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Research Paper

The selective 5-HT$_{1A}$ receptor antagonist WAY-100635 increases neuronal activity of the basolateral nucleus of the amygdala in 6-hydroxydopamine-lesioned rats

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Abstract: In the present study, extracellular recording was used to examine the neuronal activity of the basolateral nucleus (BL) of the amygdala and the effects of systemic administration of the selective 5-HT$_{1A}$ receptor antagonist WAY-100635 on the neuronal activity in the normal rats and rats with 6-hydroxydopamine (6-OHDA)-produced lesions in the substantia nigra pars compacta (SNc). The results showed that the firing rates of BL projection neurons and interneurons were (0.39±0.04) Hz and (0.83±0.16) Hz in the normal rats, and (0.32±0.04) Hz and (0.53±0.12) Hz in 6-OHDA-lesioned rats. There was no significant difference in the firing rates of BL projection neurons and interneurons between the normal and 6-OHDA-lesioned rats. In the normal rats, all BL projection neurons fired in burst; 94% of BL interneurons fired in burst and 6% fired irregularly. In 6-OHDA-lesioned rats, 85% of BL projection neurons displayed a burst firing pattern and 15% fired irregularly; 86% of BL interneurons had a burst firing pattern and 14% fired irregularly. The distribution of firing patterns of projection neurons and interneurons in the BL in 6-OHDA-lesioned rats did not differ from that in the normal rats. Systemic administration of WAY-100635 at 0.1 mg/kg body weight did not change the mean firing rates of projection neurons and interneurons in the BL in both normal and 6-OHDA-lesioned rats. However, a higher dose of WAY-100635 at 0.5 mg/kg body weight significantly decreased the mean firing rate of BL projection neurons from (0.43±0.07) to (0.15±0.02) Hz in the normal rats (P<0.01), but significantly increased the activity of BL projection neurons in 6-OHDA-lesioned rats from (0.37±0.08) to (0.69±0.18) Hz (P<0.004). The mean firing rates of BL interneurons in the normal and 6-OHDA-lesioned rats did not change after administration of a higher dose of WAY-100635 at 0.5 mg/kg body weight. These results demonstrate that the activity of BL neurons after substantia nigra dopaminergic lesion in the SNc is regulated by activation of intrinsic and extrinsic inputs, and that 5-HT$_{1A}$ receptors significantly contribute to the regulation of the activity of BL projection neurons in both normal and 6-OHDA-lesioned rats. Furthermore, WAY-100635 induced an increase in the mean firing rate of projection neurons in the BL in 6-OHDA-lesioned rats, suggesting that 5-HT$_{1A}$ receptor is likely to play a role in generating affective symptoms in Parkinson's disease.

Key words: basolateral amygdala complex; 5-HT$_{1A}$ receptor; WAY-100635; 6-hydroxydopamine; Parkinson's disease; electrophysiology

选择性 5-HT$_{1A}$ 受体拮抗剂 WAY-100635 增加 6- 羟多巴胺损毁大鼠杏仁基底外侧核的神经活动

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摘 要：本文采用玻璃微电极细胞外记录法，观察正常大鼠和 6- 羟多巴胺(6-hydroxydopamine, 6-OHDA)损毁黑质致密部大鼠杏仁基底外侧核(basolateral nucleus, BL)神经元电活动的变化，以及体循环给予选择性 5-HT$_{1A}$ 受体拮抗剂 WAY-100635 对神

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经元电活动的影响。结果显示，正常大鼠 BL 投射神经元和中间神经元的放电频率分别是(0.39±0.04) Hz 和(0.83±0.16) Hz，6-OHDA 损毁大鼠 BL 投射神经元和中间神经元的放电频率分别是(0.32±0.04) Hz 和 (0.53±0.12) Hz，与正常大鼠相比无显著差异。在正常大鼠，所有投射神经元呈现爆发式放电；94% 的中间神经元为爆发式放电，6% 为不规则放电。在 6-OHDA 损毁大鼠，85% 的投射神经元呈现爆发式放电，15% 为不规则放电；86% 的中间神经元为爆发式放电，14% 为不规则放电，与正常大鼠相比无显著差别。静脉给予 0.1 mg/kg 体重的 WAY-100635 不改变正常大鼠和 6-OHDA 损毁大鼠 BL 投射神经元和中间神经元的放电频率。然而，0.5 mg/kg 体重的 WAY-100635 却显著降低正常大鼠 BL 投射神经元的平均放电频率(P<0.01)，明显增加 6-OHDA 损毁大鼠 BL 投射神经元的平均放电频率(P<0.004)。高剂量 WAY-100635 不影响正常大鼠和 6-OHDA 损毁大鼠 BL 中间神经元的平均放电频率。结果表明，黑质多巴胺能损毁后内在和外在的传入调节 BL 神经元的活动，在正常大鼠和 6-OHDA 损毁大鼠 5-HT_{1A} 受体调节投射神经元的活动，并且在 6-OHDA 损毁大鼠 WAY-100635 诱发投射神经元平均放电频率增加。结果提示，5-HT_{1A} 受体在帕金森病情感性症状的产生中起重要作用。

