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·Original Article·

Modulation of firing activity by endogenous $GABA_A$ receptors in the globus pallidus of MPTP-treated parkinsonian mice

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ABSTRACT

The globus pallidus in rodents, equivalent to the external segment of the globus pallidus in primates, plays an important role in movement regulation. Previous studies have shown abundant γ-aminobutyric acid (GABA)ergic innervation and $GABA_A$ receptors in the globus pallidus. In this study, we investigated the effects of endogenous $GABA_A$ receptors on the spontaneous firing activity of pallidal neurons in both normal and MPTP-treated mice using multi-barrel electrodes extracellular recordings *in vivo*. We found that in normal mice, pressure ejection of 0.1 mmol/L gabazine, a specific $GABA_A$ receptor antagonist, increased the spontaneous firing rate of globus pallidus neurons by 27.6 ± 5.6%. Furthermore, in MPTP mice (14 days after MPTP treatment), 0.1 mmol/L gabazine increased the firing rates by 51.0 ± 7.9%, significantly greater than in normal mice. These results suggest that endogenous $GABA_A$ receptors modulate the activity of globus pallidus neurons**.** The present findings may provide a rationale for investigations into the potential role of $GABA_A$ receptors in Parkinson's disease.

Keywords: globus pallidus; $GABA_A$ receptor; Parkinson's disease; single unit recording

INTRODUCTION

The globus pallidus is a critical structure in the basal ganglia involved in the manifestation of parkinsonian motor symptoms. It is known that dopamine depletion in the substantia nigra pars compacta decreases the firing rate and increases the synchronized oscillation of globus pallidus neurons, and then induces the symptoms of akinesia and resting tremor observed in Parkinson's disease[1,2]. Gamma-aminobutyric acid (GABA) is an important neurotransmitter. By activating postsynaptic $GABA_A$ receptors, it inhibits most neurons in the central nervous system[3,4]. The globus pallidus contains a high concentration of GABA. It mainly receives GABAergic input from the striatum and pallidal collaterals, and in turn sends GABAergic output to all the other basal ganglia nuclei. Morphological studies have revealed a high level of $GABA_A$ receptor expression in the globus pallidus[5-7]. Much evidence has indicated that GABA and/or $GABA_A$ receptors are closely associated with Parkinson's disease. First, the release of GABA increases in the globus pallidus of parkinsonian animals[8-10]. Second, the glutamic acid decarboxylase isoform 67 (GAD67) mRNA expression increases in the external pallidum of parkinsonian monkeys and the globus pallidus of parkinsonian cats, suggesting increased GABAergic transmission in motor deficits[11,12]. Third, the expression of $GABA_A$/benzodiazepine receptors decreases in the rostral globus pallidus in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated monkeys[13,14]. Similarly, gene expression of the $GABA_A$ receptor subunit is significantly reduced in the globus pallidus of 6-hydroxydopamine (6-OHDA) parkinsonian rats[15]. Therefore, exploring the modulation of endogenous

$GABA_A$ neurotransmission in the globus pallidus is critical for understanding the etiology and treatment of Parkinson's disease. MPTP was discovered to cause a parkinsonian syndrome in 1982 and has been used extensively in monkeys and mice to produce experimental models of Parkinson's disease. In this study, we further investigated the direct modulation of firing activity by endogenous $GABA_A$ receptors in the globus pallidus of MPTP parkinsonian mice.

MATERIALS AND METHODS

Reagents

The specific $GABA_A$ receptor antagonist gabazine, MPTP, and the anti-tyrosine hydroxylase (TH) monoclonal antibody were from Sigma (St. Louis, MO).

Animals

Adult male C57BL/6 mice (20 ± 2 g) were purchased from Vital River Experimental Animal Center (Beijing, China). The mice were individually housed at 22 ± 1°C under a 12:12 h light/dark cycle, with free access to food and water throughout the experiments. All animal experiments were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care Committee. All efforts were made to minimize animal suffering and reduce the number of animals used.

Mouse Model of Parkinson's Disease

The mice were divided randomly into two groups, control and parkinsonian groups. In the parkinsonian group, the mice were injected intraperitoneally (i.p.) with 30 mg/kg MPTP each day for five consecutive days. In the control group, MPTP was replaced by the same volume of normal saline. On different days after treatment, electrophysiological recordings were made.

