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Electrophysiological Effects of Neurotensin on Globus Pallidus Neurons of 6-Hydroxydopamine-Lesioned Rats

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Key Words
Globus pallidus · Neurotensin · 6-Hydroxydopamine · Parkinson's disease · Single unit recording

Abstract
The globus pallidus is a nucleus in the indirect pathway of the basal ganglia circuits. Neurotensin has been reported to play an important role in the central nervous system. Functional study revealed that systemic administration of neurotensin produced antiparkinsonian effects. The aim of the present study was to investigate the effects of neurotensin on the firing rate of globus pallidus neurons in 6-hydroxydopamine-lesioned parkinsonian rats. Micropressure ejection of neurotensin increased the spontaneous firing rate of globus pallidus neurons on both lesioned and unlesioned sides. Furthermore, the neurotensin-induced increase in firing rate on the unlesioned side (95.9%) was stronger than that on the lesioned side (37.3%). The neurotensin receptor antagonist, SR48692, prevented neurotensin-induced increase in firing rate. Based on the excitatory effects of neurotensin in globus pallidus of parkinsonian rats, we hypothesize that the pallidal neurotensinergic system may be involved in its possible therapy in Parkinson's disease.

Introduction

Parkinson's disease is a neurodegenerative movement disorder characterized by the progressive loss of dopaminergic neurons in substantia nigra pars compacta [1]. The globus pallidus represents a key structure in the basal ganglia. There is evidence supporting the involvement of the globus pallidus in both normal motor function and movement disorders including Parkinson's disease. For example, previous electrophysiological studies have shown that the firing rate of the globus pallidus neurons decreased in parkinsonian patients and non-human primates (macaque) [2, 3]. Consistently, the average neuronal discharge rate decreased in globus pallidus of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated monkeys. In addition to the change of firing rate, MPTP treatment tended to induce pallidal neurons to discharge in oscillatory bursts [4]. The conventional anatomical models [5] have been applied to explain the abnormal hypoactivity of globus pallidus neurons, through the indirect pathway, leading to akinesia and hypokinetic symptoms of Parkinson's disease [6, 7]. Furthermore, the oscillatory bursts firing induced by the absence of normal dopaminergic innervation is related to the symptoms of parkinsonian tremor [8–11].

Neurotensin, first isolated by Carraway and Leeman [12], is an endogenous tridecapeptide that behaves as a neurotransmitter or neuromodulator in the central ner-

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vous system [13]. Three neurotensin receptors have been identified, neurotensin type-1, type-2 and type-3 receptor (NTS3/sortilin) [14–16]. Morphological studies have revealed that the globus pallidus expresses both neurotensin type-1 and type-2 receptors [17, 18]. Recently, Martorana et al. [19] reported that neurotensin type-1 receptor is localized in both parvalbumin-positive and -negative globus pallidus neurons. The globus pallidus receives neurotensinergic innervation arising from the striatum [20, 21]. Previous studies have indicated that the expression of neurotensin and neurotensin receptor in globus pallidus changed in Parkinson's disease. In 6-hydroxydopamine (6-OHDA)-treated rats and parkinsonian patients, an increase in neurotensin-immunoreactivity has been found in globus pallidus [22, 23]. However, the expression of neurotensin receptors in globus pallidus decreased markedly in parkinsonian patients [24, 25]. Recently, a functional study revealed that systemic administration of a neurotensin analog, which can cross the blood-brain barrier, produced antiparkinsonian-like effects in 6-OHDA-treated rats [26]. Our previous studies have described the electrophysiological effects of neurotensin on globus pallidus neurons of normal rat [27, 28]. In the present study, single-unit recordings were used to observe the effects of neurotensin on neuronal activity of globus pallidus neurons in 6-OHDA-lesioned parkinsonian rats.

Materials and Methods

Animals

Experiments were performed according to the university guidelines on animal ethics. All efforts were made to minimize animal suffering and to reduce the number of animals used. Adult male Wistar rats (200–250 g) were individually housed with free access to food and water in a light- (12:12 h light/dark cycle) and temperature- (22 ± 1°C) controlled room.

6-OHDA Lesion

Rats were deeply anesthetized with chloral hydrate (400 mg/kg, i.p.) and gently placed in a stereotaxic frame (Narishige SN-3, Tokyo, Japan). The skull was then exposed and a burr hole was drilled. A cranial burr hole (1 mm) was drilled into the skull over the injection site, and a microsyringe was lowered into the medial forebrain bundle: 3.2 mm posterior, 1.5 mm lateral to the bregma, 8.4 mm ventral to the skull surface [29]. A total dose of 14.5 μg 6-OHDA hydrochloride (H4381; Sigma, St. Louis, Mo., USA) in 4 μl sterile saline containing 0.01% ascorbic acid was then injected into the right medial forebrain bundle at a rate of 1.0 μl/min. The microsyringe was allowed to rest for 10 min to prevent backflow of the toxin. Rats were pretreated 30 min before the 6-OHDA infusion with 25 mg/kg desipramine to protect noradrenergic projections.