关键词：基底外侧杏仁核复合体；5-HT_{1A} 受体；WAY-100635；6-羟多巴胺；帕金森病；电生理学
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Parkinson's disease (PD) is a disorder caused by the degeneration of the dopaminergic nigrostriatal pathway, characterized by bradykinesia, resting tremor, postural instability and rigidity. However, the pathological process of the disease is by no means confined to the dopaminergic neurons in the substantia nigra pars compacta (SNc) and as the disease progresses additional non-motor manifestations develop[1,2]. Several lines of evidence have shown that the degeneration of the nigrostriatal pathway causes morphological or functional abnormalities of the limbic system in PD patients and parkinsonian animals. For instance, *in vivo* magnetic resonance volumetric analysis and postmortem studies have reported reductions in the size of the amygdala and hippocampus in PD patients[3,4]. These reductions may reflect neuropathological changes in these structures, i.e. neuronal loss, neuronal shrinkage and Lewy body formation[5]. In addition, the incidence of depression in PD patients is about 40%[1,2]. Depression can be present before locomotor difficulties, and its natural history does not parallel to the progression of physical symptoms in PD, suggesting that the degeneration of dopaminergic neurons in the SNc leads to a dysfunction of the limbic system.

The amygdala, one of the components in the limbic system, has been divided into several nuclei based on cytoarchitectural, histochemical, anatomical and functional criteria[6]. Amygdalar areas that are associated with affective conditioning and responding include the basolateral amygdala complex (BLA; comprised of lateral, basolateral and basomedial nucleus) and the central nuclei[7]. Functional abnormalities in the BLA correlate with severity of depression, and the BLA mediates anxiety and fear processing[1,2,7]. The BLA expresses dopamine and 5-hydroxytryptamine (serotonin, 5-HT) receptors and receives dopaminergic and serotonergic innervation which is reduced in PD[8-13]. Degeneration of serotonergic neurons, decreased brain 5-HT content, and alterations in various types of 5-HT receptors, have all been demonstrated in postmortem studies using neurochemical and autoradiographic techniques[14-16]. Moreover, *in vivo* studies have consistently demonstrated reduced levels of 5-hydroxyindoleacetic acid (5-HIAA), a breakdown product of 5-HT, in the cerebrospinal fluid of PD patients, with some studies reporting an additional reduction of 5-HIAA in depressed PD patients[15,16]. These findings have shown that the serotonergic dysfunction appears to be an etiologic and pathophysiological factor for depression in PD.

The basolateral nucleus (BL) is of special interest because it is a key component of the BLA, and abundant evidence suggests the relevance of the 5-HT_{1A} receptor subtype in the neurobiology of depression. Therefore, in this study, we examined *in vivo* electrophysiological effect of the administration of WAY-100635, a selective 5-HT_{1A} receptor antagonist, on the spontaneously active neurons in the BL in the normal and 6-hydroxydopamine (6-OHDA)-lesioned rats. Additionally, changes in firing rate and firing pattern of these neurons in 6-OHDA-lesioned rats were also observed.

1 MATERIALS AND METHODS

1.1 *Animals and chemicals*

Data were obtained from 85 male Sprague-Dawley rats supplied by the Experimental Animal Center of Xi'an Jiaotong University; 44 rats were for 6-OHDA lesion and 41 for control. At the beginning of the experiment (6-OHDA lesion), the weight of the rats was (240±2) g. As BL neurons were recorded 3-4 weeks later, the weight of these rats was (300±3) g. Rats were housed in a 12-hour light/

12-hour dark cycle with food and water available. All protocols were approved by the Institutional Animal Care and Use Committee of the University, and all efforts were made to minimize the number of animals used and their suffering.

The chemicals used in this study were desipramine hydrochloride, 6-OHDA hydrochloride, WAY-100635 maleate salt and apomorphine hydrochloride (Sigma). Desipramine and WAY-100635 were prepared in 0.9% saline; 6-OHDA and apomorphine were prepared in distilled water containing 1 mg/mL ascorbate. These chemicals were prepared on the day of the experiment.

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 body weight, i.p.) in order to protect noradrenergic neurons. Animals were anesthetized with 4% chloral hydrate (300 mg/kg body weight, i.p.) and mounted in a stereotaxic instrument. The scalp was incised and retracted to expose the skull and a single hole was drilled over the right SNc, then 4 μL 6-OHDA hydrochloride solution (2 μg/μL) were injected following the coordinates: AP 5.1-5.2 mm posterior to bregma, L 1.9-2.1 mm from the midline, D 7.2-7.4 mm from the dura[17]. After each injection the needle was left in place for an additional 5 min before being slowly withdrawn. During the experiment, body temperature (37-38 °C), heart rate and pupillary diameter were monitored. Two weeks post-surgery, rats were given apomorphine (0.05 mg/kg body weight, s.c.) and those exhibiting more than 20 contralateral turns per 5 min were chosen for further study[18].

1.3 *Electrophysiological recordings*

Electrophysiological recordings were performed 3 weeks after 6-OHDA lesion of the SNc. Rats were anesthetized with urethane (1.2 g/kg body weight, i.p.) and mounted in a stereotaxic frame as described previously. A tracheal cannula was inserted to facilitate spontaneous respiration, and the right jugular vein was catheterized for the administration of additional anesthetic or WAY-100635. A hole was drilled over the right BL, and glass microelectrode (10-20 MΩ) filled with 2% Pontamine Sky Blue in 0.5 mol/L sodium acetate was directed stereotaxically to the right BL (AP 2.0-2.8 mm posterior to bregma, L 4.8-5.0 mm from the midline, D 6.5-7.4 mm from the dura). The extracellular signal was amplified, bandpass-filtered and then monitored with an oscilloscope and an audio monitor. The signal was stored in a computer equipped with the Spike 2 analysis system (Cambridge Electronic Design, UK) for off-line analysis. The activity of each neuron was recorded for 6-10 min before administration to establish a baseline and 15 min after systemic administration of WAY-100635 (0.1 or 0.5 mg/kg body weight, i.v., respectively). A change of >30% of basal firing rate was considered significant for an individual neuron. Only one neuron was observed per animal for administration of WAY-100635.

