Role of Ionotopic Glutamatergic and GABAergic Inputs on the Firing Activity of Neurons in the External Pallidum in Awake Monkeys

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INTRODUCTION

The external segment of the pallidum (GPe) is located at a strategically important locus of the basal ganglia connections and plays key roles in the physiology and pathophysiology of the basal ganglia. The GPe receives major GABAergic inputs from the neostriatum (Str) and the GPe itself through axon collaterals of projection neurons and major glutamatergic projections from the subthalamic nucleus (STN) (Kita 1992; Kita and Kitai 1991; Kita et al. 1999; Parent and Hazrati 1995; Robledo and Feger 1990). The Str and STN are considered to be input-nuclei of the basal ganglia. Some neurons of the centremedian-parafascicular complex of the thalamus and the frontal cortex also innervate the GPe (Deschenes et al. 1996; Kincaid et al. 1991; Mouroux et al. 1997; Naito and Kita 1994; Sadikot et al. 1992; Yasukawa et al. 2004). The GPe sends GABAergic projections to various nuclei in the basal ganglia including the Str, the internal segment of the pallidum (GPi), STN, and the substantia nigra (Bewan et al. 1998; Hazrati et al. 1990; Kita 1994; Kita and Kita 1994; Kita et al. 1999; Nambu and Llinás 1997). These connections imply that the GPe converges information reaching the basal ganglia and that information processed in the GPe are conveyed to many of the nuclei in the basal ganglia. Thus the GPe might play a significant role in controlling the level and pattern of firing activity of neurons in various nuclei of the basal ganglia and, hence, in movement control (Bolam et al. 2000; Kita 1994; Mink 1996; Mink and Thach 1993).

GPe neurons in awake animals maintain a high level of firing activity (Anderson and Horak 1985; DeLong 1971; Matsumura et al. 1995; Nambu et al. 2000; Tremblay et al. 1989; Yoshida et al. 1993). The level and pattern of firing activity change with the development of basal ganglia diseases including Parkinson's disease and hemiballism (Beric et al. 1996; Boraud et al. 2001; Filion and Tremblay 1991; Lent et al. 1998; Nini et al. 1995; Pan and Walters 1988; Sterio et al. 1994; Tremblay et al. 1989; Vitek et al. 1999). Thus exploring the mechanisms controlling the firing activity of GPe neurons is important for understanding basal ganglia functions in normal and pathological conditions. STN neurons in awake animals are also highly active (Matsumura et al. 1992; Nambu et al. 2000). Thus it can be expected that both local GABAergic and STN-GPe glutamatergic inputs play crucial roles in the maintenance of the high level of firing activity and the generation of firing patterns of the GPe neurons. Previous studies in rodents have indicated that activation of glutamatergic and GABAergic synapses evoke ionotropic receptor-mediated responses in the GPe (Kita 1992; Nambu and Llinás 1997; Ogura and Kita 2000). The aims of the present study were to explore the role of ionotropic glutamatergic and GABAergic inputs on the level and pattern of the GPe firing activity and also to clarify the glutamatergic and GABAergic components of the responses induced in GPe neurons by the stimulation of the motor cortex in awake monkeys.

METHODS

Monkey preparation

This study was performed in compliance with the guidelines of National Institutes of Health Guide for Care and Use of Laboratory Animals.
Animals, the Tokyo Metropolitan Institute for Neuroscience, and the National Institute of Physiological Sciences for the Use and Care of Laboratory Animals in Research. The monkey preparation methods used in the present study were very similar to those reported elsewhere (Nambu et al. 2000, 2002). In short, four Japanese monkeys (Macaca fuscata), named K3, K5, K6, and K8, were trained to sit in a monkey chair quietly. The monkeys received a surgery to fix their heads painlessly to a stereotaxic frame attached to the chair. Under ketamine hydrochloride (10 mg/kg im) and pentobarbital sodium (25 mg/kg iv) anesthesia, the skull of monkeys was widely exposed, and two stainless steel pipes were mounted in parallel over the frontal and occipital areas for head fixation.