Rotational Behavior Tests

Behavioral testing was performed 14 days after the injection of 6-OHDA treatments, the rats were injected subcutaneously with 0.2 mg/kg apomorphine hydrochloride (A4393; Sigma) dissolved in 0.1% ascorbate saline solution. The numbers of contralateral turns were counted from the videotape. The lesion was considered successful in those animals that made at least 210 net contralateral rotations in 30 min.

Extracellular Single Unit Recordings

On the day of electrophysiological recordings, rats were deeply anesthetized with urethane (1 g/kg, i.p.) and placed in a stereotaxic apparatus (Narishige SN-3). An incision was made in the scalp, the skull exposed, and a burr hole drilled in the skull. Rectal temperature was maintained at 36–38°C by a heated pad.

Three-barrel microelectrodes were fastened at each end with metal tubing and prepared using a Stoelting pipette puller (Stoelting Co., Wood Dale, Ill., USA). The tips of microelectrodes were broken to 3–10 μm under a microscope. The resistance of microelectrodes ranged from 10–20 MΩ. The recording electrode was filled with 0.5 M sodium acetate containing 2% pontamine sky blue dye. The other two micropressure ejection barrels connected to a 4-channel pressure ejector (PM2000B; Micro Data Instrument, South Plainfield, N.J., USA). The two micropressure ejection barrels respectively contained neurotensin and vehicle (normal saline), SR48692 and vehicle (DMSO), SR48692 and SR48692 with neurotensin or neurotensin and neurotensin with SR48692. The electrode was then placed into the globus pallidus with coordinates of 0.8–1.2 mm posterior, 2.5–3.5 mm lateral from the bregma, 5.5–7.2 mm vertical from the dura [29]. Neurons were identified as pallidal on the basis of their location and electrophysiological features. Drugs were ejected onto the surface of firing cells with short pulse gas pressure (1,500 ms, 5.0–15.0 psi).

The recorded electrical signals were amplified by a microelectrode amplifier (MEZ-8201; Nihon Kohden, Tokyo, Japan) and displayed on a memory oscilloscope (VC-11; Nihon Kohden), while being fed to an audiomonitor. The amplified electrical signals were passed through low- and high-pass filters into a bioelectricity signal analyzer and computer. Spike times were preprocessed online and further analyzed offline using the program of Histogram Version 1.00 (Shanghai Medical University, Shanghai, China) for spike data analysis. The firing rates were recorded in 1-s bins. Drug infusion was performed only once for each recording and a period of 30 min at least was allowed to pass before another recording in the same track.

At least 5 min stable basal firing was collected from each neuron before drug ejection onto the globus pallidus. The frequency of basal firing was determined by the average frequency of 120-s baseline data before drug administration. The maximal change of frequency within 50 s following drug application was considered as drug effect. A change of at least 20% of basal firing rate during drug application was considered significant [30]. Drug application was performed only once for each recording, and a period of 30 min was allowed to pass before another recording in the same track.

Histology and Immunohistochemistry

To verify the position of single unit recording, pontamine sky blue was ejected from the recording electrode tip by iontophoresis (10 μA, 20 min). The rats were then sacrificed by chloral hydrate (600 mg/kg, i.p.) and perfused with 4% paraformaldehyde solu-

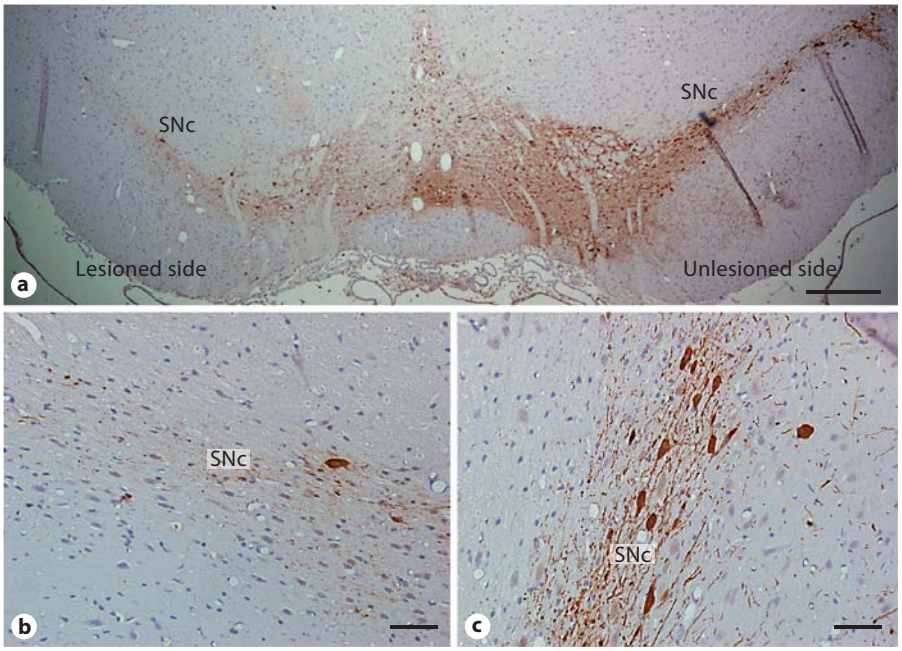

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Fig. 1. Immunostaining of tyrosine hydroxylase in rat substantia nigra pars compacta in 6-OHDA-lesioned parkinsonian rats. **a** Low-magnification photomicrograph showing the immunostaining of tyrosine hydroxylase in substantia nigra pars compacta. **b**, **c** High-magnification photomicrograph of tyrosine hydroxylase in substantia nigra pars compacta of lesioned (**b**) and unlesioned (**c**) sides. Scale bars: (**a**) 0.5 mm, (**b**, **c**) 50 μm.