The changes in the firing pattern of the BL neurons between the normal and 6-OHDA rats, and pre- and post-administration of WAY-100635 were determined by comparing the interspike interval histograms (ISIH; bin width=4 ms) together with visual inspections of the spike trains similar to the methods described previously[18,19]. According to the ISIHs and the spike trains, firing patterns were classified into the following types: (1) regular or slightly irregular neuron, with an ISIH characterized by a nearly symmetrical distribution; (2) irregular neuron, with an ISIH characterized by an asymmetrical distribution; (3) the burst firing pattern, with an ISIH exhibiting an obviously positive skewness with a long progressive decline. These patterns were determined using a minimum of 500 spikes. Visual inspection of digital neuronal activity and raster displays were useful complements to the analysis of discharge patterns of the neurons. Several parameters that reflect ISIH were quantitatively evaluated[18,19]. The mode was the most frequent interspike interval (ISI). The ratio of the mode to the mean ISI is called the asymmetry index. This ratio gives information on the shape of the ISIH. A ratio of less than one reflects an asymmetrical shape, indicating a larger fraction of short ISIs. This is expected when there is bursting activity. The degree of regularity of neuronal discharge was determined by calculating the ISI coefficient of variation, which is defined as the ratio between standard deviation and mean ISI.

1.4 *Histology and immunocytochemistry*

At the end of each experiment, the recording site was marked by the injection of Pontamine Sky Blue (-20 μA, 15 min). The rat was given an overdose of urethane, and perfused with saline followed by 4% paraformaldehyde in phosphate buffered saline (PBS). The brain was immediately removed and post-fixed in the same fixative for 4 h. Then they were placed in PBS with 20% sucrose overnight. The brains were frozen and cut into 40 μm thick coronal sections using a cryotome. The sections containing the BL were stained with cresyl violet to determine the location of Pontamine Sky Blue marks for histological verification and reconstruction of recording sites.

To determine the extent of nigral dopaminergic degeneration, sections of the SNc from rats receiving 6-OHDA injection were examined for free-floating tyrosine

hydroxylase (TH) immunohistochemistry, as previously described[20]. Briefly, sections were preincubated with 3% bovine serum albumin in PBS containing 0.3% Triton X-100 for 30 min at room temperature and then incubated at 4 °C for 48 h with anti-TH monoclonal antibody (1:250, Chemicon). Next, sections were incubated for 2 h with biotinylated anti-mouse IgG (1:200, Chemicon), and incubated for 2 h with the avidin-biotin-peroxidase complex (1:100, Vector) at room temperature. Finally they were exposed for 10-15 min at room temperature to a solution of 0.05% 3,3'-diaminobemidine (Sigma) containing 0.01% H_2O_2, which served as chromogen in the subsequent visualization reaction. Rinses were performed between each step excluding the blocking solution step and the addition of the primary antibody. Sections were rinsed, mounted onto gelatin-coated slides, dehydrated, cleared in xylene and coverslipped. Rats with a total or subtotal loss of TH immunoreactivity in the SNc were used for analysis of electrophysiological recordings.

1.5 *Statistical analysis*

Differences of the firing rates between two groups were analysed by Student's *t*-test. The mean ISI, asymmetry index and the ISI coefficient of variation, and changes in the firing rate of the BL neurons after the administration of WAY-100635 were analysed with nonparametric Mann-Whitney *U* or Wilcoxon test, as these 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 or as percent of control. Statistical analyses were performed using SPSS 10.0 and the level of significance was determined as $P<0.05$.

2 RESULTS

All the recording sites used in the normal and 6-OHDA-lesioned rats were verified to be within the BL and confirmed by the location of the Pontamine Sky Blue iontophoresis (Fig.1*A*). 6-OHDA-lesioned rats in the study turned consistently towards the side controlateral to lesion side with more than 20 turns per 5 min [(45±4) turns/5 min] after apomorphine stimulation and displayed a total loss of TH immunoreactivity in the SNc (Fig.1*B*).

2.1 Neuronal activity of BL neurons in normal and 6-OHDA-lesioned rats

A total of 41 BL neurons in 41 normal rats and 44 neurons in 44 6-OHDA-lesioned rats were recorded, respectively. Firing rates and action potential durations of BL neurons recorded extracellularly were consistent with those previously characterized[21-23]. The basal firing rate of BL neurons in the two groups was very low. The mean firing rate of BL neurons in the normal rats was (0.63±0.1) Hz (n=41, range: 0.08-2.73 Hz), and that in 6-OHDA-lesioned rats was (0.42±0.06) Hz (n=44, range: 0.08-2.34 Hz). Similar to previous studies in which action potential durations were measured, a cut-off level of 2.5 ms action potential duration was used to segregate the two populations[21,23]. Hence slow-firing neurons displayed long action potential durations (>2.5 ms), whereas fast-firing neurons displayed short action potential durations (<2.5 ms; Fig.2*A* and *B*). This segregation is consistent with evidence presented here and indicates that slow-firing neurons with long action potential durations are excitatory projection neurons, whereas the fast-firing neurons with short action potential durations are inhibitory interneurons[21-23]. Action potential