A few days after the surgery, the primary motor cortex (M1) along the anterior bank of the central sulcus was mapped by observing body part movements induced by cortical microstimulation and also by unitary responses to somatosensory stimuli (skin touch and passive joint movement) to identify the forelimb region. After mapping, two pairs of stimulating electrodes (made of 200 μm diam enamel-coated stainless steel wire; intertip distance, 2 mm) were implanted chronically in the forelimb region of the M1. In monkey K8, the supplementary motor area (SMA) was also mapped, and a pair of stimulating electrode was implanted in the forelimb region of the SMA. To access the pallidum and STN, two holes (10–15 mm diam) were drilled in the skull. A plastic chamber covering both holes was fixed onto the skull with acrylic resin. Recordings of GPE units began after recovery from the surgery. During the experimental sessions, the monkeys were seated in a monkey chair with their heads restrained. The forelimb region of the GPE was determined by cortical stimulation.

Electrode assembly for local injection of drugs

Single-unit recordings of GPE neurons in combination with local applications of drugs were performed with an electrode assembly consisting of a platinum-iridium wire (No. 7675, A-M Systems, Carlsborg, WA) placed in a silica tube (No. 2000018, 147 μm OD, 74 μm ID, Polymicro Technologies, Phoenix, AZ) for unit recordings, two other silica tubes for drug delivery, and a protective stainless-steel tubing (Fig. 1). These three silica tubes were assembled with a 600 to 700 μm separation between the orifices of the drug-injection tubes and the tip of the metal recording electrode and were glued together with epoxy resin (Fig. 1). The two silica tubes for drug injection, −25 cm long, were connected with epoxy resin to the needles of 10- or 25-μl Hamilton microsyringes. These syringes contained the α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA)/kainate blocker 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzo[f]quinoxaline-7-sulfonamide (NBQX, 1–2 mM), the N-methyl-D-aspartate (NMDA) antagonist 3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP, 1–2 mM), or the GABA A antagonist gabazine (1 mM) dissolved in saline. A total volume of 0.1–0.2 μl was injected at a rate of 0.03 μl/min by advancing the plungers with computer controlled stepping motor-driven actuators.

The method for the muscimol blockade of the STN was the same as that described elsewhere (Nambu et al. 2000). In short, a tungsten wire attached to the 30-gauge needle of a 10-μl Hamilton microsyringe was penetrated vertically in to the STN using a hydraulic microdrive (Fig. 1). Using single-unit recordings, the STN was identified by firing patterns and responses to cortical stimulation and to passive joint movements. The response pattern of STN neurons to M1 or SMA stimulation, in particular, provided important information for locating...
the nucleus. The STN responses consisted of an early excitation, an inhibition, and a late excitation as reported previously (Nambu et al. 2000). The two clearly distinguishable excitatory responses were observed in the STN but not in the zona incerta or the lateral hypothalamus. Strong responses to passive joint movements were also unique to the STN but not to the zona incerta, hypothalamus, or the substantia nigra. To block the unitary activity of the STN, the GABA_A receptor agonist, muscimol (0.5 μg/μl, 0.5–1.0 μl), was injected in the nucleus.

Unit recordings and data analysis

The electrode assembly was penetrated obliquely in to the GPe using a hydraulic microdrive (Narishige Scientific Instrument). The unitary activity was amplified, converted into digital data with a window discriminator and sampled using a computer for on-line data analysis. The unitary activity and converted digital data were also stored on videotapes using a Neurocorder (Neurodata, Delaware Water Gap, PA) for further analysis. The pattern of spontaneous activity and responses to the cortical electrical stimulation (300-μs duration single pulse, strength of 100–800 μA and interval of 1.4–2 s) were recorded in the GPe before and after the injection of drugs. Once an injection was made, the next injection site was separated by 1–1.5 mm from the previous injection site.

The rates and patterns of firing were analyzed by calculating the mean rates and autocorrelagrams (bin width, 0.5 ms) from 50 s of digitized recordings. The regularity of the firing was assessed by the existence of multiple peaks and their height in the autocorrelagrams. Slow sweep traces of digitized unitary activity were also used to assess the firing patterns. Cortical stimulation induced responses of GPe neurons were assessed by constructing peristimulus time histograms (PSTHs, bin width: 1 ms) for 100 stimulation trials. Mean values and SDs of the firing rate during 100 ms preceding the time of stimulation were calculated from PSTHS and were considered to be the values for base discharge. The changes in the firing activity in response to cortical stimulation were judged to be significant if the firing rate during at least two consecutive bins (2 ms) reached the statistical level of $P < 0.05$ (1-tailed t-test). The latency of the response was defined as the time at which the firing rate first exceeded this level.

