removing cortical inputs. Cortical neurons are highly sensitive to ischemia. Approximately 10–15 min forebrain ischemia induces cell death in cerebral cortex (Pulsinelli et al., 1982). The more severe ischemia in the present study (~21 min) silences the cortical neurons as shown in Fig. 3 and causes the disappearance of the up state in spiny neurons. Therefore, the depression of spontaneous activity in spiny neurons after ischemia most likely results from the depression of cortically driven monosynaptic transmission. Together with the depression of spontaneous activities, all the measurements on cortically evoked initial EPSPs indicate the suppression of excitatory monosynaptic transmission after ischemia. This result is not a surprise as previous studies have shown that synaptic transmission in spiny neurons was depressed during hypoxia/hypoglycemia *in vitro* (Calabresi et al., 1997a,b) and after ischemia *in vivo* (Xu, 1995). Depression of synaptic transmission after hypoxia/ischemia has also been observed in other regions of the brain such as hippocampus and cerebral cortex (Buzsaki et al., 1989; Urban et al., 1989; Luhmann and Heinemann, 1992; Rosen and Morris, 1993; Gao et al., 1998).

Surprisingly, despite the depression of excitatory

monosynaptic transmission and the reduction of neuronal excitability, the cortically evoked L-PSPs significantly increase in spiny neurons after ischemia. The L-PSPs are polysynaptic events and resemble the second EPSP component of thalamostriatal responses in decorticated animals (Wilson et al., 1983a). The thalamic transection experiment in the present study (Fig. 4C) has demonstrated that the generation of L-PSP after ischemia indeed requires intact thalamostriatal pathway. The existence of L-PSP neurons in control animals, though small in number, suggests that this is not a new component induced by ischemia but a component masked by stronger inhibition in intact animals. The inhibition in striatum were abolished after ischemia and never returned to the control levels after ischemia (Fig. 5). It has also been shown in hippocampus that anoxia depressed GABAergic IPSPs *in vitro* (Krnjevic et al., 1991; Congar et al., 1995) and the ischemia reduces the amplitude of IPSPs in CA1 neurons *in vivo* (Gao et al., 1998). A L-PSP that is similar to the L-PSPs described in the present study has also been observed in CA1 pyramidal neurons after severe forebrain ischemia (Gao and Xu, 1996). Although the neural circuit is different in the hippocampus and neostriatum, it is possible that the depression of the inhibitory component may be, at least in part, responsible for the facilitation of excitatory polysynaptic potentials in these two regions after ischemia.

Spiny neurons in the neostriatum receive massive glutamatergic inputs from cortex and thalamus (Fonnum et al., 1981; Kemp and Powell, 1971; Parent and Hazrati, 1995) and glutamate excitotoxicity has been indicated as the major player in cell death following ischemia (Rothman and Olney, 1986; Choi and Rothman, 1990). The severe ischemia in the present study produces more than 90% of cell death in striatum 24 h following ischemia (Ren et al., 1997). The facilitation of cortically evoked excitatory polysynaptic potentials in spiny neurons may therefore trigger the process of cell death through glutamate excitotoxicity.

Complete removal of IPSPs after ischemia

One of the most striking observations in the present study is the abolishment of IPSPs in spiny neurons after ischemia. The short-lasting IPSPs in striatum are masked by initial EPSPs and can be detected by a paired-pulse test (Park et al., 1980; Wilson et al., 1983a,b) or by pharmacological manipulations (Flores-Hernandez et al., 1994). The IPSPs may come from reciprocal collaterals of GABAergic spiny neurons and/or from the GABAergic inhibitory aspiny interneurons (Park et al., 1980; Kita et al., 1990; Flores-Hernandez et al., 1994; Jaeger et al., 1994; Koos and Tepper, 1999). Shortly after severe ischemia the IPSPs completely abolished and never returned to the control levels up to 12 h after ischemia. It has been shown that the IPSPs are more susceptible to ischemia than the EPSPs. The amplitude of IPSPs in cortical neurons was more strongly suppressed than the EPSPs during hypoxia *in vitro* (Luhmann and Heinemann, 1992). The IPSPs in striatal neurons disappeared earlier than initial EPSPs after a mild ischemia and recovered later than initial EPSPs after ischemia (Xu, 1995). Depression of inhibition has also been reported in different regions using various ischemia models (Mittmann et al., 1994; von Giesen et al., 1994; Schiene et al., 1996). The mechanisms underlying the depression of IPSPs following ischemia is not clear. Some studies have indicated that it is due to the functional disconnection of inhibitory interneurons from excitatory inputs (Rosen and Morris, 1993; Congar et al., 1995). Other investigators, in contrast, have suggested that it may be a failure of GABAergic neurons to release GABA from their terminals (Krnjevic et al., 1991) or a result of alteration of GABA reversal potential (Katchman et al., 1994). It is well established that the interneurons are more resistant to ischemia than the principal projection neurons such as CA1 pyramidal neurons in hippocampus and spiny neurons in striatum (Francis and Pulsinelli, 1982; Pulsinelli, 1985; Zoli et al., 1997). Most likely the activities of these ischemia-vulnerable projection neurons are more severely damaged by the ischemic insult than the ischemia-resistant interneurons. Therefore, it is conceivable that the depression of IPSPs after ischemia is due to the failure of presynaptic terminals to excite inhibitory neurons rather than the failure of interneurons to conduct the inhibitory transmission.

The normal function of the brain needs a delicate balance of excitation and inhibition. A small deviation from this balance will cause abnormal function and the severe disturbances may produce pathological conditions such as epilepsy and excitotoxic cell death. In pathological conditions such as ischemia, the depression of inhibition causes excessive excitation and finally results in excitotoxic neuronal injury. This notion is supported by the observation that the application of GABA agonist protects the neuronal damage following ischemia (Sternau et al., 1989; Schwartz et al., 1995).

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