Extracellular Single Unit Recording

After anesthesia with urethane (1 g/kg, i.p.), the mice were placed in a stereotaxic frame and a hole was drilled in the skull. In all experiments, rectal temperature was maintained at 37 ± 1°C. The tip diameter of three-barrel microelectrodes ranged from 3 to 10 μm and their resistances were 10–20 MΩ. One microelectrode filled with 0.5 mol/L sodium acetate containing 2% pontamine sky blue dye was used for recording. The other two were used for drug application and were connected to a 4-channel pressure ejector. The coordinates of the globus pallidus were 0.22–0.34 mm posterior, 1.5–2.5 mm lateral to bregma, and 3.5–4.5 mm vertical from the dura[16]. Globus pallidus neurons were identified based on location and firing properties. Before drug application, a 2-min stable recording was made as the basal firing rate. The maximal change of firing rate within 50 s after drug ejection was considered as the effect.

Histological Controls

To identify the sites of extracellular recordings, pontamine sky blue in the recording barrel was iontophoresed (10 μA, 20 min) into the globus pallidus. The mice were then sacrificed with chloral hydrate (600 mg/kg, i.p.) and perfused transcardially with 4% paraformaldehyde. The brains were frozen and sectioned to identify recording sites under a light microscope (Fig. 1)

To confirm the success of the parkinsonian model, mice receiving MPTP were examined for TH staining. Monoclonal anti-TH antibody (1:5 000) was applied to coronal sections containing the substantia nigra. The number of TH-positive neurons in each section was counted in a blind manner. Loss of TH-positive neurons was observed in the substantia nigra pars compacta of MPTP-treated mice (Fig. 2).

Statistics

Data are expressed as mean ± SEM. The paired *t*-test was used to compare the difference in firing rate before and after gabazine/vehicle application. Statistical comparisons between or among groups were determined with Student's *t*-test and one-way ANOVA. $P < 0.05$ was set as the level of significance.

RESULTS

Electrophysiological Effects of Gabazine on Spontaneous Firing of Globus Pallidus Neurons in Normal Mice

All the neurons recorded showed a biphasic positive/negative waveform, and were characterized as type II globus pallidus neurons. To identify the effects of endogenous $GABA_A$ receptors, we monitored the spontaneous activity of 43 pallidal neurons in normal mice.