Before examining the effects of locally applied glutamate and GABA antagonists on cortically evoked responses in each neuron, the stimulating site that produced the largest early response sequence was chosen from the aforementioned stimulating sites. The data obtained by M1 and SMA stimulation were grouped together because the components of responses induced by the stimulation of these two cortices were considered to be the same. As mentioned in DISCUSSION, cortical stimulation induced responses in the GPe consist of multiple overlapping excitatory and inhibitory responses. Because of this complexity, the changes in cortically-stimulation-induced responses by local injections of glutamate and GABA antagonists were assessed by simply measuring the peak response amplitude, an average of the two highest for excitation or of the two lowest for inhibition from the mean prestimulus firing level. We considered these measurements to be a simple yet sensitive, although not precise, method for the assessment of excitation and inhibition changes. The changes in the spontaneous firing rate, the amplitudes, and the durations of the responses to cortical stimulation were evaluated by paired t-test.

Histology

Several of the recording and drug injection sites were marked by passing a cathodal DC (20 μA for 30 s) through the recording electrode. At the end of the final experiment, the monkeys were killed with pentobarbital sodium (50 mg/kg iv) and perfused transectionally with 2 L of phosphate buffered saline, pH = 7.3, followed by 5 L of 4% paraformaldehyde in 0.1 M phosphate buffer. The monkeys were then perfused with 3 L of 0.1 M phosphate buffer containing 10% sucrose and, finally, with 2 L of the phosphate buffer containing 30% sucrose. The brains were cut serially into 60 μm thick frontal sections on a freezing microtome. These sections were mounted onto gelatin-coated glass slides and stained with 1% Neutral Red. The recording and drug injection sites were reconstructed according to the lesions made by current injections and the traces of the electrode tracks (data not shown).

RESULTS

Pallidal local drug injection procedure

The distance between the orifices of the drug injection tubes and the tip of the metal unit-recording electrode was ~600–700 μm. This distance was determined after testing various injection tube and electrode assemblies to find the optimum conditions to observe a consistent gabazine blockade of the cortical-stimulation-induced inhibitory responses in GPe neurons. The concentration of gabazine was 1 mM, which was 100–200 times higher than that used for bath application in rat brain-slice experiments (Matsui and Kita 2003; Seutin et al. 1997). A volume of 0.2 μl was slowly injected at a rate of 0.03 μl/min for each injection. The assemblies with a distance between drug tube orifices and the tip of the recording electrode <500 μm gave quicker and stronger gabazine effects than did assemblies with a longer orifice-electrode tip distance. However, with this type of electrode assembly, unit recordings during the injection of gabazine were frequently lost. With the 600 to 700 μm orifice-electrode distance, a total blockade of the inhibitory response was observed 3–10 min after the initiation of the gabazine injection. Even a partial recovery from the gabazine effect took >40–60 min after injection, and many neurons were lost before that time (data not shown).

Because of the time restrictions associated with the use of awake monkeys, we opted not to attempt to obtain the drug-recovery data. Instead, the neuronal activity obtained before drug injection was used as a control. All the drugs used in this study were dissolved in saline. Local injection of saline did not change the neuronal activity of any pallidal neurons tested ($n = 5$). The radius of the effective area of the gabazine was estimated to be ~1 mm from the orifice of the drug-injection tube because neurons evoking clear inhibitions to cortical stimulation were recorded by advancing the electrode by 300–500 μm.

Firing rate, firing patterns, and responses to cortical stimulation

Previous unit recording studies described two types of GPe neurons in awake monkeys (Anderson and Horak 1985; DeLong 1971; Tremblay et al. 1989). The neurons included in the present report were the high-frequency firing with pause type of GPe neurons, which were the most numerous in monkey GPe (DeLong 1971). The spontaneous firing rate of 35 GPe neurons recorded was 62.6 ± 25.8 (SD Hz). Stimulation of the M1 evoked a sequence of responses in GPe neurons typically consisting of an early excitation, an inhibition, and a late excitation with latencies shown in Table 1. In monkey K8, electrical stimulation of both the M1 and SMA was performed. SMA stimulation evoked a sequence of responses that was very similar to those evoked after M1 stimulation except that the
activity, NBQX (1 mM in saline). Effects of NBQX

As described previously, stimulation of the forearm area of the supplementary motor area evoked stronger responses in the neurons that responded to these stimuli than those published elsewhere (Nambu et al. 2000; Yoshida et al. 1993). In some GPe neurons, a slow inhibition and a slow excitation followed the late excitation (e.g., Fig. 5). The changes in the cortical stimulation induced responses of GPe neurons by NBQX described in the preceding text were very similar to those observed after the muscimol blockade of the STN (compare Fig. 2D with Figs. 7A and 8A).