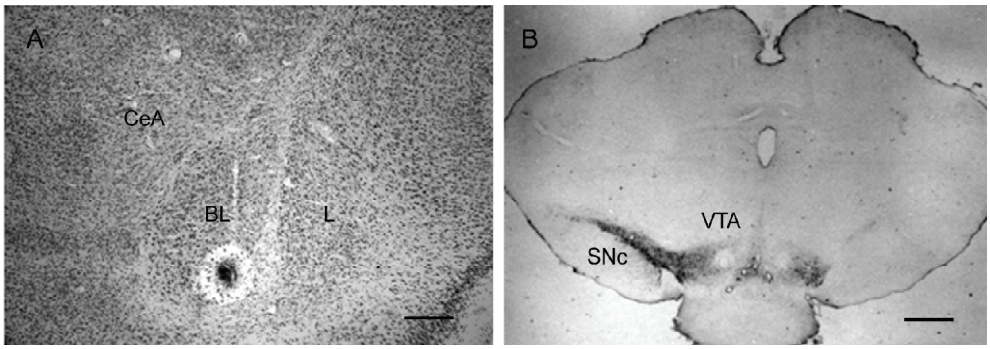

Fig. 1. Cresyl violet staining showing the recording site in the right BL marked with iontophoretically injected Pontamine Sky Blue (*A*). TH immunoreactivity showing the total degeneration of dopaminergic neurons in the SNc on the 6-OHDA injected side (right) compared to normal side (left; *B*). CeA, central nucleus of the amygdala; BL, basolateral nucleus; L, lateral nucleus; SNc, substantia nigra pars compacta; VTA, ventral tegmental area. Scale bar, *A*: 0.5 mm; *B*: 2 mm.

duration was used instead of firing rate in the present study because it allowed better discrimination between the two populations of neurons, in particularly in 6-OHDA-lesioned rats.

In the normal rats, BL projection neurons displayed a mean action potential duration of (2.91±0.09) ms (n=19, range: 2.50-3.50 ms), and a mean firing rate of (0.39±0.04) Hz (n=19, range 0.08-0.64 Hz; Fig.2*C*). The mean action potential duration of BL interneurons was (1.94±0.08) ms (n=22, range: 1.1-2.4 ms), and the mean firing rate was (0.83±0.16) Hz (n=22, range: 0.09-2.73 Hz; Fig.2*C*). All of the 19 projection neurons fired in burst. 94% of interneurons had a burst firing pattern and 6% fired irregularly. Firing pattern parameters of these neurons were summarized in Table 1. In 6-OHDA-lesioned rats, the mean action potential duration of BL projection neurons was (2.90±0.05) ms (n=23, range: 2.60-3.50 ms), and the mean firing rate was (0.32±0.05) Hz (n=23, range: 0.08-0.94 Hz; Fig.2*C*). No significant differences were found in the mean firing rate of the projection neurons between the normal and 6-OHDA-lesioned rats. BL interneurons displayed a mean action potential duration of (2.10±0.06) ms (n=21, range: 1.80-2.40 ms), and a mean firing rate of (0.53±0.12) Hz (n=21, range: 0.11-2.34 Hz; Fig.2*C*). No significant differences were observed in the mean firing rate of the interneurons between the normal and 6-OHDA-lesioned rats. 85% of projection neurons had a burst firing pattern and 15% fired irregularly. 86% of interneurons showed a burst firing pattern and 14% fired irregularly. There were no significant differences in the distribution of firing patterns in both groups. Concerning firing pattern parameters, no significant differences were detected either in the mean ISI, asymmetry index or ISI coefficient of variation between the normal and 6-OHDA-lesioned rats (Table 1).

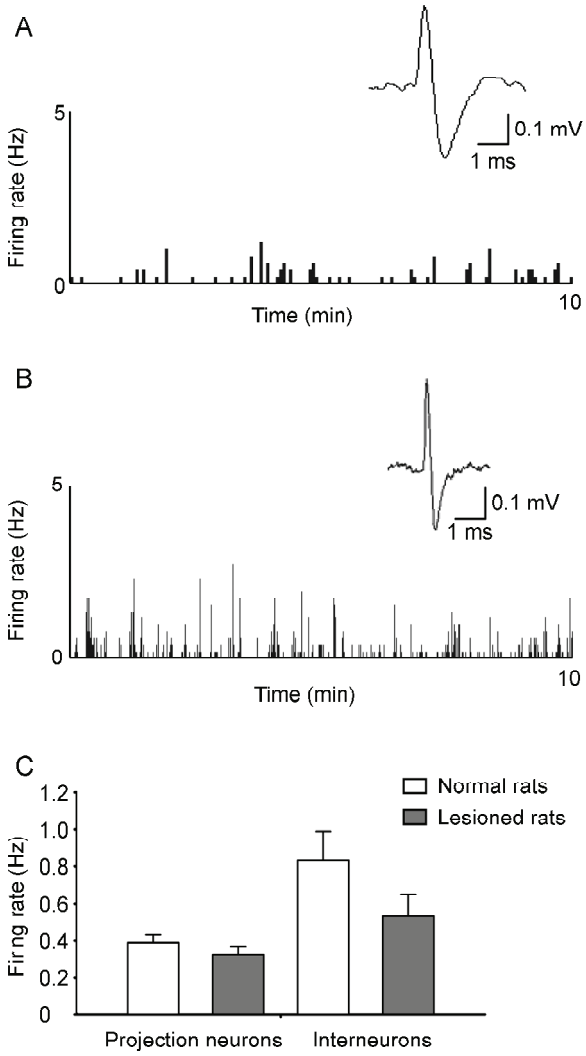

Fig. 2. Representative firing rate histograms and waveforms of neurons recorded in the BL in 6-OHDA-lesioned rats. *A*: A projection neuron. *B*: An interneuron. *C*: Comparison of the mean firing rates of projection neurons and interneurons recorded in the BL in the normal and 6-OHDA-lesioned rats. means±SEM. Student's t test showed there were no significant differences between the two groups.