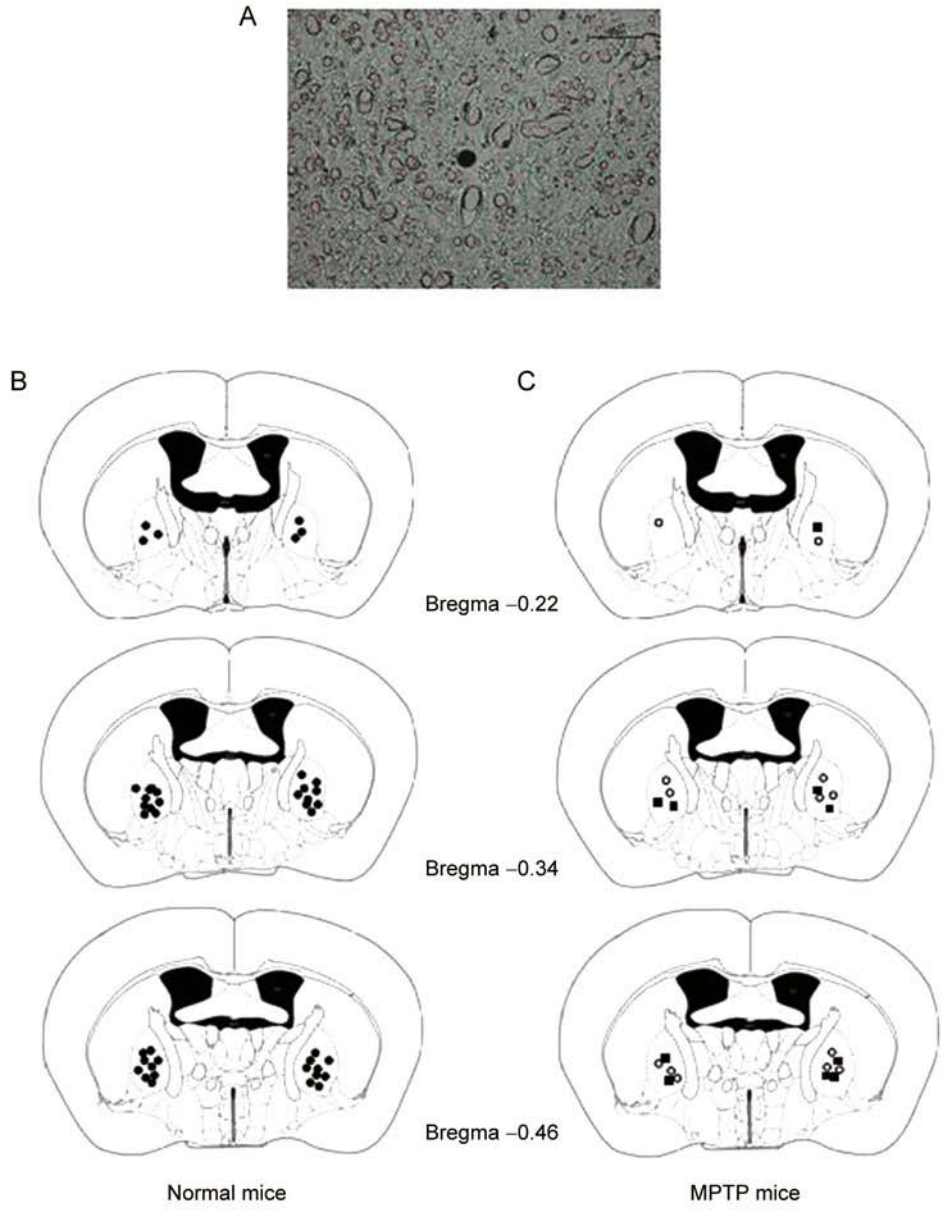

Fig. 1. Confirmation of the location of recorded neurons in normal and MPTP-treated mice. A: Example of pontamine sky blue staining in the globus pallidus. Scale bar, 100 μm. B: Maps of recorded neurons in normal mice (black circles, *n* = 43). C: Maps of recorded neurons in MPTP-treated mice (white circles: 3 days after MPTP, *n* = 13; black squares: 14 days after MPTP, *n* = 10).

The basal spontaneous firing rate ranged from 0.8 Hz to 32.2 Hz (average, 11.7 ± 1.2 Hz). Pressure ejection of normal saline did not change the firing rate (basal, 11.8 ± 1.9 Hz; saline, 12.0 ± 1.9 Hz; increase, 2.7 ± 1.8%; $n = 18$, $P > 0.05$, Fig. 3). But pressure ejection of 0.1 mmol/L gabazine increased the frequency of spontaneous firing from 11.7 ± 1.7 Hz to 13.7 ± 1.7 Hz ($n = 25$, $P < 0.001$, Fig. 3). The average increase was 27.6 ± 5.6%, significantly different from that of vehicle ejection ($P < 0.001$).

Electrophysiological Effects of Gabazine on Spontaneous Firing of Globus Pallidus Neurons in MPTP-treated Mice

To further investigate the modulation of firing activity by

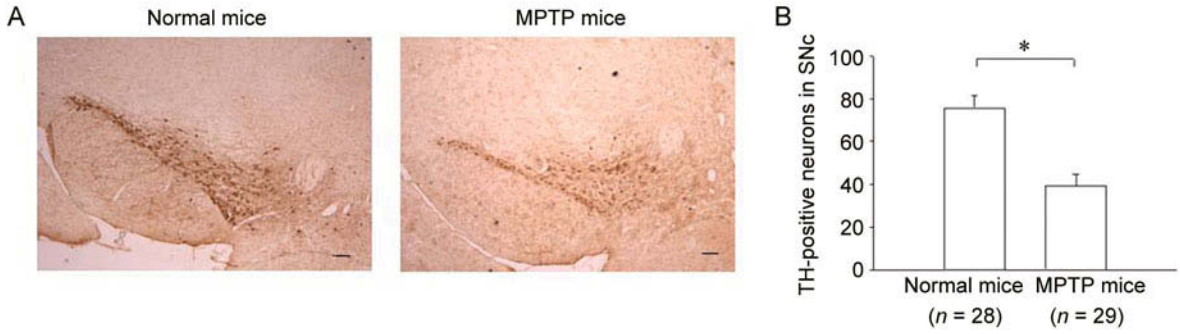

Fig. 2. Confirmation of the MPTP parkinsonian mouse model. A: Immunostaining for tyrosine hydroxylase (TH) in the substantia nigra pars compacta (SNc) of normal and MPTP-treated mice. Scale bars, 100 μm. B: Numbers of TH-positive neurons in SNc of normal and MPTP-treated mice. *P <0.05.

