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Enhanced excitatory synaptic transmission in spiny neurons of rat striatum after unilateral dopamine denervation

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Abstract

The synaptic transmission and intrinsic membrane properties of spiny neurons in rat neostriatum were studied after unilateral dopamine depletion using in vivo intracellular recording and staining techniques. Two to four weeks after dopamine denervation, the spontaneous firing rate of spiny neurons increased and the spontaneous membrane potential fluctuation stayed at a more depolarized state for longer periods of time. The amplitude of cortically evoked initial excitatory postsynaptic potentials increased and a late excitatory postsynaptic potential that was occasionally found in control neurons was elicited from 23% of spiny neurons after dopamine denervation. No significant changes in intrinsic membrane properties of spiny neurons were observed after dopamine denervation. These results suggest that dopamine inhibits excitatory synaptic transmission of spiny neurons in naïve animals. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Dopamine; Excitatory synaptic transmission; Spiny neuron; Striatum; Intracellular recordings

Spiny neurons in neostriatum are innervated by dopaminergic inputs from substantia nigra (SN) and glutamatergic projections from cerebral cortex and thalamus. Dopaminergic inputs to spiny neurons synapse in close relation to glutamate terminals [13]. Accumulating evidence indicates that dopamine (DA) acts as a modulator altering the efficiency of neuronal responses to excitatory glutamatergic transmission [14]. However, the neurophysiological outcomes of DA modulation are complicated and controversial. Studies using in vitro preparation have shown that DA could have distinct effects on excitatory postsynaptic potentials (EPSPs) of spiny neurons depending on the subtype of DA receptors activated. D_2 receptor activation reduces (±)-αamino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor mediated EPSPs, whereas D_1 receptor activation enhances AMPA and NMDA receptor mediated response [14]. Although most studies have shown that DA depresses spontaneous neuronal firing in striatum [5], enhancement of spontaneous discharge has also been observed in striatal neurons upon DA release [7].

Parkinson's Disease (PD) is characterized by the degeneration of DA containing neurons in SN. Neurophysiological studies have provided a great deal of information regarding the effects of dopamine denervation on striatal activity using 6-hydroxydopamine (6-OHDA) lesioned animal models [3]. It has been shown that DA denervation increases the spontaneous membrane potential depolarization and the excitability of striatal neurons to glutamate [2–4]. However, most of these data are obtained from in vitro preparations. Few studies on DA denervated neurons in striatum have been conducted using in vivo intracellular recording techniques. To further characterize the electrophysiological changes of striatal neurons and corticostriatal synaptic transmission after DA denervation, the present study compared the spontaneous activities and cortically evoked EPSPs of spiny neurons in naïve and DA denervated rats using intracellular recording and staining techniques in vivo. Such preparation preserves the integrity of the nervous system and at the same time has the power to study the synaptic transmission and intrinsic membrane properties of individually identified neurons.

Animals were used in accordance with institutional and federal guidelines. Adult male Wistar rats (200–350 g) were used in the present study. For DA denervation, 6-OHDA was delivered into the right SN with two injections (2 μg/μl each site) [16]. Seven to ten days after SN lesion, the animals were injected with apomorphine (0.5 mg/kg, s.c.)

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for rotation test. Only the rats exhibited consistent contralateral rotation (10–20 turns/min) for at least 20 min were selected for the electrophysiological study. The denervation of DA was further confirmed by immunocytochemistry using anti-tyrosine hydroxylase (TH) antibodies (Chemicon). Electrophysiological experiments were performed 2–4 weeks after 6-OHDA injections.

Intracellular recording in vivo was performed in the right striatum as described previously [21]. In brief, the animals were anesthetized with 1–2% halothane and fixed on stereotaxic frame. The skull was opened to expose the recording site and for placement of stimulus electrodes. One pair of bipolar stimulating electrodes was made from 000 stainless steel insect pins. The electrodes were separated by 1.0 mm and placed into 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 tip resistance of 50–80 MΩ when filled with 3% neurobiotin (Vector) in 2 M potassium acetate. After impalement, neurons with a stable membrane potential of −60 mV or greater were selected for further study. Electrophysiological data were digitized using Axodata (Axon Instruments). After each successful recording, neurons were intracellularly stained with neurobiotin.