Table 1. Firing pattern parameters of BL neurons of the amygdala in the normal and 6-OHDA-lesioned rats

Groups	Number of neurons	Mean ISI (s)	Asymmetry index	ISI coefficient of variation
Normal rats				
Projection neurons	19	3.77±0.75	0.006±0.001	1.30±0.04
Interneurons	22	3.55±0.68	0.012±0.002	1.41±0.07
6-OHDA-lesioned rats				
Projection neurons	23	5.22±0.70	0.015±0.008	1.24±0.04
Interneurons	21	3.70±0.63	0.010±0.003	1.41±0.06

No significant differences were observed between projection neurons or interneurons in the normal and 6-OHDA-lesioned rats. means±SEM. Mann-Whitney U test.

2.2 Effect of WAY-100635 on BL neurons in normal and 6-OHDA-lesioned rats

Systemic administration of the selective 5-HT_{1A} receptor antagonist WAY-100635 (0.1 or 0.5 mg/kg body weight, respectively) had variable effects on the firing rate of spontaneously active neurons in the BL in the normal and 6-OHDA-lesioned rats. Results of the responses of BL neurons to different doses of WAY-100635 were summarized in Table 2. No changes in the firing pattern in the normal and 6-OHDA-lesioned rats were observed after administration of WAY-100635.

In the normal rats, administration of WAY-100635 at 0.1 mg/kg body weight did not change the mean firing rates of projection neurons and interneurons in the BL [projection neurons, n=7; pre-WAY-100635: (0.37±0.06) Hz; post-WAY-100635: (0.38±0.12) Hz; mean change: (14±34)% of basal rates; P>0.05; interneurons, n=8; pre-WAY-100635: (1.03±0.28) Hz; post-WAY-100635: (1.57±0.59) Hz; mean change: (24±22)% of basal rates; P>0.05; Fig.3*A*]. On an individual neuron basis, WAY-100635 (0.1 mg/kg body weight) increased the firing rates of 3 projection neurons and decreased the firing rates of 4 such neurons. The firing rates of 5 interneurons examined in the BL were increased by the administration of WAY-100635 at 0.1 mg/kg body weight, while 2 neurons showed a decrease and 1 was unaltered. Increasing the dosage of WAY-100635 (0.5 mg/kg body weight) did not induce a significant change in the mean firing rate of BL interneurons [n=10; pre-WAY-100635: (0.57±0.22) Hz; post-WAY-100635: (0.65±0.27) Hz; mean change: (114±131)% of basal rates; P>0.05; Fig. 3*B*]. Of the 10 interneurons examined in the normal rats, 4 increased, 5 decreased and 1 did not change the firing rate after administration of WAY-100635 at 0.5 mg/kg body weight. However, 9 projection neurons examined in the BL in the normal rats decreased their mean firing rates after administration of higher dose of WAY-100635 [n=9; pre-WAY-100635: (0.43±0.07) Hz; post-WAY-100635: (0.15±0.02) Hz; mean change: (-59±9)% of basal rates; P<0.01; Fig.3*B* and 4*A*]. WAY-100635 (0.5 mg/kg body weight) decreased the firing rates of 8 neurons, and did not alter the firing rate of 1 neuron.

In 6-OHDA-lesioned rats, the mean firing rates of BL projection neurons and interneurons after administration of WAY-100635 at 0.1 mg/kg body weight did not change compared to that before administration [projection neurons, n=7; pre-WAY-100635: (0.21±0.04) Hz; post-WAY-100635: (0.19±0.05) Hz; mean change: (-1±19)% of basal rates; P>0.05; interneurons, n=8; pre-WAY-100635: (0.71±0.26) Hz; post-WAY-100635: (1.47±0.55) Hz; mean change: (119±68)% of basal rates; P>0.05; Fig.3*C*]. Of the 7 projection neurons examined in 6-OHDA-lesioned rats, 3 neurons increased and 3 neurons decreased their firing rates, and 1 was unaltered after administration of WAY-100635 at 0.1 mg/kg body weight. WAY-100635 (0.1 mg/kg body weight) increased the firing rates of 5 of 8 interneurons examined in the lesioned rats, decreased the firing rates of 2 neurons and did not alter the firing rate of 1 neuron. A higher dose of WAY-100635 (0.5 mg/kg body weight) significantly increased the mean firing rates of BL projection neurons in 6-OHDA-lesioned rats [n=11; pre-WAY-100635: (0.37±0.08) Hz; post-WAY-100635: (0.69±0.18) Hz; mean change: (64±21)% of basal rates; P<0.004; Fig.3*D* and 4*B*]. WAY-100635 (0.5 mg/kg body weight) increased the firing rates of 8 of 11 projection neurons examined in

Table 2. Responses of BL neurons of the amygdala to systemic administration of the selective 5-HT_{1A} receptor antagonist WAY-100635 in the normal and 6-OHDA-lesioned rats