In six GPe neurons that were pretreated with local NBQX, the effect of gabazine was examined. Gabazine increased the firing rate of all NBQX-treated neurons (Fig. 2, G and M). The mean increase was 116.5 ± 16.7% and was significant. The pauses and silent phases were completely eliminated, and the firing became very regular and oscillatory (Fig. 2, H and I). Gabazine also abolished all responses induced by cortical stimulation (Fig. 2G).

**Effects of CPP**

To assess the role of NMDA receptors on the unitary activity, the NMDA antagonist CPP (1–2 mM in saline ≤0.2 µl) was injected in the vicinity of the recording neurons. CPP greatly decreased (56.7 ± 35.6%, n = 16) the firing rate of all GPe neurons tested (Fig. 2, A, D, and J). The autocorrelograms of these neurons indicated that NBQX increased the regularity of firing slightly as judged from the prominent humps in the autocorrelogram (compare Fig. 2, B with E). The slow traces of digitized spikes indicated that pauses and group discharges were still present after NBQX injection (Fig. 2, C and F). In 4 of 16 neurons, a few seconds of grouped discharges, referred to as an active phase hereafter, and a few seconds of a completely silent phase began to appear alternately in fairly regular intervals although the interval slowly changed over time (Fig. 3 A).

The development of the active and silent phases was similar to that observed after muscimol blockade of the STN (described in the following text) (see also Nambu et al. 2000). In five other neurons, active and silent phases occurred alternately in more irregular intervals as shown by the minor peaks in the autocorrelogram (Fig. 3B). The intervals between peaks were 2–16 s long and changed slowly over time.

Local injection of NBQX greatly attenuated or totally abolished the early and late excitations induced by cortical stimulation (Fig. 2, A and D). The mean attenuation of the late excitation was 84.3 ± 23.2% (n = 16) and was significant (Fig. 2K). In 7 of 16 GPe neurons, the late excitation was totally abolished and the remaining inhibitory response was of a very long duration (365 ± 68.8 ms, P < 0.0001 paired t-test, n = 7, Fig. 2D). In the remaining nine GPe neurons, NBQX greatly reduced but did not completely abolish the late excitation. In these neurons, the duration of the inhibition was significantly increased from 13.8 ± 2.4 to 45.3 ± 25.3 ms (P < 0.01 paired t-test, n = 9). The mean attenuation of the late excitation for all 16 neurons was 84.4 ± 21.8% (Fig. 2L).

NBQX also attenuated or abolished the slow inhibition-excitation sequence that was evoked after the late excitation in some GPe neurons. However, these changes were not evaluated statistically because of a very small sample size (data not shown). The changes in the cortical stimulation induced responses of GPe neurons by NBQX described in the preceding study were very similar to those observed after the muscimol blockade of the STN (compare Fig. 2D with Figs. 7A and 8A).

In six GPe neurons that were pretreated with local NBQX, the effect of gabazine was examined. Gabazine increased the firing rate of all NBQX-treated neurons (Fig. 2, G and M). The mean increase was 116.5 ± 16.7% and was significant. The pauses and silent phases were completely eliminated, and the firing became very regular and oscillatory (Fig. 2, H and I). Gabazine also abolished all responses induced by cortical stimulation (Fig. 2G).

**Effects of gabazine**

Local application of gabazine (1 mM in saline ≤0.2 µl) increased the firing rate of 10 of the 11 GPe neurons tested (Fig. 5, A, D, and G). One GPe neuron had a slightly decreased firing rate after the gabazine application. The average increase in the firing rate was 115.8 ± 81.5% (P < 0.0005, paired t-test, n = 11). In six GPe neurons, the pauses became more apparent after the gabazine application (e.g., Fig. 5F) although these pauses disappeared in other four neurons.

Local application of gabazine totally abolished the inhibition induced by cortical stimulation in all GPe neurons tested (Fig. 5, D and I). Gabazine had no effects on the early excitation (Fig. 5, D and H, P > 0.5). However, gabazine greatly attenuated or abolished the late excitation (Fig. 5, D and J). The mean attenuation of the late excitation was 77.0 ± 29.2% (P < 0.001, n = 9). Gabazine had no effect on the slow inhibition-excitation sequence occurring after the late excitation in some GPe neurons (e.g., Fig. 5, A and D).