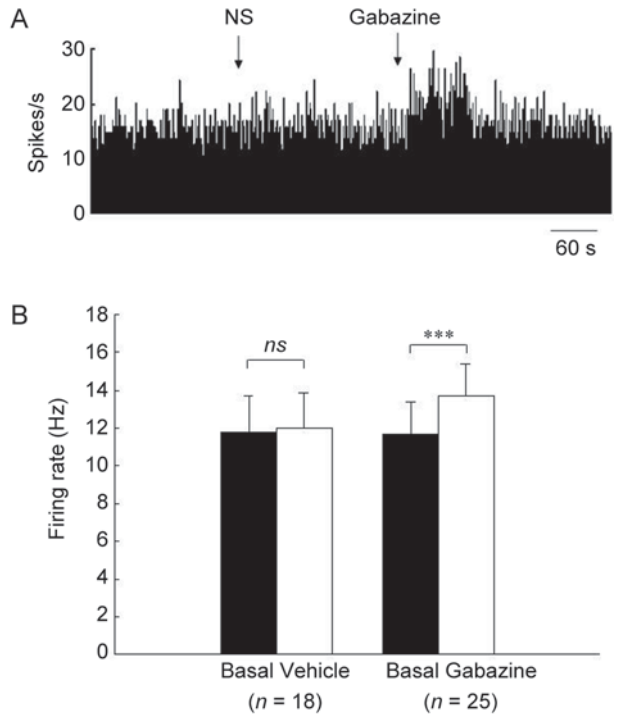

Fig. 3. Effects of gabazine on the spontaneous firing rate of globus pallidus neurons in normal mice. A: Raw frequency histogram showing that intrapallidal pressure ejection of 0.1 mmol/L gabazine increased the firing rate in this neuron. B: Summary of the effects of gabazine on the firing rates of globus pallidus neurons. ns, not significant; ***P <0.001.

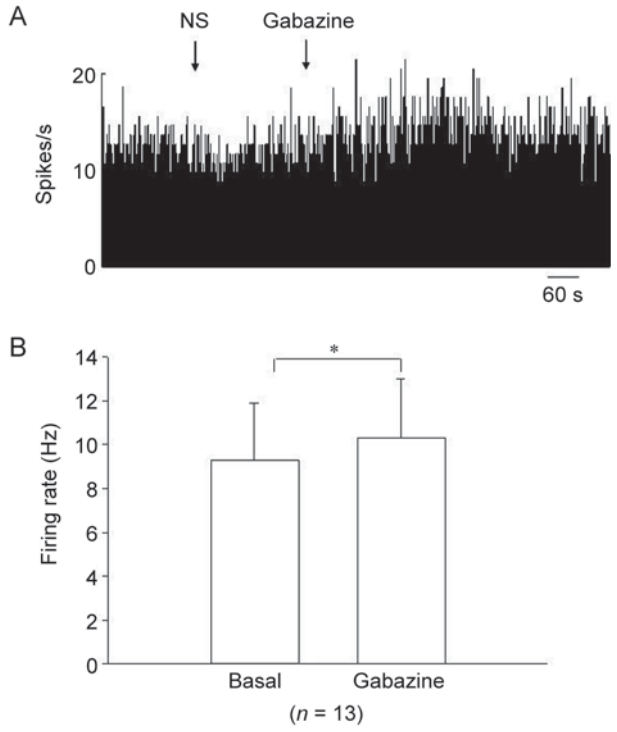

Fig. 4. Effects of gabazine on the firing rate of pallidal neurons in mice three days after MPTP treatment. A: Raw frequency histogram showing that 0.1 mmol/L gabazine increased the firing rate in this pallidal neuron. B: Pooled data summarizing the effects of gabazine on the firing rate of globus pallidus neurons. *P <0.05.