Quantification of the membrane potential fluctuation was accomplished by sampling the spontaneous activity at 1 kHz for 20 s and counting the number of samples at each membrane potential. The data were presented as all-point histograms. This gives the proportion of the time spent by the neurons at each membrane potential. The distribution was normalized and the weighted sum of 3 Gaussian's distribution was used to fit the data [17] using Axograph 4.0 (Axon Instruments, Inc):

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

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

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

where: *a, d* and (*1*-(*a* + *d*)) refer to weighting factor (i.e. proportion of time spent) for Down state, transitional zone and Up states, respectively. *b, e, g,* are average potentials and *c, f, h,* are referred as the variance of the three states. The values derived from the Gaussian's distribution of each neuron in naïve animals will be pooled and compared with those from DA denervated animals.

All data were presented as mean ± SEM. Student's *t*-test or analysis of variance (ANOVA) were used for statistical analysis using StatView 4.1 (Abacus Concepts).

A total of 56 striatal neurons were successfully recorded from 30 animals. 41 neurons were morphologically identified as spiny neurons after intracellular staining (Fig. 1). One neuron was identified as aspiny neuron and excluded from the present study. Fourteen unidentified neurons were considered as spiny neurons based on the stereotaxic parameters and electrophysiological characteristics. No obvious morphological difference was observed in intracellularly stained spiny neurons in naive and DA-denervated rats. The TH-staining fibers in the ipsilateral striatum dramatically decreased after DA denervation (Fig. 1B), especially in dorsal parts of the striatum. Intrinsic membrane properties of striatal neurons were similar between naïve and DA-denervated animals (Table 1).

The baseline membrane potential of spiny neurons spontaneously shifted between two relatively constant subthreshold levels, a hyperpolarized Down state (−61~−94 mV) and a depolarized Up state (−71~−40 mV) [18,20]. The membrane potential also spent time at transition between

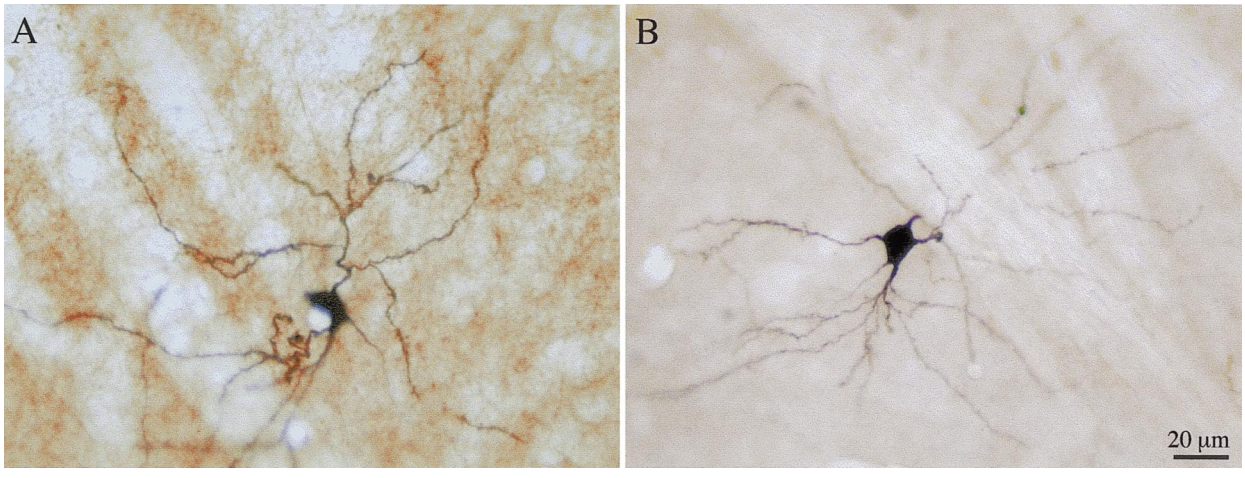

Fig. 1. Photomicrographs of intracellularly labeled spiny neurons in striatum after recording. The sections were counter-stained with tyrosine hydroxylase (TH)-immunocytochemical staining. (A) A spiny neuron recorded from a naïve rat. Dense TH positive fibers were observed throughout the striatum. The soma is not in focus because the focus plane was adjusted to show the dendritic spines. (B) A spiny neuron recorded from a DA-denervated rat. No TH immunoreactive fibers were found in the dorsal striatum.