tion transcardially. The brains were dissected out and incubated in paraformaldehyde overnight. After that, the brains were frozen and sectioned to identify recording and microinjection sites under light microscope.

To determine the extent of nigral dopaminergic degeneration, rats receiving unilateral injection of 6-OHDA were examined for immunohistochemical staining of tyrosine hydroxylase. After perfusion, the brain containing the part of substantia nigra was removed from the cranium and fixed overnight. Tissues were fixed in 4% paraformaldehyde for 24 h and were dehydrated in a graded series of ethanol concentrations before paraffin embedding. Coronal sections (5 μm) were made using a microtome and mounted on slides coated with 3-aminopropyltriethoxysilane and dried at 37°C for 24 h. The sections were deparaffinized and rehydrated. Endogenous peroxidase was quenched with 0.3% H_2O_2 (30 min). After washing in distilled water (3 × 5 min), the sections were incubated with 5% normal goat serum for 1 h. This was followed by application of monoclonal anti-tyrosine hydroxylase antibody (1:5,000; Sigma) incubated overnight at 4°C. After washing in PBS (3 × 5 min), the sections were incubated with the biotinylated secondary antibody (1:100) for 60 min, followed by washes with PBS (3 × 5 min). The immunoreactive sites were revealed by incubation in 0.05% 3,3-diaminobenzidine tetrahydrochloride (DAB; Sigma) in 0.1 M Tris buffer. The sections were then washed with tap water, dehydrated, cleared with xylene and coverslipped using mountant. Quantification of cells under the microscope was done. The loss of tyrosine hydroxylase-positive neurons on the lesioned side was more than 90% in the present study (fig. 1).

Drugs and Statistics

Neurotensin was obtained from Sigma. SR48692{2-[(1-(7-chloro-4-quinolinyl)-5-2(2,6-dimethoxyphenyl)pyrazol-3-yl)carbonylamino]-tricyclo(3.3.1.1.3.7)-decan-2-carboxylic acid} was kindly provided by Dr. Danielle Gully (Sanofi Recherche, Toulouse, France). 6-OHDA hydrochloride, apomorphine hydrochloride and monoclonal anti-tyrosine hydroxylase antibody were obtained from Sigma.

The data are expressed as means ± SDs. Paired t test was used to compare the difference of firing rate before and after treatment. Statistical comparisons between or among groups were determined with Student's t test and one-way ANOVA. The level of significance was preset by using a p value of 0.05.

Results

All the neurons recorded in the present study showed a biphasic positive/negative waveform, which are characterized as type II globus pallidus neurons [31, 32]. In normal rats, the basal spontaneous firing rate of globus pallidus neurons ranged from 3 to 40 Hz (13.6 ± 7.2 Hz, n = 21). Micropressure ejection of 0.1 mM neurotensin significantly increased the spontaneous firing rate of pallidal neurons in normal rats. In 11 out of 21 pallidal neurons, 0.1 mM neurotensin increased the firing rate from 14.5 ± 4.7 to 21.3 ± 7.2 Hz (n = 11, $p < 0.001$, fig. 2a). The average increase was 48.3 ± 20.7%, which was significantly different compared to that of vehicle (normal saline) injection (basal: 14.8 ± 4.4 Hz; vehicle: 15.1 ± 4.2 Hz; increase: 2.7 ± 4.4%, n = 11, $p < 0.01$ compared to neurotensin).