endogenous $GABA_A$ receptors, we assessed the gabazine-induced increase in firing rate of pallidal neurons in MPTP-treated mice. In all 23 recorded neurons, the average basal firing rate was 9.7 ± 1.7 Hz, which was not significantly different from that of normal mice (11.7 ± 1.2 Hz). No clear change in the firing pattern occurred in MPTP mice. In these mice (three days after MPTP treatment), gabazine increased the frequency of spontaneous firing from 9.3 ± 2.6

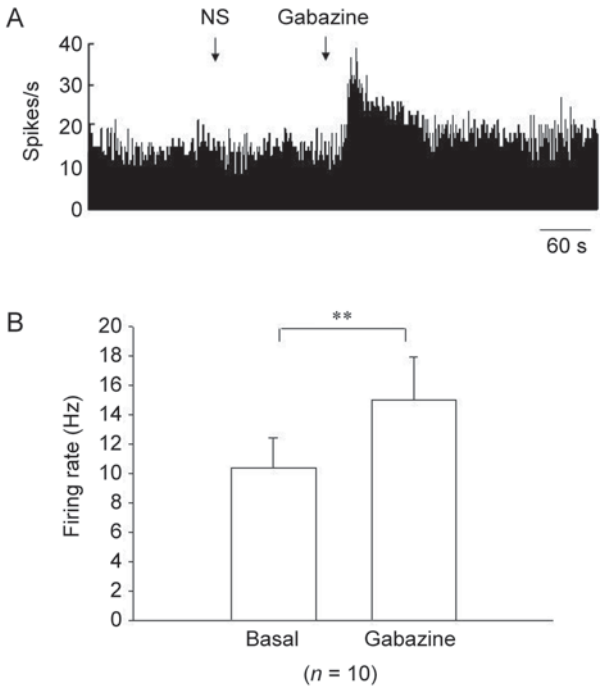

Fig. 5. Effects of gabazine on the firing rate of pallidal neurons in mice at 14 days after MPTP treatment. A: Raw frequency histogram showing that 0.1 mmol/L gabazine increased the firing rate in this pallidal neuron. B: Pooled data summarizing the effects of gabazine on the firing rate of globus pallidus neurons. **P <0.01.

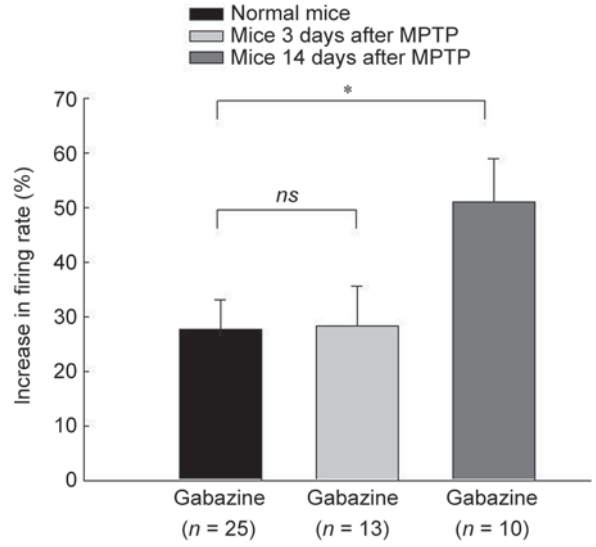

Fig. 6. Comparisons of gabazine-induced increases in firing rate of pallidal neurons between normal and MPTP-treated mice. ns, not significant; *P <0.05.

Hz to 10.3 ± 2.7 Hz (n = 13, P <0.05, Fig. 4). The average increase was 28.4 ± 7.2%, which was not significantly different from that in normal mice. Furthermore, in mice 14 days after MPTP treatment, gabazine increased the spontaneous firing rate from 10.3 ± 2.1 Hz to 15.0 ± 2.9 Hz (n = 10, P <0.01, Fig. 5). The average increase was 51.0 ± 7.9%, which was greater (P <0.05) than that in normal mice. The gabazine-induced increases in firing rate between normal and MPTP mice are compared in Figure 6. In addition to testing at 3 and 14 days after MPTP treatment, we assessed the gabazine-induced increase in firing rate of pallidal neurons at 1, 5 and 7 days, and found no significant difference between the MPTP and normal groups (data not shown).