Groups	Neuronal types	Dose of WAY-100635 (mg/kg body weight)	Excitation		Inhibition		No effect		Total
			n	(%)	*n*	(%)	*n*	(%)	
Normal rats	Projection neurons	0.1	3	(43)	4	(57)	0	(0)	7
		0.5	0	(0)	8	(89)	1	(11)	9
	Interneurons	0.1	5	(63)	2	(25)	1	(12)	8
		0.5	4	(40)	5	(50)	1	(10)	10
6-OHDA-lesioned rats	Projection neurons	0.1	3	(43)	3	(43)	1	(14)	7
		0.5	8	(73)	1	(9)	2	(18)	11
	Interneurons	0.1	5	(63)	2	(25)	1	(12)	8
		0.5	4	(44)	2	(22)	3	(34)	9

A change of >30% of basal firing rate was considered as a significant alteration for an individual neuron, and only one neuron was observed per animal for administration of WAY-100635.

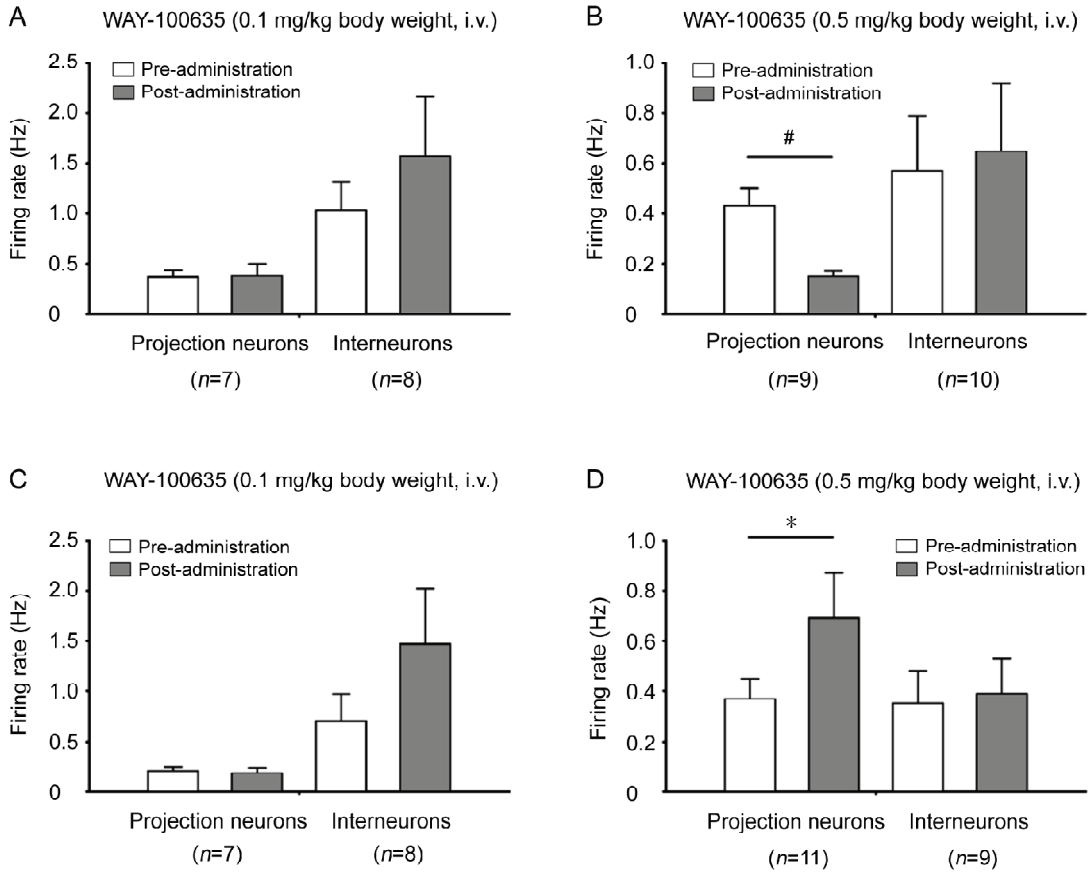

Fig. 3. Effect of the selective 5-HT_{1A} receptor antagonist WAY-100635 on firing rates of projection neurons and interneurons recorded in the BL in normal (*A*, *B*) and 6-OHDA-lesioned (*C*, *D*) rats. WAY-100635 at 0.1 mg/kg body weight (i.v.) did not change the mean firing rates of projection neurons and interneurons in the BL in both two groups. WAY-100635 at 0.5 mg/kg body weight (i.v.) significantly decreased the mean firing rate of projection neurons in the normal rats (*B*), whereas the same dose of WAY-100635 produced a significant increase in the mean firing rates of projection neurons in 6-OHDA-lesioned rats compared to pre-administration (*D*). The mean firing rates of interneurons in two groups did not alter after administration of 0.5 mg/kg body weight of WAY-100635. Only one neuron was observed per animal for administration of WAY-100635. means±SEM. $^{\#}P<0.01$, $^{*}P<0.004$, Wilcoxon test.