**Effects of NBQX, CPP, and gabazine on GPe neurons after muscimol blockade of the STN**

We have shown in a previous study that injection of muscimol in the STN completely blocked the unitary activity of the STN (Nambu et al. 2000). The present study also employed muscimol blockade of the STN to eliminate the glutamatergic STN-pallidal input. The STN blockade was considered suc-
FIG. 2. Effects of local 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzo[f]quinoxaline-7-sulfonamide (NBQX) and gabazine on GPe neurons. A–I: peristimulus time histograms (PSTHs) showing responses to M1 stimulation, autocorrelograms, and digitized spike traces with a slow-sweep speed of a GPe neuron recorded in control (A–C), 10 min after injection of NBQX (D–F), and 10 min after an additional injection of gabazine (G–I). NBQX (1 mM, 0.2 μl) decreased the spontaneous firing rate, abolished the early and late excitations, and prolonged the inhibition. An additional application of gabazine (1 mM, 0.2 μl) blocked the inhibition, greatly increased the firing rate, and regularized the firing intervals. All PSTHs in this and subsequent figures were constructed from 100 stimulation trials with a bin width of 1 ms. Cortical stimulation was given at time = 0. Autocorrelograms were constructed from 50 s of digitized recordings with a bin width of 0.5 ms. J–L: summaries of NBQX effects on the spontaneous firing (J), the peak amplitude of the early excitation (K), and the peak amplitude of the late excitation (L) of 16 GPe neurons examined. M: gabazine effects on the spontaneous firing of 6 GPe neurons pretreated by NBQX. Paired t-tests indicate the significance in the changes.

J Neurophysiol • VOL 92 • NOVEMBER 2004 • www.jn.org
cessful when the cortical stimulation no longer evoked excitatory responses (e.g., Fig. 7) (also see Nambu et al. 2000). The STN blockade resulted in the following time-dependent changes in the firing pattern of GPe neurons. The STN blockade greatly decreased the firing rate, to complete silence in some neurons. However, 5–10 min after the muscimol injection, the activity began to increase with repeated occurrences of short grouped spike discharges. As time progressed, the activity further increased and developed into repeated occurrences of 2–12 s of a very high-frequency active phase and then 2–12 s of a completely silent period, as has been reported previously (Nambu et al. 2000) (Figs. 6B and 7D). In most of neurons, the intervals between active phases were fairly regular, as shown in the autocorrelograms with clearly identifiable multiple peaks, although the duration of each active and silent phase varied in some degree (Figs. 6A and 7B). The intervals between the peaks in the autocorrelograms differed from neuron to neuron with the shortest and the longest intervals being 1.8 and 22 s, respectively (n = 22). Also, the intervals slowly changed over time for each neuron. In the GPe neurons with alternately occurring active and silent phase activity, cortical stimulation induced different responses depending on the phase of the neurons at the time of stimulation (Fig. 6, C and D). Stimulation applied during the silent phase triggered the active phase 400–800 ms after the stimulation (Fig. 6E). Stimulation applied during the active phase induced different responses depending on the intensity and timing of the stimulation. Low-intensity stimulation and stimulation applied during an early part of the active phase often induced a short inhibition (Fig. 6F). Conversely, strong stimulation and stimulation ap-
plied during a late part of the active phase often terminated the active phase (Fig. 6G).

After the muscimol blockade of the STN, six neurons were tested with local application of CPP. CPP caused insignificant changes in the mean firing rate in these neurons with the firing rate decreasing in three neurons, increasing in one, and remaining unchanged in two (Fig. 7M). Seven GPe neurons were tested with NBQX, three of which were tested with CPP prior to NBQX. NBQX decreased the firing rate of all GPe neurons (Fig. 7, I and N). The mean decrease in the firing rate was 51.2 ± 42% and was significant (P < 0.02, paired-t-test). The decrease in the firing rate was accompanied by a decrease in the duration and frequency of the active phases, an increase in the irregularity of the firing during the active phases, and an increase in the irregularity of the intervals of the alternately occurring active and silent phases as judged by the decrease in the height of the prominent peaks in the autocorrelograms (Fig. 7, F–H and J–L). The decrease in the firing rate also appeared to be accompanied by an increase in the duration of the inhibition to cortical stimulation, although precise measurement of the duration was often difficult because of a low spontaneous firing rate (e.g., Fig. 7, E and I).

