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Research Paper

Changes in the firing activity of serotonergic neurons in the dorsal raphe nucleus in a rat model of Parkinson's disease

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Abstract: In the present study, changes in the neuronal activity of serotonergic neurons in the dorsal raphe nucleus (DRN) and the effect of the selective 5-HT_{1A} receptor antagonist WAY-100635 in a rat model of Parkinson's disease (PD) were investigated by using extracellular single unit recording. Rat model of PD was produced by microinjection of 6-hydroxydopamine (6-OHDA) into the substantia nigra pars compacta on the right side of the brain. The results showed that the mean spontaneous firing rate of DRN serotonergic neurons in the control and 6-OHDA-lesioned rats were (1.76±0.11) spikes/s (n=24) and (2.43±0.17) spikes/s (n=21), respectively. The firing rate of serotonergic neurons in 6-OHDA-lesioned rats was significantly higher than that in the control rats (P< 0.001). In the control rats, 92% (22/24) of the neurons fired regularly and 8% (2/24) fired in bursts. In rats with 6-OHDA lesions, 9% (2/21) of neurons fired regularly, 43% (9/21) exhibited irregular pattern and 48% (10/21) fired in bursts. The percentage of DRN serotonergic neurons firing in bursts was obviously higher in 6-OHDA-lesioned rats than that in the control rats (P<0.001). Local injection of WAY-100635 (3 μg in 200 nL) into the DRN significantly increased the firing rate of serotonergic neurons with no change in firing pattern in the control rats (n=19, P<0.002), but did not change the firing rate and firing pattern of serotonergic neurons in 6-OHDA-lesioned rats (n=17, P>0.05). These results suggest the dysfunction of 5-HT_{1A} receptor in 6-OHDA-lesioned rats and the involvement of the DRN in the pathophysiological mechanism of PD.

Key words: dorsal raphe nucleus; 5-HT_{1A} receptor; WAY-100635; Parkinson's disease

帕金森病大鼠中缝背核 5- 羟色胺能神经元电活动的变化

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摘 要： 本实验采用玻璃微电极细胞外记录法，观察了帕金森病(Parkinson's disease, PD)大鼠中缝背核(dorsal raphe nucleus, DRN) 5-羟色胺(5-hydroxytryptamine, 5-HT)能神经元电活动的变化。在大鼠右侧中脑黑质致密部内微量注射 6-羟多巴胺(6-hydroxydopamine, 6-OHDA)制作 PD 模型。结果显示，对照组和 PD 组大鼠 DRN 中 5-HT 能神经元的放电频率分别是(1.76±0.11) spikes/s (n=24)和(2.43±0.17) spikes/s (n=21)，PD 组大鼠的放电频率显著高于对照组(P<0.001)。在对照组大鼠，92% (22/24)的神经元呈规则放电，8% (2/24)为爆发式放电；在 PD 组大鼠，具有规则、不规则和爆发式放电的神经元比例分别为 9% (2/21)、43% (9/21)和 48% (10/21)，爆发式放电的 5-HT 能神经元比例明显高于对照组(P<0.001)。在对照组大鼠，DRN 内局部注射 5-HT_{1A} 拮抗剂 WAY-100635 (3 μg/200 nL)显著增加 5-HT 能神经元的放电频率而不影响其放电形式(n=19, P<0.002)；而 WAY-100635 不改变 PD 组大鼠 5-HT 能神经元的放电频率和放电形式(n=17, P>0.05)。结果提示，用 6-OHDA 损毁黑质致密部造成的 PD 模型大鼠中神经元 5-HT_{1A} 受体功能失调，并且 DRN 参与 PD 的病理生理学机制。