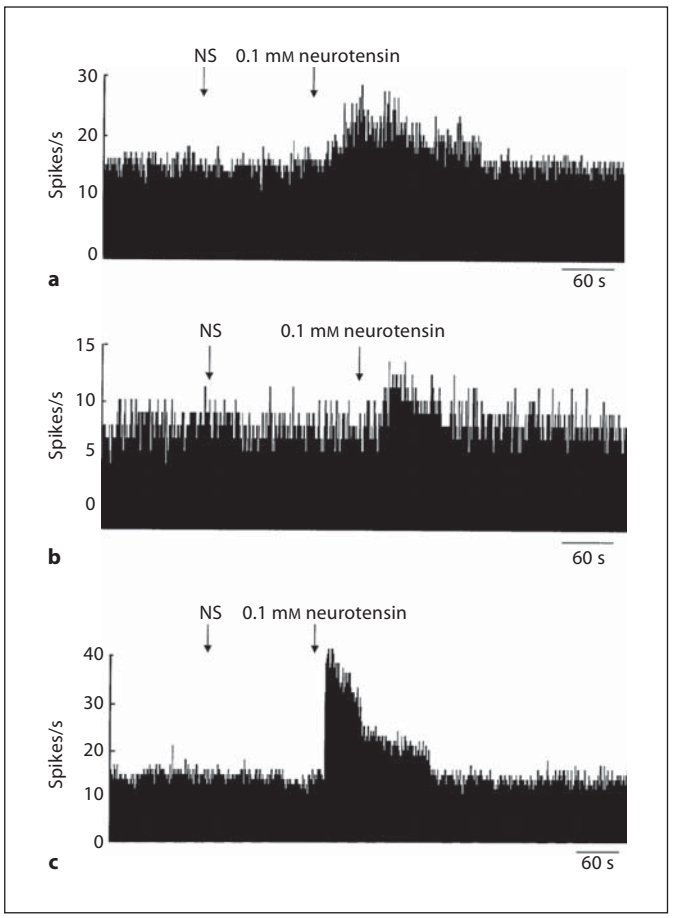

Fig. 2. Effects of intrapallidal micropressure ejection of neurotensin on the spontaneous firing rate of globus pallidus neurons. Typical frequency histograms showing that neurotensin (0.1 mM) increased the firing rate of pallidal neurons in normal rats (**a**), lesioned side (**b**) and unlesioned side (**c**) of 6-OHDA parkinsonian rats. NS = Normal saline.

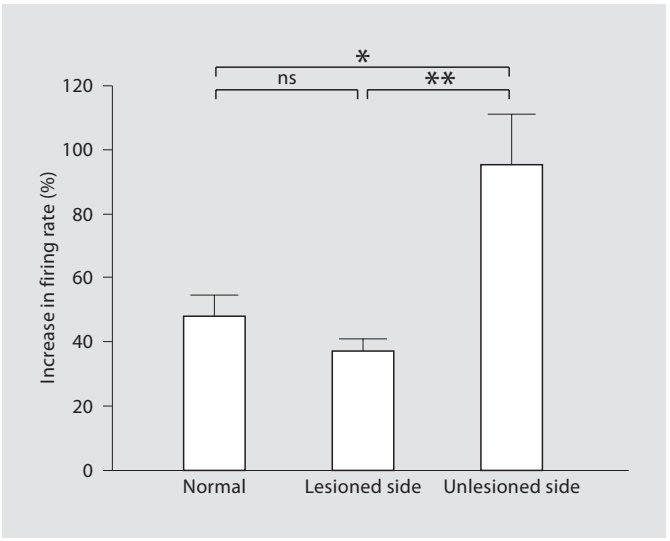

Fig. 3. Comparison of neurotensin-induced increase in firing rate between 6-OHDA-lesioned rats and normal rats. * $p < 0.05$, ** $p < 0.01$, ns = not significant.

Effects of Neurotensin on Spontaneous Firing of Globus Pallidus in 6-OHDA-Lesioned Rats

To clarify the effects of neurotensin on globus pallidus neurons of 6-OHDA-lesioned parkinsonian rats, we monitored the spontaneous activity of 59 pallidal neurons sampled from 15 parkinsonian rats. On the lesioned side, pallidal neurons discharged with a mean firing rate of 14.4 ± 7.0 Hz (n = 30), which was not significantly different from that of normal rats (13.6 ± 7.2 Hz, n = 21, $p > 0.05$). In 13 out of 30 pallidal neurons, 0.1 mM neurotensin increased the frequency of spontaneous firing from 11.1 ± 6.7 to 15.1 ± 8.7 Hz ($p < 0.001$, fig. 2b). The average increase was 37.3 ± 12.6%, which was significantly different from that of vehicle (normal saline) injection (basal: 11.1 ± 6.6 Hz; vehicle: 11.5 ± 7.0 Hz; increase: 3.9 ± 8.1%, n = 9, $p < 0.01$ compared to neurotensin). There was no significant difference in neurotensin-induced excitation of pallidal neurons between the lesioned side (37.3 ± 12.6%, n = 13) of parkinsonian rats and that of normal rats (48.3 ± 20.7%, n = 11, $p > 0.05$). In another 17 neurons, local administration of neurotensin had no significant effect on the firing rate of pallidal neurons.

On the unlesioned side, pallidal neurons discharged with a mean firing rate of 13.5 ± 13.2 Hz (n = 29), which was not different from that of normal rats (13.6 ± 7.2 Hz, n = 21, $p > 0.05$). In 17 out of 29 pallidal neurons, 0.1 mM neurotensin increased the spontaneous firing from 11.3 ± 9.4 to 20.2 ± 14.7 Hz ($p < 0.001$, fig. 2c). The average increase was 95.9 ± 65.9%, which was significantly different compared to vehicle (normal saline) injection (basal: 10.6 ± 8.5 Hz; vehicle: 10.9 ± 8.2 Hz, increase: 6.5 ± 9.2%, n = 10, $p < 0.001$ compared to neurotensin). The increase in firing rate on the unlesioned side (95.9 ± 65.9%, n = 17) was much stronger than that on the lesioned side (37.3 ± 12.6%, n = 13, $p < 0.01$), as well as that in normal rats (48.3 ± 20.7%, n = 11, $p < 0.05$, fig. 3). There was no correlation found between the rotational behavior in each rat, or the size of the lesion, and the effect of neurotensin on the unlesioned side of the brain.