After the muscimol blockade of the STN, local application of gabazine greatly increased the firing rate of all five GPe neurons tested (Fig. 8G). The mean increase was 198 ± 138% and was significant (P < 0.005, paired-t-test, n = 5). The increase in the firing rate was associated with a great increase in the regularity of the spike intervals (Fig. 8F) as noted by several humps in the autocorrelogram (Fig. 8E). Gabazine also totally abolished the cortical stimulation induced inhibition and the silent periods of the GPe neurons (Fig. 8, A, C, D, and F).

**DISCUSSION**

**Limitations of the local drug application method**

Local application of glutamate and GABA_A antagonists should have affected not only the neuron being recorded but also other nearby neurons. Because all GPe projection neurons have local axon collaterals, it is possible that the synaptic actions of these nearby neurons on the recording neuron obscure the direct effects of the antagonists on the neuron. However, we considered this not to be a significant problem. If gabazine, a GABA_A antagonist, increased the activity of the nearby neurons by removing GABAAergic inhibition to these neurons, the activity of the local axon collaterals innervating the recording neuron might increase. This would not affect the recording neuron, however, because the GABAAergic inputs had already been blocked. On the other hand, if the glutamate antagonists, CPP and NBQX, decrease the activity of the surrounding neurons, it would disinhibit the recording neuron.
and act to increase its activity. However, injection of CPP and NBQX decreased the activity of most of the neurons examined, indicating that the direct effect of the antagonists on the recording neurons was stronger than the effects through the axon collaterals.

The electrode assembly had 600 to 700 \( \mu \)m separations between the orifices of the drug ejection tubes and the tip of the metal recording electrode. This distance was chosen after a preliminary experiment to obtain a consistent and reliable gabazine effect on the inhibitory responses induced by cortical stimulation in GPe neurons. The electrode assembly provided stable recording and also increased the chance of covering the entire somatodendritic space of recorded neurons by the injected drugs. However, the dendrites located far from the drug tube orifice might not be within the effective reach of the drugs. For this reason, we infer that locally injected drugs might have failed to produce maximum effects in some of the neurons. Another limitation of the present method was that the number of neurons recorded was small because of the necessity of the distance and time separations between the drug injections.

Another limitation of the local antagonist application method is that assessment of the actual concentrations of the applied antagonists at receptor sites of the recorded neurons is not possible. However, we assumed that the concentration of the antagonists at the active sites was not excessively high.
based on the observation of the efficacy of blocking the cortically evoked responses and also by the application method of using a small volume with a very slow injection speed. Nevertheless, we tested the possible effects of NBQX and gabazine that are unrelated to the antagonistic action on rat GPe neurons in slice preparations (Kaneda and Kita, unpublished observations). Bath application of 10 and 100 μM NBQX abolished internal capsule stimulation-induced excitatory responses without altering spontaneous firing of all four neurons tested. Application of 1 mM NBQX depolarized these four neurons ~10 mV and increased the spontaneous firing. Bath application of gabazine ≤1 mM abolished inhibitory responses to striatum stimulation without altering the excitatory responses, spontaneous firing frequencies or firing pattern of all four neurons tested. The development of long active and silent phases was not observed in any of neurons recorded. These observations suggested that the primary effects of the NBQX and gabazine in the present study were the blockade of AMPA/kainate and GABA<sub>A</sub> receptor-mediated responses, respectively.

**Spontaneous activity**

Based on the results of the present study, we assume that the major forces driving the spontaneous activity of GPe neurons include the glutamatergic synaptic inputs, the GABAergic synaptic inputs, and other drives. In the very simplified schematic model provided in Fig. 9B, these three forces were given...
equal strength, as the relative strength of each was not determined in the present study. A sum of these forces determines the level of spontaneous activity.

GLUTAMATERGIC INPUTS. Previous studies using rat brain slice preparations indicated that rat GPe neurons have AMPA/kainate and NMDA receptors that can be activated by the stimulation of STN-pallidal glutamatergic inputs (Hanson and Jaeger 2002; Ogura and Kita 2000). The present study revealed that local application of NBQX greatly reduced the level of spontaneous activity of GPe neurons. CPP also reduced the activity, although the effect was not as strong as with NBQX. These results suggest that the high rate of spontaneous activity was not determined in the present study. A sum of these forces determines the level of spontaneous activity.