关键词： 中缝背核；5-HT_{1A} 受体；WAY-100635；帕金森病
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Parkinson's disease (PD) is a progressive neurodegenerative disease of which the principal pathological characteristics are the loss of dopaminergic neurons of the substantia nigra pars compacta (SNc) in the midbrain. Low level of dopamine in the striatum leads to such symptoms as slow movement (bradykinesia) and impaired motor control (resting tremor, postural instability, rigidity). However, other neurotransmitter systems, e.g., serotonergic, noradrenergic and cholinergic systems, also show signs of degeneration[1,2]. The exact role of serotonin (5-hydroxytryptamine, 5-HT) in the pathogenesis of PD remains unclear. The dorsal raphe nucleus (DRN) is a main source of serotonergic neurons in the brain and gives rise to an extensive serotonergic innervation of the forebrain. Thus, the central serotonergic nervous system consequently plays an important role in a wide variety of brain functions[3,4]. Neurochemical and neuropathological studies have demonstrated that 5-HT concentration is significantly reduced in some areas of the brain[2,5,6] and that there is a loss of serotonergic neurons in the raphe nuclei of Parkinsonian patients [2,7]. In addition, studies have also observed a reduction of 5-HT_{1A} binding sites in the raphe nuclei of patients with PD and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated monkeys[5,8]. These findings suggest that serotonergic system is involved in the pathophysiology of PD. However, at present, there are no *in vivo* electrophysiological studies that have investigated the neuronal activity of serotonergic neurons in the DRN and 5-HT_{1A} receptor function after unilateral lesion of the nigrostriatal pathway in rats. To address this issue, we investigated the changes in firing rate and firing pattern of serotonergic neurons in the DRN in 6-hydroxydopamine (6-OHDA)-lesioned rats using electrophysiological techniques. In order to evaluate the 5-HT_{1A} receptor function, the effects of the selective 5-HT_{1A} receptor antagonist WAY-100635 on serotonergic neuronal firing in the DRN were observed in the control and 6-OHDA-lesioned rats.

1 MATERIALS AND METHODS

1.1 *Animals and biochemicals*

Fifty-one adult male Sprague-Dawley rats, weighing 260-280 g, were housed in a 12-hour light/12-hour dark cycle with food and water available. The experiments were performed according to the Guideline of the Institutional Animal Care Committee of Xi'an Jiaotong University. All efforts were made to minimize the number of animals used and their suffering. The biochemicals used in the experiments were desipramine, 6-OHDA hydrochloride, apomorphine hydrochloride and WAY-100635 maleate salt (Sigma). Desipramine, apomorphine and WAY-100635 were prepared in 0.9% saline; 6-OHDA in distilled water containing 0.01% ascorbic acid.

1.2 *6-OHDA lesion of the SNc*

Thirty minutes prior to the injection of 6-OHDA, rats were pretreated with desipramine (25 mg/kg, i.p.) in order to protect noradrenergic neurons. Then 2 μL of 6-OHDA hydrochloride solution (4 μg/μL) were injected into the right SNc following the coordinates: AP 4.9-5.2 mm posterior to bregma, L 1.8-2.2 mm from the midline, D 7.2-7.4 mm from the dura[9]. After each injection, the micropipette was left in place for an additional 5-10 min before being slowly withdrawn. Two weeks post-surgery, rats were given apomorphine (0.05 mg/kg, s.c.) and those exhibiting more than 20 contralateral turns per 5 min were chosen for the electrophysiological investigation[10].

1.3 *Electrophysiological recordings and firing pattern analysis*

Electrophysiological recordings were performed 3 weeks after 6-OHDA lesion of the SNc. Extracellular single unit recordings were made in rats anaesthetized with urethane (1.2 g/kg, i.p.). Glass microelectrodes (10-20 MΩ) filled with 1% pontamine sky blue in 0.5 mol/L sodium acetate were directed stereotaxically to the right DRN (AP 7.7-8.0 mm posterior to bregma, L 1.6-1.8 mm from the midline, D 5.2-5.7 mm from the dura)[9]. The neuronal firings were amplified, bandpass-filtered, displayed on an oscilloscope and stored in a computer equipped with the Spike2 analysis system (Cambridge Electronic Design, UK) for off-line analysis. Local injection of saline or WAY-100635 into the right DRN was done via a glass micropipette (external diameter of 50 μm) connected to a 1-μL Hamilton microsyringe. The micropipette was lowered toward the DRN with an oblique lateromedial orientation. A total volume of 200 nL of saline or WAY-100635 (3 μg in 200 nL saline) was injected over a 2-minute period to the control or 6-OHDA-lesioned rats. Only one neuron was recorded per animal for injection of WAY-100635. The neurons which met the following criteria were included for off-line analysis in this study: (1) the neuron with spontaneous firing rate of 0.1-5.0 spikes/s, a wide action potential duration [(1.5-2.5) ms] and a single or bursting firing pattern[11-13]; (2) the neuron recorded was histologically confirmed from the DRN and the tip of the injection micropipette was correctly placed in the DRN. The baseline activity of each neuron was recorded for 5 min before any treatment, and changes in neuronal firing were observed for 10-20 min after intra-DRN injection of saline or WAY-100635. The firing patterns

were determined by interspike interval histograms (ISIHs) and the coefficient of variation[14].