Consistent with the previous morphological study that neurotensin receptor was expressed in about 56% of

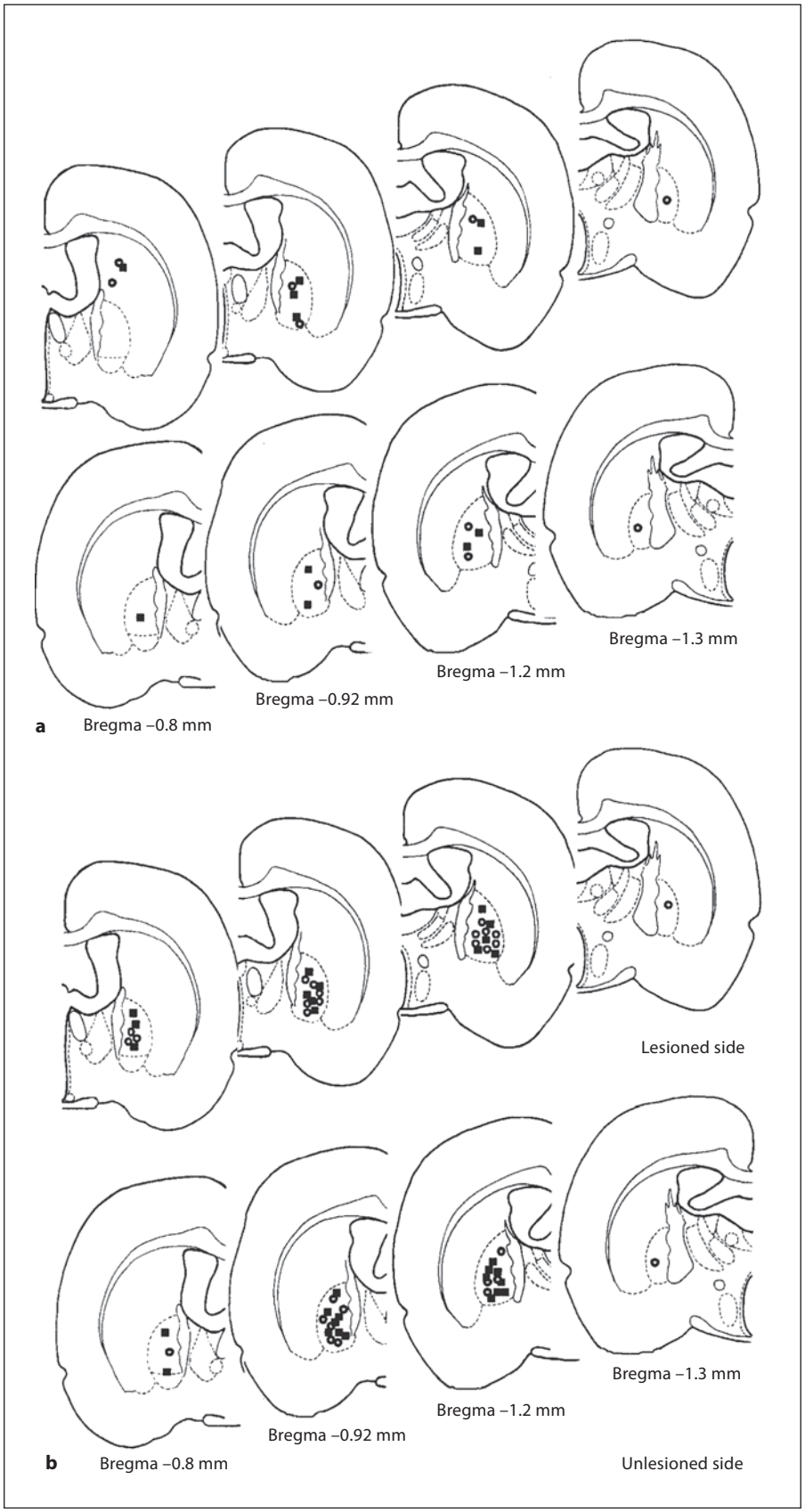

Fig. 4. Maps of recorded pallidal neurons with or without response to neurotensin. **a** Maps of recorded neurons in normal rats with (black square, n = 11) or without (white circle, n = 10) response to neurotensin. **b** Maps of recorded neurons in 6-OHDA-lesioned rats with (black square, n = 30) or without response to neurotensin (white circle, n = 29).

globus pallidus neurons [19], the present data showed that neurotensin only increased the firing rate of a proportion of pallidal neurons. Figure 4 showed the maps of recorded neurons with or without response to neurotensin in globus pallidus.