FIG. 7. Effects of CPP and NBQX on GPe neurons observed after muscimol (0.5 μg/μL, 1.0 μL) injection in the STN. A–L: PSTHs show M1-stimulation-induced responses, autocorrelograms with long and short time scales, and digitized spike traces with a slow-sweep speed of a GPe neuron obtained in the control (A–D), 10 min after injection of CPP (2 mM, 0.2 μL; E–H), and 10 min after an additional injection of NBQX (1 mM, 0.2 μL; I–L). Note the long-duration inhibition without any excitation to M1 stimulation (A) and a slow oscillation with ~0.3 Hz (B). CPP increased the duration of the M1-stimulation-induced inhibition (E) and the duration of the silent period in the slow oscillation (H). NBQX further increased the duration of the M1-stimulation-induced inhibition (I) and of the silent period in the slow oscillation (L). CPP and NBQX did not abolish the slow oscillation. M and N: summaries of CPP and NBQX effects on the spontaneous firing of GPe neurons after the STN blockade.
of GPe neurons in awake monkeys is in part due to glutamatergic inputs activating both AMPA/kainate and NMDA receptors. The major origin of the glutamatergic innervations to the GPe is the STN. STN neurons fire at 20–40 Hz in awake monkeys and can provide continuous inputs to the GPe (Matsumura et al. 1992; Nambu et al. 2000). Indeed, unit recording studies in awake monkeys, including ours, showed that the blockade of the STN by local muscimol injection greatly decreased the firing rate of GPe neurons (Hamada and DeLong 1992; Hamada and Hasegawa 1994; Nambu et al. 2000). However, some GPe neurons started to fire grouped discharges a few minutes after the muscimol blockade and slowly developed into a few seconds of an active phase alternating with a few seconds of a silent phase, as mentioned earlier.

The results of the present study further indicated that local application of NBQX decreased the firing rate of most of the GPe neurons even after the muscimol blockade of the STN. This observation suggested that GPe neurons received sustained glutamatergic inputs not only from the STN but also from other sources. Other possible minor origins include the centromedian-parafascicular nuclear complex of the thalamus, the cerebral cortex, and the pedunculopontine tegmentum (De-schenes et al. 1996; Kincaid et al. 1991; Mouroux et al. 1997; Naito and Kita 1994; Sadikot et al. 1992; Yasukawa et al. 2004). Another possibility was an incomplete blockade of the STN by muscimol injection. Muscimol very effectively silenced the unitary activity of the STN for several hours and blocked the cortical stimulation-induced disynaptic excitations mediated through the STN (Nambu et al. 2000; present data). Based on these observations, we speculate that a wide area of the STN was blocked by the muscimol injection, including even the nuclei surrounding the STN.