1.4 *Histology and immunocytochemistry*

At the end of each experiment, the recording site was marked by the ejection of pontamine sky blue (-20 μA, 15 min). The rat was given an overdose of urethane, and perfused with saline followed by 4% paraformaldehyde, the brain was removed, frozen, sectioned at 40 μm and stained with cresyl violet for histological verification of the recording and injecting sites (Fig.1*A*). The sections of the SNc from rats receiving 6-OHDA injection were examined by immunocytochemical staining of tyrosine hydroxylase (TH) to determine the extent of nigral dopaminergic degeneration[15] (Fig.1*B*), and rats with a total or partial loss of TH immunoreactivity in the SNc were used to analyze the results of electrophysiological recordings.

1.5 *Statistical analysis*

Differences of the firing rates were obtained by using Student's *t*-test. The mean interspike interval (ISI) and coefficient of variation were analysed using the Mann-Whitney U-test, as the data were not normally distributed. The proportions of different firing patterns under all the experimental conditions were compared using χ^2 test. All data were expressed as means±SEM. Statistical analyses were performed using SPSS 10.0 for Windows. $P<0.05$ was considered statistical significance.

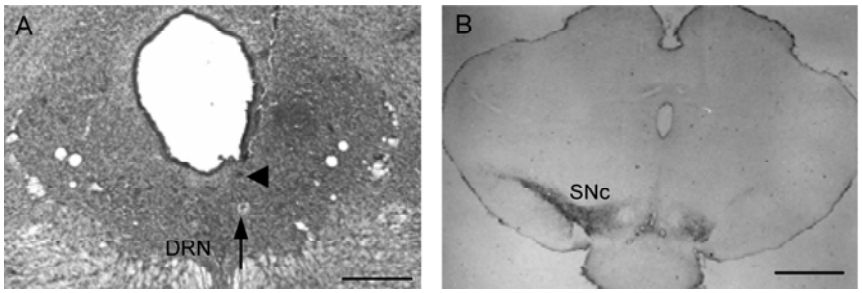

Fig. 1. Histological photomicrograph and TH immunocytochemistry. *A*: Histological photomicrograph showing the recording site marked with iontophoretically injected pontamine sky blue (arrow) and the trace of glass micropipette injection (arrowhead) in the DRN. Scale bar, 0.5 mm. *B*: Immunocytochemistry of TH showing the total degeneration of dopaminergic neurons in the SNc on 6-OHDA-injected side (right) compared to the normal side (left). Scale bar, 2 mm.

2 RESULTS

All the recording sites used in the control and 6-OHDA-lesioned rats were verified to be within the DRN. The neurons recorded had a typical long action potential duration of 1.5-2.5 ms and a slow spontaneous firing rate of 0.97-3.75 spikes/s. The action potential displayed a prominent positive deflection followed by a negative or negative/positive transient. Therefore, these neurons had electrophysiological characteristics of serotonergic neurons[11-13].

2.1 Neuronal activity of DRN serotonergic neurons in control and 6-OHDA-lesioned rats

A total of 24 serotonergic neurons in the DRN were recorded in 24 control rats. The firing rate of these neurons ranged from 0.97 to 2.55 spikes/s with a mean of (1.76± 0.11) spikes/s (n=24; Fig.2*A*). The mean ISI was (533±36) ms with a mean coefficient of variation of 0.37±0.03 (Fig.