Table 1
Membrane properties of spiny neurons in naive and DA-denervated rats[a]

	RMP (mV)	H_{spk} (mV)	W_{spk} (ms)	T_{spk} (mV)	Rin (MΩ)	Tcon (ms)	Rheobase (nA)	FAHP (mV)
Control	−74.4 ± 0.9 (29)	77.5 ± 2.5 (28)	1.16 ± 0.06 (28)	−50.8 ± 1.0 (28)	28.8 ± 1.7 (26)	6.82 ± 0.69 (26)	0.36 ± 0.04 (28)	5.7 ± 0.6 (28)
DA-denerv.	−75.4 ± 1.5 (23)	78.0 ± 2.1 (23)	1.19 ± 0.05 (23)	−52.9 ± 0.7 (23)	29.8 ± 2.5 (23)	6.39 ± 0.61 (20)	0.40 ± 0.06 (23)	6.3 ± 0.7 (23)

[a] Values are mean ± SEM; with number of neurons in parentheses. RMP, resting membrane potential. Hspk, spike height is measured from the resting membrane potential. Wspk, spike width is measured at half of the peak amplitue of the action potential. Tspk, spike threshold, is measured at the beginning of the upstroke of the action potential Rin. 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 after-hyperpolarization is measured as deviation from the beginning of the upstroke of an action potential within 5 ms after the peak of a single spike.

these two states (Fig. 2A,B arrows). Action potentials were intermittently triggered from the noisy membrane potential fluctuations in the Up state (Fig. 2A,B). Spontaneous firing rate of spiny neurons increased significantly from 0.47 ± 0.11 Hz in naïve animals to 1.18 ± 0.49 Hz after DA-denervation ($P < 0.05$, unpaired t-test, control: $n = 21$, DA-denervated: $n = 18$). Quantitative description of the spontaneous membrane potential fluctuation for each neuron was obtained by fitting a 3-Gaussian's distribution to the all-point frequency histogram of membrane potentials (Fig. 2A,B). After DA denervation, the proportion of the Up state increased significantly from 50.6 ± 4.3% to 81.7 ± 2.9% ($P < 0.01$, unpaired t-test, control: $n = 26$, DA-denervated: $n = 13$, Fig. 2C) while the proportion of the transition zone decreased dramatically from 32.0 ± 2.9% to 7.2 ± 1.8% ($P < 0.01$). The average membrane potential at different states showed no obvious changes after denervation (Fig. 2D).

Ipsilateral cortical stimulation elicited EPSPs from spiny neurons. The threshold stimulation for inducing initial