Effects of SR48692 on Neurotensin-Induced Excitation

The neurotensin receptor antagonist SR48692, which has a higher affinity for neurotensin type-1 receptor compared to neurotensin type-2 receptor, was used in the present study. On the lesioned side, in 19 pallidal neurons, 1 mM SR48692 alone had no effect on firing rate (basal: 13.2 ± 9.6 Hz; SR48692: 13.7 ± 9.2 Hz; increase: 6.1 ± 12.2%, $n = 19$, $p > 0.05$). In 12 neurons that then received SR48692 with neurotensin, the firing rate did not change significantly (basal: 13.1 ± 10.5 Hz; neurotensin+SR48692: 14.3 ± 12.0 Hz, $p > 0.05$, fig. 5a). The average change of firing rate was 6.6 ± 9.7%, which was significantly decreased compared to that of neurotensin alone ($p < 0.001$). In a separate set of rats, administration of vehicle (DMSO) did not alter the spontaneous firing rate of pallidal neurons (basal: 16.6 ± 8.9 Hz; vehicle: 17.9 ± 10.3 Hz; increase: 6.2 ± 6.7%, $n = 8$, $p > 0.05$). On the unlesioned side, however, the effects of SR48692 were different from those on the lesioned side. In 5 out of 19 pallidal neurons, SR48692 decreased the spontaneous firing rate from 13.6 ± 8.9 to 7.2 ± 7.6 Hz. The average decrease was 48.3 ± 26.8%. In 10 neurons, co-ejection of SR48692 with neurotensin did not alter the firing rate of pallidal neurons (basal: 13.7 ± 8.6 Hz; neurotensin+SR48692: 14.5 ± 9.3 Hz, $p > 0.05$, fig. 6a). The average change was 5.1 ± 8.5%, which was significantly decreased compared to that of neurotensin alone ($p < 0.001$). In the control group, administration of vehicle (DMSO) did not alter the spontaneous firing rate of pallidal neurons (basal: 16.8 ± 11.9 Hz; vehicle: 17.6 ± 12.7 Hz; increase: 4.6 ± 5.1%, $n = 6$, $p > 0.05$).

Considering that only a proportion of pallidal neurons responded to neurotensin, we further performed another group of experiments to apply neurotensin first and then SR48692 with neurotensin. On the lesioned side, in 7 pallidal neurons with response to micropressure ejection of 0.1 mM neurotensin (basal: 11.8 ± 3.3 Hz; neurotensin: 15.6 ± 3.9 Hz; increase: 33.6 ± 9.1%), co-ejection of neurotensin with SR48692 did not change the firing rate significantly (basal: 11.3 ± 3.0 Hz; neurotensin+SR48692: 12.0 ± 3.3 Hz, $p > 0.05$). The average change was 6.3 ± 7.3%, which was significantly decreased compared to that of neurotensin alone (33.6 ± 9.1%, $p < 0.001$, fig. 5b). On the unlesioned side, in 7 pallidal neurons with response to neurotensin (basal: 9.6 ± 5.7 Hz; neurotensin: 17.3 ± 11.2 Hz; increase: 86.3 ± 34.2%), co-ejection of neurotensin with SR48692 did not change the firing rate significantly (basal: 10.9 ± 7.2 Hz; neurotensin+SR48692: 10.8 ± 6.4 Hz, $p > 0.05$). The average change was 1.7 ± 5.1%, which was significantly decreased compared to that of neurotensin alone (86.3 ± 34.2%, $p < 0.001$, fig. 6b). In control experiments, neurotensin was applied twice to another group of pallidal neurons. On the lesioned side, in 10 neurons with response to neurotensin, neurotensin increased the firing rate by 40.0 ± 11.8% (basal: 12.4 ± 6.7 Hz; neurotensin: 17.0 ± 8.6 Hz, $p < 0.001$) on the first time, and 36.1 ± 11.5% (basal: 13.1 ± 6.9 Hz; neurotensin: 17.7 ± 9.0 Hz, $p < 0.001$) on the second time ($n = 10$, $p = 0.16$ compared to that on the first time, fig. 5c). On the unlesioned side, in 8 neurons with response to neurotensin, neurotensin increased the firing rate by 98.0 ± 63.3% (basal: 12.2 ± 8.7 Hz; neurotensin: 22.0 ± 14.4 Hz, $p < 0.001$) on the first time, and 70.0 ± 42.7% (basal: 12.4 ± 8.0 Hz; neurotensin: 20.3 ± 13.1 Hz, $p < 0.001$) on the second time ($n = 8$, $p = 0.098$ compared to that on the first time, fig. 6c).

Discussion

The present findings revealed that the spontaneous firing rate of globus pallidus neurons in 6-OHDA-lesioned rats did not change significantly compared to that in normal rats, which is in line with the previous studies that there was no significant difference in pallidal firing between nigral 6-OHDA-induced parkinsonian rats and normal rats [33]. However, Chang et al. [34] have shown that unilateral 6-OHDA lesion decreased the spontaneous firing of globus pallidus neurons. Consistently, a decreased discharge rate of globus pallidus neurons was reported in parkinsonian monkey [4]. The anesthesia may play a potential role in this discrepancy on firing rate under parkinsonian state. The decreased firing of globus pallidus neurons was performed in freely-moving rats or monkey [4, 34], while unchanged firing of globus pallidus neurons was collected in anesthetized animals [33] including the present recording.