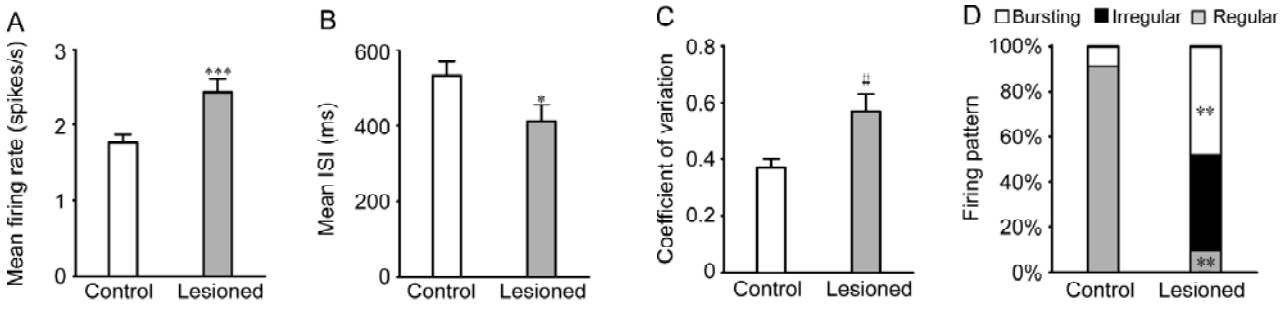

Fig. 2. Comparisons of mean firing rate (*A*), mean ISI (*B*), mean ISI coefficient of variation (*C*) and distribution of firing patterns (*D*) of the DRN serotonergic neurons in the control rats (n=24) and 6-OHDA-lesioned rats (n=21). $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, $^{\#}P<0.004$ *vs* the control rats.

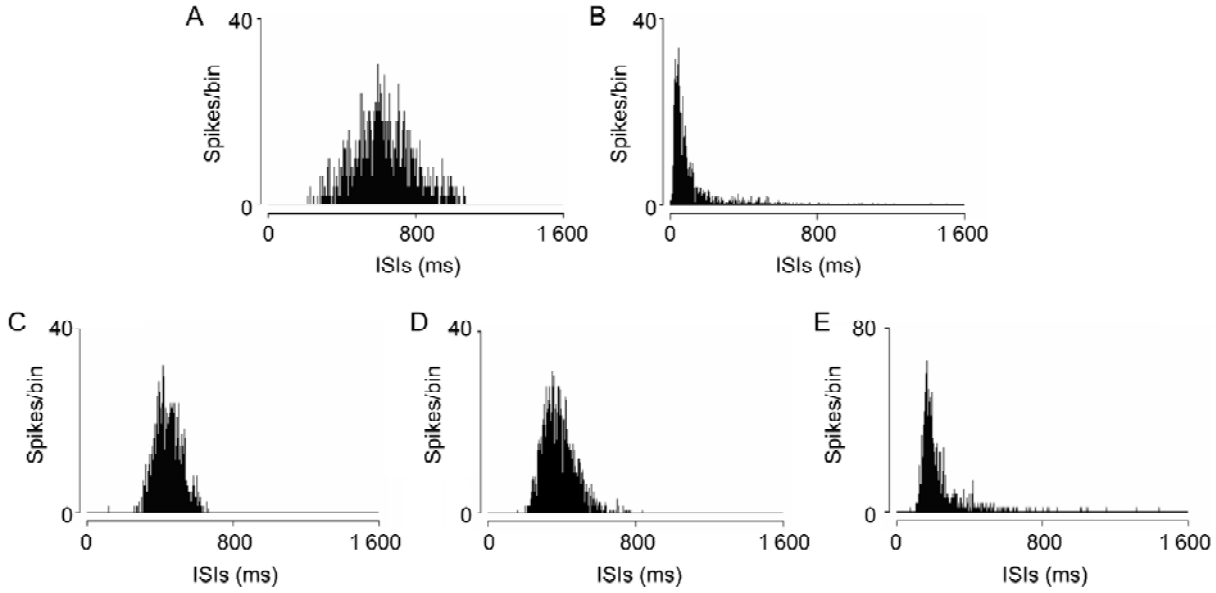

Fig. 3. The interspike interval histograms (ISIHs) (bin=4 ms) showing the different firing patterns of the DRN serotonergic neurons in the control (*A*, *B*) and 6-OHDA-lesioned (*C*-*E*) rats. *A*, *C*: Regular firing neurons, the ISIHs exhibiting a symmetrical distribution of the ISIs. *B*, *E*: Burst-firing neurons, the ISIHs exhibiting an obviously positive skewness with a long progressive decline. *D*: An irregular firing neuron, the ISIH exhibiting a random distribution.