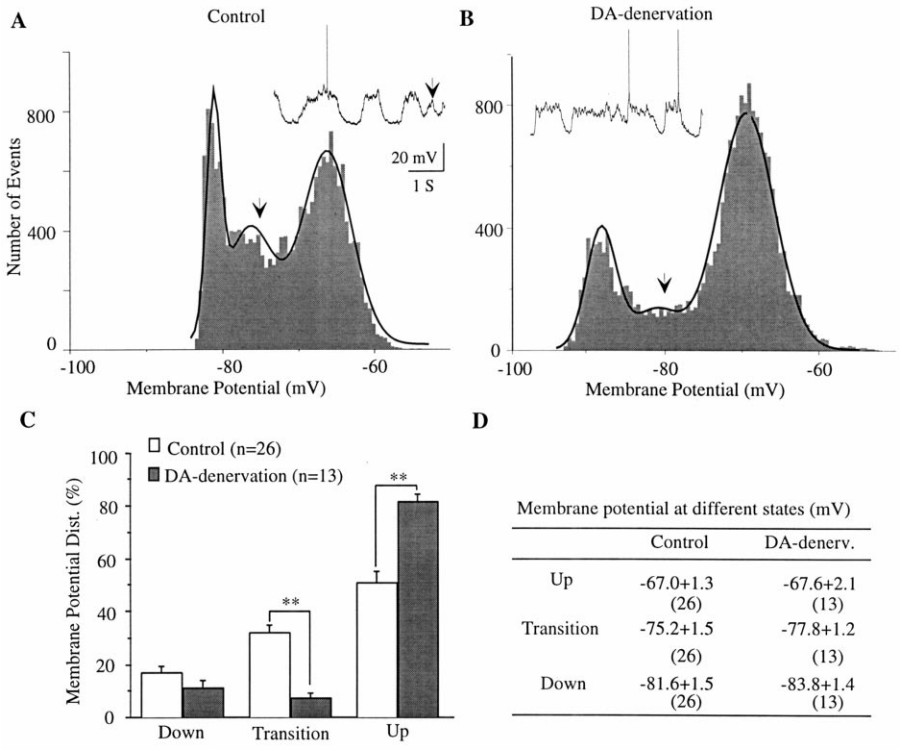

Fig. 2. The changes of spontaneous membrane potential fluctuation before and after DA-denervation. (A,B) All-point histograms showing the number of events at various membrane potentials of representative spiny neurons in naïve (A) and DA-denervated rats (B). Each histogram derives from recording of 20 s spontaneous neuronal activities and the bin width is 0.5 mV. Histograms do not include the action potentials. The three peaks of the histogram represent the Down state, transition zone (arrows) and Up state of the membrane potential fluctuation. Thick lines are the fittings of three-Gaussians. The inserts are the intracellularly recorded traces. (C) Histogram showing the proportional distribution of the Up state, transition zone and Down state before and after DA-denervation. The proportion of Up state increased significantly while the proportion of transition zone decreased accordingly after DA denervation. $^{**}P < 0.01$, unpaired t-test. (D) Table showing the average membrane potential at different membrane states. No significant difference in membrane potential at different states was found before and after DA denervation. Values are mean ± SEM, with number of neurons in parentheses.

EPSPs was 0.38 ± 0.03 mA in naïve animals and 0.33 ± 0.02 mA in DA denervated animals ($P > 0.05$, $n = 26$ in both groups). The stimulus intensity was step increased to 1.5, 2, 2.5, 3, 4, 5 times (T) as strong as the threshold stimulus intensity. The amplitude of the initial EPSPs increased accordingly with increasing stimulus intensities (Fig. 3A). In comparison with naïve animals, the amplitudes of the initial EPSPs in spiny neurons increased at all stimulus intensities after DA-denervation but the statistic significance was detected only at 5T stimulus intensity ($P < 0.05$, unpaired t-test, $n = 25$ for control; $n = 26$ for DA denervated). The latency of the initial EPSPs remained unchanged. In addition to the initial EPSPs, a late excitatory postsynaptic potential (L-PSP) was occasionally evoked from spiny neurons of naïve animals by cortical stimulation (Fig. 3C). The L-PSPs had more than one component with the latency to peak of 30–50 ms and 55–75 ms for the 1st and 2nd component, respectively. The latency of L-PSPs decreased with increasing stimulus intensity indicating that they were polysynaptic events. The incidence of L-PSPs in spiny neurons increased from 8% of control animals (2/26) to 23% after DA-denervation (6/26).

The present study has demonstrated that the spontaneous activities in spiny neuron significantly increase and the cortically evoked excitatory synaptic transmission is facilitated after DA denervation. These results suggest that DA has strong inhibitory effects on spiny neurons in naïve animals. The potentiation of excitatory synaptic transmission after DA denervation may be responsible for the hypokinesia in Parkinson's disease.

The membrane potential of spiny neurons fluctuates between the depolarized Up state and hyperpolarized Down state [18,20]. In responses to temporally coherent excitatory synaptic inputs from cerebral cortex, spiny neurons depolarize from Down state to Up state during which the neurons stay at a membrane potential close to the threshold for spike generation [18]. Although the membrane potentials spend most of the time in the Up and Down states, they also spend time in transition between these two states. The time spent and the shape of the transition could be altered by the experimental manipulation [20]. In the present study, the proportion of Up state in spiny neurons significantly increases after DA denervation while the proportion of transition zone reduces. The shift from transition zone to Up state probably is not due to the changes of membrane properties of spiny neuron after DA denervation. Because the spiny neurons are not intrinsically bistable [14] and the membrane properties of spiny neurons remain unchanged after DA denervation as shown in the present study and by other investigators [4,15]. Such change most likely is due to the potentiation of corticostriatal synaptic transmission after DA denervation so the weak inputs that are responsible for the transition zone are strengthened and drive the membrane potential to the more depolarizing Up state level. In support of this notion, it has been shown that the blockage of D_1 receptors or DA denervation increases the neuronal activity in striatum suggesting that the activation of D_1 receptors exerts a strong restraining influence on the spontaneous activity of striatal neurons [4,12].