Previous morphological evidence indicated a decreased neurotensin binding in the globus pallidus of parkinsonian patients [25]. However, the present electrophysiological recordings showed that neurotensin-induced excitation on the lesioned side was slightly, but not significantly, decreased compared to that in normal rats, suggesting no significantly functional loss of neurotensin receptor

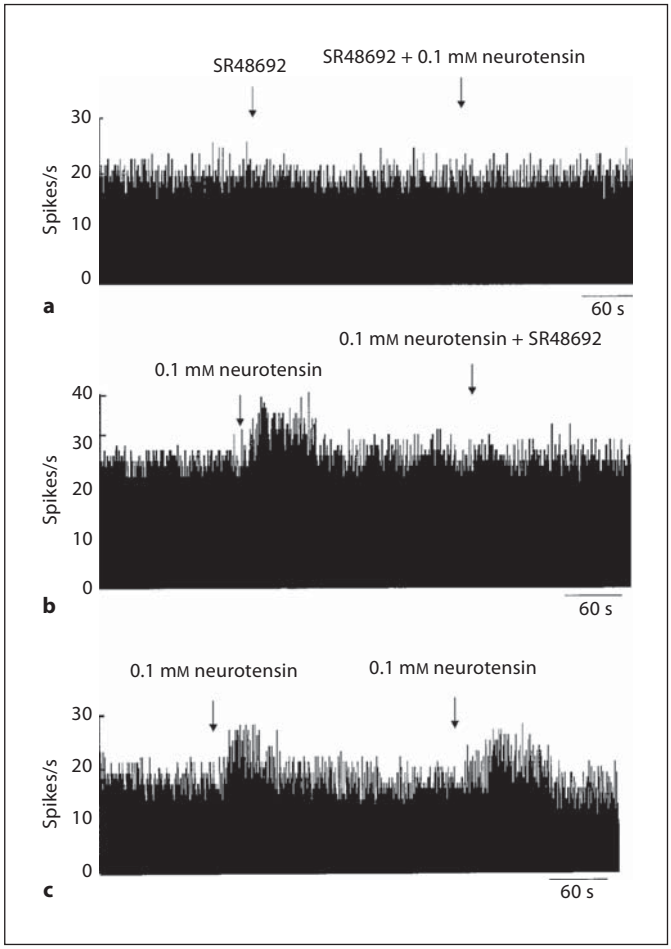

Fig. 5. Effects of SR48692 on neurotensin-induced increase in firing rate of pallidal neurons of 6-OHDA-lesioned side. **a** In this cell, SR48692 alone had no effect on the spontaneous firing rate. Co-ejection of SR48692 with neurotensin did not alter the firing rate. **b** In this cell which was activated by neurotensin, co-ejection of SR48692 with neurotensin prevented neurotensin-induced excitation. **c** In this cell, neurotensin was applied twice to the globus pallidus neuron with the same application interval as for the experiments **a** and **b**.

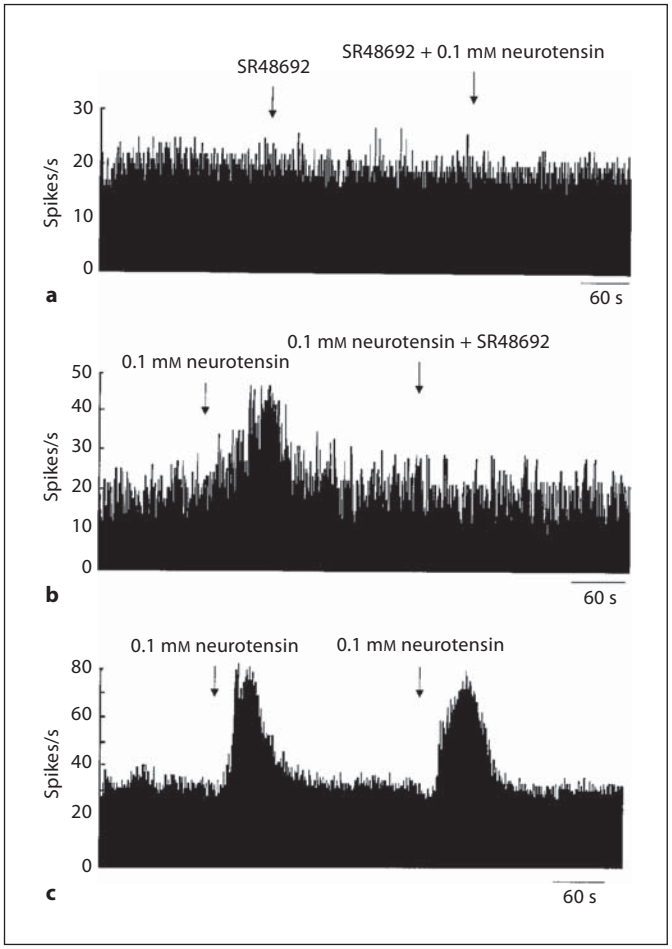

Fig. 6. Effects of SR48692 on neurotensin-induced excitation of pallidal neurons of 6-OHDA unlesioned side. **a** In this cell, SR48692 alone had no effect on the spontaneous firing rate. Co-ejection of SR48692 with neurotensin did not alter the firing rate. **b** In this cell which was activated by neurotensin, co-ejection of SR48692 with neurotensin did not change the firing rate. **c** In this cell, neurotensin was applied twice to the globus pallidus neuron with the same application interval as for the experiments **a** and **b**.