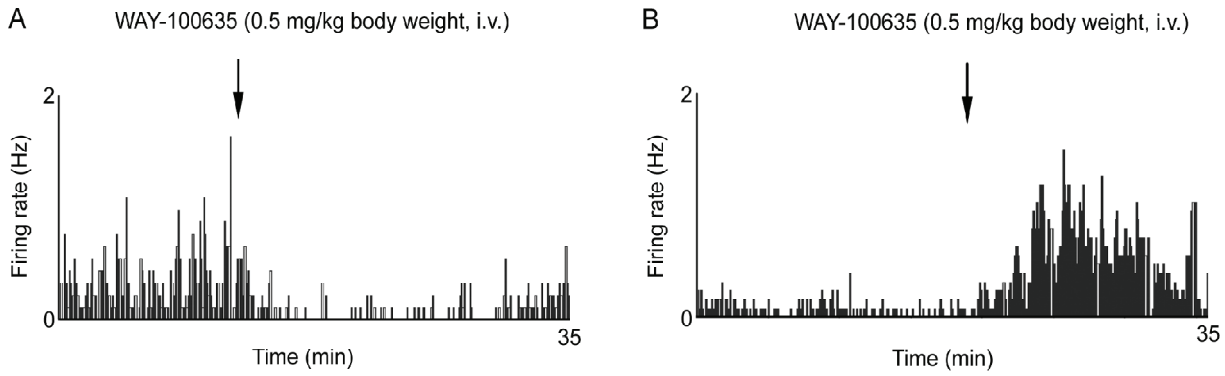

Fig. 4. Representative firing rate histograms of two BL projection neurons recorded showing the mean firing rate significantly decreased in the normal rats (*A*) and significantly increased in 6-OHDA-lesioned rats (*B*) after systemic administration of the selective 5-HT_{1A} receptor antagonist WAY-100635 at 0.5 mg/kg body weight.

6-OHDA-lesioned rats, decreased the firing rate of 1 neuron and did not alter the firing rates of 2 neurons. However, the mean firing rates of 9 interneurons in 6-OHDA-lesioned rats did not change after administration of WAY-100635 at 0.5 mg/kg body weight [n=9, pre-WAY-100635: (0.35±0.13) Hz; post-WAY-100635: (0.39±0.14) Hz; mean change: (36±45)% of basal rates; P>0.05; Fig.3*D*]. Only 4 interneurons increased, 2 neurons decreased and 3 neurons did not change in the firing rates.

3 DISCUSSION

In the present study we examined the changes in BL neuronal activity in the BLA after 6-OHDA lesion of the SNc in rats and the effect of 5-HT_{1A} receptor antagonist WAY-100635 on BL neuronal activity in the normal and 6-OHDA-lesioned rats. The results indicate that: (1) lesion of the SNc did not induce significant changes in the firing rate and firing pattern of projection neurons and interneurons in the BL compared to that in the normal rats, and (2) systemic administration of the specific 5-HT_{1A} receptor antagonist WAY-100635 (0.5 mg/kg body weight) induced a significant increase in the firing rate of BL projection neurons in 6-OHDA-lesioned rats, but a significant decrease in the normal rats.

The BL is a main component in the BLA, which receives afferent projections from areas including the cortex, thalamus, ventral tegmental area (VTA), SNc, and dorsal raphe nucleus (DRN)[12,13,24,25]. These afferent inputs regulate the activity of BL neurons through different receptors. In addition, the anatomic organization of the BL suggests that local circuitry is an important aspect of neuronal activity in this nucleus. The interneurons in the BL display extensive axonal arborization, with many terminals onto the soma of the projection neurons, and thus can exert powerful modulation over projection neuron firing[26]. Therefore, the neuronal activity of the BL is regulated by complex intrinsic and extrinsic mechanisms.

The BL receives dense innervation by dopaminergic terminals from the VTA and SNc and expresses both D_1 and D_2 receptors[11,12]. Systemic administration and microiontophoresis of dopamine receptor agonist in the BL cause a decrease in the firing rate of projection neurons and an increase in the firing rate of interneurons. Moreover, electrical stimulation of the VTA and SNc in rats produces results similar to the effects observed with systemic administration of dopamine receptor agonist[21,27]. Dopamine receptor activation decreases firing in BL projection neurons through direct inhibition and also indirectly via interneurons[21]. These results indicate that the effect of dopamine receptor activation depends upon the type of neuron. Several studies have reported a roughly 20% reduction of TH-positive fiber densities in the BL, lateral and central nucleus of the amygdala in 1-methy-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated mice, and the degeneration of dopaminergic neurons in the SNc produces a dramatic decrease in dopamine and its major metabolites in dopaminergic projection areas[15,28,29]. Therefore, the level of dopamine in the BL after lesioning the SNc was decreased, leading to an increase in excitation of BL projection neurons and a decrease in interneurons. The medial prefrontal cortex (mPFC) and sensory association cortical areas such as entorhinal, temporal area 3 and perirhinal cortices receive dopaminergic innervation mainly arising from the VTA. Dopamine exerts both direct and indirect effects on the excitability of the pyramidal neurons via D_1 and D_2 receptors[30]. *In vivo* extracellular recordings indicate that local administration of dopamine and VTA stimulation predominantly decrease the spontaneous firing of single pyramidal neurons[31]. Furthermore, mPFC stimulation potently activates interneurons that can inhibit BL projection neurons, and sensory association cortical area inputs drive BL projection neurons[22,32,33]. Lesioning the SNc induces a decrease of VTA and downregulation of D_1 and D_2 receptors in the prefrontal cortex and consequently a reduction of excitatory glutamatergic outputs from the cortex that results in a decrease of the mPFC output on BL interneurons as well as sensory association cortex outputs on BL projection neurons[15,34]. In addition, the mediodorsal nucleus (MD) of the thalamus also sends glutamatergic projections to the BL[25]. However, the effect of MD inputs on the two basic neuronal subtypes in the BLA still remains unclear. As mentioned above, these data indicate that the activity of neurons in the BL is regulated by activation of intrinsic and extrinsic inputs[21,22,33,35,36]. Therefore, the balance between these excitatory and inhibitory inputs could be responsible for the activity of BL neurons in 6-OHDA-lesioned rats, the net result being an unchanged neuronal activity compared to that in the normal rats.