2*B* and *C*). In the control rats, the serotonergic neurons exhibited two different firing patterns, i.e., the majority of the neurons (92%, 22/24) showed a regular firing pattern and 8% (2/24) fired in bursts (Fig.2*D*, 3*A* and *B*). A total of 21 serotonergic neurons in the DRN were recorded in 21 6-OHDA-lesioned rats. The firing rate of the serotonergic neurons varied from 1.24 to 3.75 spikes/s. The mean firing rate increased significantly to (2.43±0.17) spikes/s compared with that in the control rats (n=21, P<0.001; Fig.2*A*). The mean ISI of these neurons was (413±42) ms, significantly lower than that in the control rats (P<0.05), and the mean coefficient of variation, 0.57±0.06, was significantly higher than that in the control rats (P<0.004; Fig.2*B* and *C*). The firing pattern of the neurons in 6-OHDA-lesioned rats changed significantly towards a more irregular or bursting firing. The percentage of burst-firing neurons increased to 48% (10/21, P <0.01), whereas that of regularly firing neurons decreased to 9% (2/21, P< 0.01). In addition, 43% (9/21) of the neurons displayed an irregularly firing pattern (Fig.2*D* and 3*C*-*E*).

2.2 Effect of WAY-100635 on DRN serotonergic neurons in control and 6-OHDA-lesioned rats

In order to observe the effect of the intra-DRN injection on the neuronal activity, saline (200 nL) was injected into the DRN in 12 control rats. There was no change in either firing rate or pattern of the DRN neurons. The mean firing rate of the DRN neurons was (1.55±0.16) spikes/s before and (1.60±0.17) spikes/s after saline injection (P>0.05). In 19 control rats, local injection of WAY-100635 (3 μg in 200 nL), a selective 5-HT_{1A} receptor antagonist, into the DRN significantly increased the mean firing rate of serotonergic neurons to (2.80±0.35) spikes/s (P<0.002; Fig. 4*A* and 5*A*). The mean firing rate of these neurons before

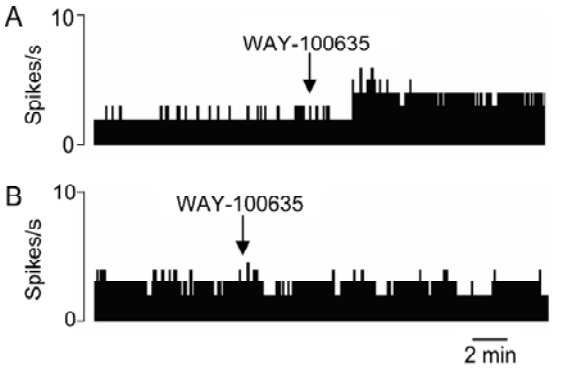

Fig. 4. The rate histograms showing the effects of intra-DRN injection of 5-HT_{1A} receptor antagonist WAY-100635 (3 μg in 200 nL) on the firing rate of the DRN serotonergic neurons in the control and 6-OHDA-lesioned rats. Injection of WAY-100635 increased the firing rate of the DRN serotonergic neurons in the control rats (*A*), but did not change the firing rate of the neurons in 6-OHDA-lesioned rats (*B*). The arrows indicate the onset of the injection.