in the globus pallidus of 6-OHDA parkinsonian rats. Furthermore, the present study also showed that neurotensin exerted stronger excitatory effects in globus pallidus of the 6-OHDA unlesioned side, which may suggest the compensation in the globus pallidus of unlesioned side. The finding was supported by some morphological evidence. For example, unilateral striatal lesions which destroyed a certain proportion of dopaminergic terminals in the lesioned striatum led to slight, but significant, increase of neurotensin-binding site densities in the contralateral striatum [35]. Furthermore, in bilateral 6-OHDA-lesioned rats, the number of neurotensin-binding sites was increased in the lateral part of the prefrontal cortex [36]. However, Masuo et al. [37] reported that there was no significant change of neurotensin receptors in the contralateral striatum and substantia nigra of 6-OHDA-lesioned rats, although the expression on contralateral striatum and substantia nigra was much higher than that on the lesioned side.

Another finding of the present experiments was that SR48692 inhibited the spontaneous firing in some of the pallidal neurons on the unlesioned side, which indicated that endogenous neurotensinergic system may mod-

ulate the cellular activity of pallidal neurons. Consistently, functional studies revealed that intrapallidal microinjection of SR48692 modulated motor symptoms in tardive dyskinesia model [38]. Interestingly, SR48692 only decreased the firing rate of pallidal neurons on the unlesioned side rather than lesioned side. Our previous results have shown that SR48692 also decreased the basal firing of globus pallidus neurons in normal rats. One of the possibilities for the lack of the effect of SR48692 on the lesioned side may be that the tonic activity of neurotensinergic system on the lesioned side was decreased compared to that on the unlesioned side. Destruction of dopaminergic terminals may change the activity of the neurotensinergic system in globus pallidus. Previous studies have revealed that the neurotensin-immunoreactive territory of the globus pallidus on the 6-OHDA-lesioned side was larger than that on the unlesioned side. This accumulation may be caused by the decrease in release of neurotensin from striatopallidal terminals [21]. If this is true, the decreased tonic activity of endogenous neurotensinergic system in globus pallidus of 6-OHDA parkinsonian rats may be involved in the pathophysiology of Parkinson's disease. According to the functional scheme of basal ganglia circuit [7], hypoactivity of globus pallidus causes disinhibition of the subthalamic nucleus, leading to overactivity of the entopeduncular nucleus and the substantia nigra pars reticulata. The resulting inhibition of thalamocortical activity leads to bradykinesia and rigidity in Parkinson's disease.

Previous functional study revealed that systemic administration of a neurotensin analog produced antiparkinsonian effects in 6-OHDA-treated rats [26]. The present findings that neurotensin directly excited globus pallidus neurons under parkinsonian state may provide a rationale for further investigations into its potential in the treatment of motor disorders originating from the basal ganglia. However, it is well known that neurotensin has a close relationship with the dopaminergic system. Morphological studies have revealed that neurotensin receptors, particularly neurotensin type-1 receptor, were highly prevalent in midbrain neurons. Approximately 80% of dopamine cells in the ventral tegmental area and substantia nigra pars compacta expressed neurotensin receptors [17, 39, 40]. In addition, there was a wide distribution of neurotensin-like immunoreactivity and neurotensin-binding sites on the surface of midbrain dopamine neurons [39, 41]. Microinjection of neurotensin into the substantia nigra pars compacta caused an increase in dopamine and dopamine metabolites in the globus pallidus and striatum [42]. Furthermore, intracerebroventricular administration of neurotensin elevated concentrations of dopamine in the striatum and amygdale [43]. The extracellular dopamine concentration in the nucleus accumbens of 6-OHDA-treated rats was significantly increased following neurotensin microinjection into the ventral tegmental area [44]. Therefore, the therapeutic effect of systemic administration of neurotensin would depend on the interaction of its effects on the whole basal ganglia nuclei.

In conclusion, our present findings demonstrated that neurotensin excited globus pallidus neurons in 6-OHDA-lesioned rats. The lack of tonic activity of endogenous neurotensinergic system in the globus pallidus of 6-OHDA-lesioned side may be involved in the pathophysiology of Parkinson's disease, while the excitatory effects of neurotensinergic system in globus pallidus of 6-OHDA-lesioned rats may provide a rationale for further investigations into its possible therapy in Parkinson's disease.

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