DISCUSSION

GABA is the major inhibitory neurotransmitter and exerts its functions through two receptor subtypes: $GABA_A$ and $GABA_B$[17,18]. By activating $GABA_A$ receptors, it mediates fast synaptic inhibition through the opening of chloride channels. Previous studies have shown that activation of $GABA_A$ receptors inhibits firing activity in the globus pallidus[19-22]. The present results that gabazine increased the firing rates further demonstrated that endogenous $GABA_A$ receptors modulate the activity of pallidal neurons in mice. Our findings are consistent with the previous reports that local application of $GABA_A$ receptor antagonists increases the firing rate of globus pallidus neurons in monkeys[23,24].

According to the basal ganglia circuit, dopamine depletion in the substantia nigra decreases the spontaneous firing rate of neurons in the globus pallidus. Hypoactivity of the globus pallidus in turn contributes to the akinesia and hypokinetic symptoms of Parkinson's disease. One example is that the basal firing rate of globus pallidus neurons decreases in parkinsonian patients and non-human primates[25,26]. Here, the basal firing rate of pallidal neurons in MPTP mice tended to be decreased compared to that in normal mice, but there was no statistical difference. However, in MPTP mice, it was very difficult to find a pallidal neuron with spontaneous firing, which suggests that some or most pallidal neurons became silent in the parkinsonian state. This is in line with the report by Chan and colleagues[27] that ~60% of globus pallidus neurons lose their normally robust autonomous pacemaking

in parkinsonian animals. Since the globus pallidus neurons receive abundant GABAergic innervation, the increase in firing rate induced by blockade of $GABA_A$ receptors may have a potential therapeutic effect in Parkinson's disease.

Moreover, the present results showed that gabazine exerted a stronger excitation on the globus pallidus neurons of MPTP parkinsonian mice. Two possible reasons, increase of GABA release and/or enhancement of $GABA_A$ receptor activity, may explain the gabazine-induced stronger excitation. Much evidence shows that GABA or $GABA_A$ receptors change in Parkinson's disease, such as the increase of GABA release in the globus pallidus of parkinsonian animals[8-10] and the increase of mRNA of the GABA synthesis enzyme GAD67 in the globus pallidus of various animal models of Parkinson's disease[28-30]. It is usually expected that the increased GABA release from striatopallidal GABAergic terminals in the parkinsonian state may down-regulate $GABA_A$ receptor expression in the globus pallidus. Gnanalingham and Robertson[31] reported that $GABA_A$ receptor binding decreases in the globus pallidus of 6-OHDA parkinsonian rats. Similarly, the mRNA levels of $GABA_A$ receptors decrease in the globus pallidus of 6-OHDA lesioned rats and parkinsonian patients[15]. Furthermore, Chadha *et al.*[15] revealed that after 6-OHDA lesioning, the mRNA levels of both the α1 and β2 subunits are reduced by only 18 ± 3% and 16 ± 3%, respectively, in the globus pallidus. Therefore, gabazine could still increase the firing rate of neurons by blocking the remaining $GABA_A$ receptors. However, in contrast with the reported down-regulation of $GABA_A$ receptors, previous studies also revealed no statistical decrease or subunit-specific changes of these receptors in the globus pallidus. Calon *et al.*[14] reported no statistical decrease in the binding sites of the $GABA_A$/benzodiazepine receptor complex in the globus pallidus in MPTP parkinsonian animals. Other *in vivo* studies revealed a region- and subunit-specific regulation of $GABA_A$ receptor subunit mRNA levels by endogenous GABA[32]. Increased GABA concentration results in a decrease in mRNA expression of the α1 subunit, but an increase in the β2 and γ2 subunits in the globus pallidus[32]. Therefore, we hypothesized that increased GABA release and probably enhanced expression of some $GABA_A$ receptor subunits underlie the enhancement of gabazine-induced increase in firing in MPTP parkinsonian mice.

In conclusion, our electrophysiological study showed that blockade of $GABA_A$ receptors increased the spontaneous firing rate of globus pallidus neurons in both normal and parkinsonian mice. Furthermore, the stronger excitation induced by gabazine in MPTP mice implies a role for $GABA_A$ receptors in the etiology and treatment of Parkinson's disease.

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