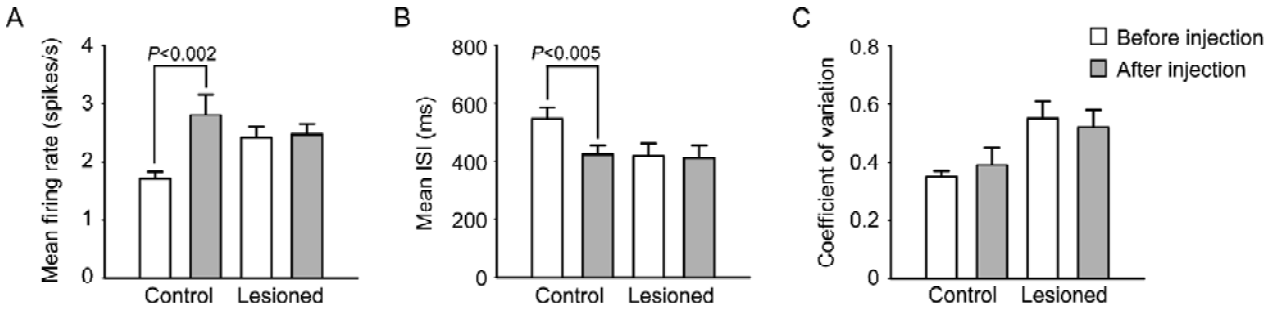

Fig. 5. The changes of mean firing rate (*A*), mean ISI (*B*) and mean ISI coefficient of variation (*C*) after local injection of 5-HT_{1A} receptor antagonist WAY-100635 in the control rats (n=19) and 6-OHDA-lesioned rats (n=17).

injection was (1.71±0.12) spikes/s. The mean ISI significantly decreased to (423±32) ms (P<0.005), but the coefficient of variation (0.39±0.06) was not significantly different from that before injection (P>0.05; Fig.5*B* and *C*). In 17 6-OHDA-lesioned rats, the mean firing rate of the DRN serotonergic neurons before WAY-100635 injection was (2.42±0.18) spikes/s. After injection of WAY-100635 (3 μg in 200 nL) into the ipsilateral DRN of the lesioned rats, the mean firing rate of these neurons changed to (2.47±0.18) spikes/s (P> 0.05; Fig.4*B* and 5*A*). The mean ISI slightly changed [(411±44) ms, P>0.05], and the coefficient of variation (0. 52±0.06) did not change significantly compared with that before administration (P>0.05; Fig.5*B* and *C*). No changes in the firing pattern of the DRN serotonergic neurons were observed after intra-DRN injection of WAY-100635 in the control and 6-OHDA-lesioned rats.

3 DISCUSSION

All the recorded neurons located in the DRN displayed the electrophysiological characteristics of serotonergic neurons in this study corresponding to those in previous reports[11-13]. The results of the present study showed that: (1) unilateral lesion of the nigrostriatal pathway induced an obvious increase in the firing rate with change in the firing pattern of serotonergic neurons recorded in the DRN of rats, and (2) local injection of selective 5-HT_{1A} receptor antagonist WAY-100635 did not change neuronal activity of the DRN serotonergic neurons in rats with 6-OHDA-lesioned SNc, while WAY-100635 increased the neuronal activity in the control rats.

In the DRN, the 5-HT_{1A} receptors are localized on the soma and dendrites of serotonergic neurons where they act as autoreceptors[13,16]. Thus, their activation inhibits neuronal activity of serotonergic neurons and reduces 5-HT release in the cell body area and the target structures of the serotonergic projections[17,18]. Therefore, it seems that the physiological role of 5-HT_{1A} receptors is to function as sensors that respond with a change in serotonergic neuronal firing and 5-HT release when the concentration of endogenous transmitter in the extracellular space is changed. Serotonergic projections originating from the DRN innervate the forebrain and control the release of 5-HT in terminal areas of the forebrain[4]. Results from neurochemical studies have shown that the levels of 5-HT and its main metabolite 5-hydroxyindolacetic acid decrease significantly in the basal ganglia, raphe nuclei, cerebral cortex and cerebrospinal fluid in PD patients and MPTP-treated monkeys[2,5,6,19]. The reduction of 5-HT is attributable to the loss of serotonergic neurons in the raphe nuclei, because several postmortem studies have shown that an average loss of more than 50% of Nissl-stained large neurons in the DRN and a similar loss of immunocytochemically identified serotonergic neurons in the median raphe nucleus in PD patients[7]. Furthermore, a marked reduction of 5-HT_{1A} binding sites in the raphe nuclei in MPTP-treated monkeys has been reproted[5]. In addition, PET imaging study has also shown a reduction of 27% in 5-HT_{1A} binding in the midbrain raphe nuclei using ^{11}C-WAY-100635 as a ligand in PD patients, suggesting a dysfunction of 5-HT_{1A} receptors[8]. These studies demonstrate that serotonergic system is severely affected in PD. On the basis of these studies and our results, we postulate that 6-OHDA lesion of the SNc causes a loss of serotonergic neurons, leading to a decrease in concentration of 5-HT in the extracellular space, and 5-HT_{1A} receptor dysfunction in the DRN of rats. Consequently, the hyperactivity of residual serotonergic neurons causes a release of 5-HT in order to maintain the homeostasis of the extracellular 5-HT level in the DRN. Concerning the firing pattern, we found that the percentage of the DRN serotonergic neurons with irregular and bursting firing patterns increased significantly in rats with