The BL also receives prominent innervation from serotonergic terminals originating from the DRN and expresses moderate to high levels of binding sites for several 5-HT receptor subtypes[8-10,13]. The effect of 5-HT is mediated through activation of these receptor types. These receptor subtypes are differentially distributed in the amygdala. The 5-HT_{1A} receptor couples to a K^+ channel through a G protein, therefore, the activiation of 5-HT_{1A} receptors results in an enhanced K^+ conductance that inhibits neuronal

activity[37]. Accumulated evidence indicates that the complex interaction and the input-output relationship of the BL can be regulated by 5-HT. Our data support this view in that excitations, inhibitions and unaltered responses were all observed with systemic administration of WAY-100635 in projection neurons and interneurons in the BL in the normal and 6-OHDA-lesioned rats. Projection neurons in normal rats displayed a decrease in firing rate after WAY-100635 administration. Autoradiography of 5-HT receptor subtypes indicated that the BL has only a low density of 5-HT_{1A} receptors[10,38]. Stimulation of the DRN results in the facilitation of firing in a subset of amygdala neurons and iontophoretic administration of 5-HT and 5-HT_{1A} receptor agonist in all nuclei of the amygdala resulted in the observation of excited, inhibited and non-responsive neurons, although inhibition was most predominant[36,39]. Furthermore, the BL receives glutamatergic mPFC inputs that drive or regulate the BL neuronal activity[22]. However, the activity of neurons in the mPFC is not changed by systemic administration of WAY-100635[40]. Therefore, we postulated that systemic administration of WAY-100635 in the normal rats increases the activity of serotonergic neurons in the DRN, which increases 5-HT inputs of the BL, exciting the GABAergic interneurons via activation of postsynaptic 5-HT_2 receptors, while also inhibiting the activity of projection neurons. In addition, it is possible that WAY-100635 increases γ-aminobutyric acid (GABA) release by presynaptic 5-HT_{1A} receptors on the GABAergic terminals in the BL and then leads to an inhibition of the projection neurons in the normal rats, although the density of 5-HT_{1A} receptors is low in the BL[10,35,38,41].

In contrast to that in the normal rats, projection neurons in the BL in 6-OHDA-lesioned rats showed an increase in firing rate after WAY-100635 administration. Neurochemical studies have shown that the levels of 5-HT and its main metabolite 5-HIAA were reduced by as much as 57% in the basal ganglia, cerebral cortex and cerebrospinal fluid in PD patients[15,16]. The reduction of 5-HT is attributable to loss of 5-HT neurons in the raphe nuclei, because several postmortem studies have found an average loss of more than 50% of serotonergic neurons in the median raphe nucleus in PD patients[16,42]. Furthermore, a recent study has also shown a reduction of 27% in 5-HT_{1A} binding in the midbrain raphe using ^{11}C-WAY 100635 as a ligand in PD patients by PET imaging, indicating 5-HT_{1A} receptor dysfunction[43]. These studies indicate that the serotonergic system is severely affected in PD patients. It is known that there is a marked reduction in 5-HT_{1A} receptor density coupled with 5-HT_{1A} receptor dysfunction in the DRN and hippocampus in MPTP-treated monkeys and PD patients[43,44]. If these situations also exist in the BL, WAY-100635 is likely to indirectly regulate the activity of the projection neurons through the cortex in 6-OHDA-lesioned rats, given that mPFC inputs potently elicit firing of BLA interneurons, and PD patients exhibit increased cortical expression of postsynaptic 5-HT_{1A} receptors[14,22]. Therefore, with regard to the current experiment it is likely that WAY-100635 acts on 5-HT_{1A} receptors located on the pyramidal neurons in the mPFC, and increases the glutamatergic outputs, leading to an increase in the firing rate of projection neurons in the BL in 6-OHDA-lesioned rats. Systemic administration of WAY-100635 in both two groups showed that excited, inhibited and non-responsive neurons were present in the BL, and the mean firing rate of the interneurons increased slightly without significant difference. These results could be explained by the hypothesis that the subpopulations of interneurons within the BL may express different 5-HT receptor subtypes and not one 5-HT receptor subtype is expressed in all interneurons, and 5-HT_{1A} receptor expression is rather low in BL neurons[8-10,38].

In conclusion, the present study indicates that degeneration of the nigrostriatal pathway with 6-OHDA did not affect the firing pattern of projection neurons and interneurons in the BL. Systemic administration of the selective 5-HT receptor antagonist, WAY-100635, produced opposite effects in the normal and 6-OHDA-lesioned rats. WAY-100635 inhibited neuronal activity of BL projection neurons in the normal rats, whereas increased the mean firing rate of BL projection neurons in 6-OHDA-lesioned rats. The results suggest that the activity of BL projection neurons is regulated by complicated mechanism. Furthermore, the abnormal responses of BL neurons to 5-HT_{1A} receptor stimulation in 6-OHDA-lesioned rats may provide some link between the locomotor and affective symptoms of PD.

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