The present study has demonstrated that the amplitude of cortically evoked monosynaptic initial EPSPs increases after DA denervation. The cortically evoked initial EPSPs in spiny neurons consist of AMPA receptor mediated fast component and NMDA receptor mediated slow component [10,11]. However, when neurons are in magnesium-contain-

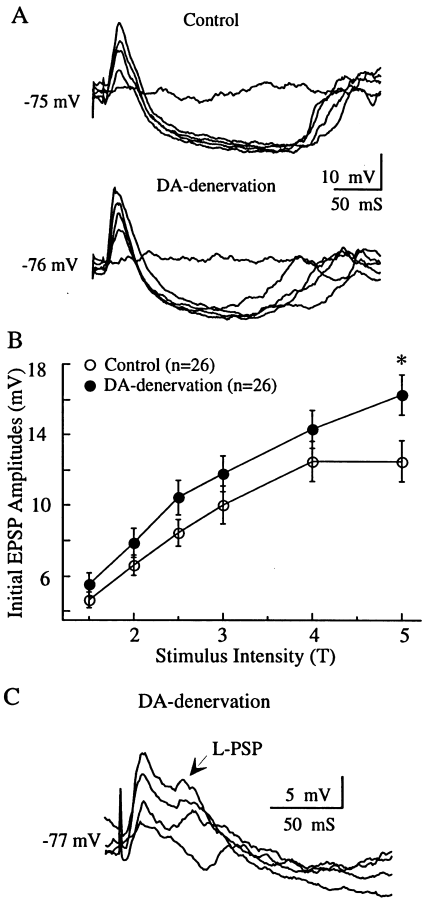

Fig. 3. The changes of cortically evoked EPSPs from spiny neurons before and after DA-denervation. All traces are the averages of four recordings. (A) Examples of cortically evoked initial EPSPs from spiny neurons in a naive (upper panel) and a DA-denervated animal (lower panel). The stimulus intensity was 0, 1.5, 2, 3, 4 times as strong as the threshold stimulus intensity. The amplitude of initial EPSPs increased with step increments of stimulus intensities accordingly. (B) Plot showing the amplitudes of the initial EPSPs at different stimulus intensities. The stimulus intensities were 1.5–5 times (T) as strong as the threshold stimulus intensity for inducing initial EPSPs. The amplitude of initial EPSPs significantly increased at 5T stimulus intensity after DA-denervation. $*P < 0.05$, unpaired t-test. (C). Traces showing the late-depolarizing postsynaptic potentials (L-PSP, indicated by arrow) evoked by cortical stimulation. In addition to the initial EPSPs, a late-depolarizing component was occasionally evoked from spiny neurons by cortical stimulation. The incidence of L-PSP neurons in striatum increased after DA denervation.

ing environment, which is the case as in vivo preparation, and at the resting membrane potential, the NMDA component is very small and the initial EPSPs are mainly contributed by AMPA receptor mediated component [11]. It has been shown that D_2 receptor activation reduces AMPA receptor mediated EPSPs [5,9]. After DA denervation, such tonic restraint on excitatory corticostriatal transmission is removed and therefore the amplitude of initial EPSPs in spiny neurons increases.

Based on their appearance and latency, the L-PSPs evoked from spiny neurons resembled the second EPSPs elicited by thalamic stimulation in decorticated animals [19]. Although L-PSPs were evoked by cortical stimulation in animals with intact cerebral cortex, evidence indicates that thalamostriatal circuits are involved in the generation of L-PSPs. It has been shown that the L-PSPs can no longer be induced after the thalamostriatal pathway is disconnected by acute thalamic knife cut [6]. The increase of L-PSP neurons from ~8% in naïve animals to ~23% in DA denervated animals suggests that the L-PSP is an unmasked excitatory polysynaptic event rather than a new component induced by DA denervation. It is conceivable that the DA inhibitory effect via D_2 receptor is removed after DA denervation. The polysynaptic cortico-thalamo-striatal excitatory synaptic transmission is disinhibited after DA denervation, which results in the facilitation of L-PSP generation in striatum.

Under physiological conditions the activity of spiny neurons is controlled by the cortical glutamatergic inputs and by the SN dopaminergic pathway [8]. The GABAergic spiny neurons in turn project to SN and globus pallidus and regulate the functional activity of these structures and the fine adjustment of motor system [1]. DA provides a strong restraining influence on spontaneous activity and evoked synaptic potentials of spiny neurons in naïve animals. In the absence of endogenous DA, this inhibitory mechanism is altered and causes hyperexcitability of striatal neurons. Such alteration will enhance the inhibitory GABAergic control of the striatum on the output structures of the basal ganglia and may be associated with the abnormal motor symptom (such as hypokinesia) observed in PD patients.

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