6-OHDA lesion, whereas that of the regular firing neurons decreased significantly compared with that in the control rats. Burst firing is of particular importance for serotonergic neurons, which has an extensive arborization of their terminal field, because it could increase the probability of action potentials arriving at the terminal region, and thus 5-HT release in this region is enhanced[3,20]. Therefore, the increased neurons with burst-firing pattern after degeneration of dopaminergic nigral neurons may help increase 5-HT release in the DRN and its projection areas in order to restore 5-HT in the extracellular space. These results suggest that the role of the hyperactivity of residual serotonergic neurons in the DRN may represent a compensatory mechanism for the dopaminergic dysfunction in PD.

WAY-100635 has been proven to be a potent and selective antagonist for pre- and most post-synaptic 5-HT_{1A} receptors, and lacks the affinity for other subtypes of 5-HT receptors[21]. In the present study, the firing rate of serotonergic neurons in the DRN increased following intra-DRN injection of WAY-100635 in the control rats, however, the firing pattern of the neurons did not change. Several studies have demonstrated that systemic administration of WAY-100635 does not modify the mean firing activity of serotonergic neurons of the DRN in the normal rats, but inhibits that of locus coeruleus (LC) noradrenergic neurons[22-24]. However, in LC-lesioned rats using either 6-OHDA or *N*-2-chloroethyl-*N*-ethyl-2-bromobenzylamine hydrochloride (DSP-4), WAY-100635 significantly increased the mean firing rate of serotonergic neurons[23], suggesting that the noradrenergic system tonically activates the firing activity of serotonergic neurons. Thus, systemic administration of WAY-100635 inhibits the firing activity of LC noradrenergic neurons, thereby decreases the endogenous noradrenergic input to excitatory α_1-adrenoceptors on serotonergic neurons in the DRN. This action attenuates the enhancing effect of WAY-100635 on serotonergic neuronal firing by the blockade of 5-HT_{1A} autoreceptors[22,23], the net result being an unchanged firing rate for most serotonergic neurons. Therefore, local injection of WAY-100635 in this study induces an increase in mean firing rate of the DRN serotonergic neurons in the control rats. The mechanism of the increase in neuronal firing is likely to be the blockade of 5-HT_{1A} autoreceptor by WAY-100635 in the the DRN that leads to membrane depolarization of serotonergic neurons, and then an increase in neuronal firing[13,25]. In addition, the blockade of 5-HT_{1A} receptors on the DRN GABA interneurons could decrease GABA-mediated inhibitory tone of serotonergic neurons and thus contributed to the observed increase in serotonergic neuronal firing[26,27]. From the above-mentioned findings, local injection of WAY-100635 in the DRN did not change the firing rate of serotonergic neurons in 6-OHDA-lesioned rats, a more possible explanation is that the degeneration of the nigrostriatal pathway results in 5-HT_{1A} receptor dysfunction in the DRN, which decreases the responsiveness of somatodendritic 5-HT_{1A} receptors to WAY-100635. In particular, this finding is supported by the findings that 5-HT_{1A} binding sites in the raphe nuclei in PD patients and MPTP-treated monkeys are significantly decreased[5,8].

In conclusion, the results of our study demonstrate that the firing rate of the DRN serotonergic neurons increases and that the majority of these neurons exhibit an irregular and burst-firing pattern in 6-OHDA-lesioned rats; the activity of the DRN serotonergic neurons in 6-OHDA-lesioned rats does not change by local injection of WAY-100635, a selective 5-HT_{1A} receptor antagonist, although the antagonist excites the neuronal activity in the control rats. These data indicate a dysfunction of the 5-HT_{1A} receptors after 6-OHDA-induced dopamine depletion in the brain, suggesting that the DRN is possibly implicated in the pathophysiology of PD.

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