GABAERGIC INPUTS. The results of local gabazine application experiments indicated that GABAergic inputs are continuously suppressing the firing of GPe neurons. The major sources of GABAergic innervations to the GPe are the Str and the GPe itself. An in vitro study suggested that unitary Str-GPe inhibitory postsynaptic potentials (IPSPs) recorded at the somata of GPe neurons are very small (Ogura and Kita 2000). In addition, the activity of Str projection neurons is very low in awake animals. However, a large number of Str neurons project to the GPe, and their axons form ~80% of the total synaptic boutons on the dendrites of GPe neurons. It is possible that these inputs to the dendrites effectively shunt the driving forces generated...
FIG. 9. Simplified representation of the driving forces of the level of spontaneous activity and responses to the cortical stimulation in the GPe. A: the diagram shows the major connections involved in controlling the activity of the GPe. B: a diagrammatic representation of the time sequence and the major driving forces that evoke a sequence of early excitation, inhibition, and late excitation in GPe neurons. The thickness of base bars represents the level of spontaneous firing that is set by the sum of the strengths of the glutamatergic excitatory component (red column), the GABAergic inhibitory component (blue column), and other excitatory components (orange column). The pink cones and blue patches represent excitation and inhibition, respectively. C: a typical response to cortical stimulation in the GPe. D: a simplified numerical model of the level of spontaneous activity and responses to M1 stimulation. Two excitatory and 3 inhibitory driving forces form the responses. Each of these forces was mimicked by an amplitude factor and a simple dual exponential function, $DE = e^{-it/\tau_a} - e^{-it/\tau_b}$, in which $\tau$ represents the time constants and all the driving forces are expressed with a same time constant, $t = 0$ at the beginning of each driving force and $DE = 0$ at $t < 0$. The rise and the fall times were chosen to mimic the experimental data (C). We assumed that the early and late excitations of the STN generated the early and late excitatory drives to the GPe. The early excitatory drive was mimicked by $EE = a \cdot DE$. The strength of the late excitatory drive $EL$ should reflect following 2 factors. One is the amplitude of the early excitation ($E1$) that should hyperpolarize and set up a condition able to evoke a rebound excitation in STN neurons. The other is the inhibition ($I$) that should disinhibit STN neurons (Nambu et al. 2002). Although the relative contributions of these 2 factors are unknown, we gave a higher contribution for the disinhibition than for the rebound, based on the observations that Str stimulation can evoke the late excitation without evoking the early excitation (Tremblay and Filion 1989; Yoshiida et al. 1993). The late excitation was mimicked by $EL = b \cdot (f + 0.1 \cdot E1) \cdot DE$. A disinhibition is mimicked by $DI = c \cdot I \cdot DE$. We assumed 2 sources of the inhibitory drives, the Str and GPe local collateral inputs. The strength of local collateral inhibition was dependent on the amplitude of the early and late excitation. The inhibition due to the early excitation was mimicked by $IE = d \cdot E1 \cdot DE$. The strength of the Str-GPe inhibitory drive was set constant and was mimicked by $IS = e \cdot DE$. The strengths of the recurrent inhibition to early excitation and the Str-GPe inhibition in control conditions were set equal as no data for setting these values was available. The inhibition due to the late GPe excitation was mimicked by $IL = f \cdot E2 \cdot DE$. The sum of these forces is shown as black curves with shaded areas. Finally, in control conditions, the strength of each driving force was set to mimic a typical cortical stimulation induced response by adjusting arbitrary constants $a$, $b$, $c$, $d$, $e$, and $f$. A computer program Excel (Microsoft) was used for calculations. E and F mimic effects of an 80% blockade of GABA$_A$ or glutamatergic inputs on the level of spontaneous activity and the responses to M1 stimulation.
at the dendrites. In contrast, GPe local axon collaterals generate large unitary inputs to the postsynaptic neurons (Ogura and Kita 2000). In addition, GPe neurons maintain much higher rates of spontaneous activity than Str neurons in awake animals (Anderson and Horak 1985; DeLong 1971; Matsumura et al. 1995; Nambu et al. 2000; Tremblay et al. 1989; Yoshida et al. 1993; present data). However, the population of local collateral boutons in the GPe is relatively small. Thus the observed gabazine effects on the spontaneous activity of GPe neurons might be due to the blockade of the inhibitions by both Str-GPe axons and GPe local axon collaterals. If this assumption is correct, the degree of the tonic inhibition should correlate positively with the level of the spontaneous activity. In the model, the GABAergic inhibitory force is represented by the height of a blue column, which consists of 50% of each of the Str-GPe and GPe local collateral inhibition. Figure 9E mimics a reduction of the GABAergic driving force by gabazine. The application of a local glutamate antagonist also reduces the inhibitory force because the decrease in the spontaneous activity should decrease the local GABAergic synaptic inputs as mentioned in the preceding text. This makes the effects of glutamate blockers on spontaneous activity less apparent in the GPe (Fig. 9F).

**OTHER FORCES.** The application of gabazine to the neurons that had been treated with NBQX or the application of gabazine after muscimol blockade of the STN greatly increased the firing rate of GPe neurons. These observations suggested that GPe neurons have intrinsic mechanisms to support high-frequency activity and/or that sustained nonionotropic glutamatergic inputs continuously activate GPe neurons. GPe neurons in rat brain slice preparations have intrinsic mechanisms to support a low level, <20 Hz, of spontaneous firing (Nambu and Llinás 1997; Ogura and Kita 2000; Stanford 2003). Although it is possible that primate GPe neurons have ionic mechanisms that support a higher level of spontaneous firing than rodent GPe neurons, the effects of non glutamatergic inputs acting in the GPe of unanesthetized animals cannot be ruled out. Such possible inputs include the metabotropic glutamatergic and serotoninergic inputs (Hashimoto and Kita 2002; Poisil et al. 2003). The GPe receives a heavy serotoninergic projection from the dorsal raphe nucleus (Hortnagl et al. 1983; Pasik et al. 1984). Application of serotonin depolarizes and increases the firing rate of GPe neurons in rat brain slice preparations (Hashimoto and Kita 2002). In the model, these other forces are presented as an orange column and provide a constant excitatory force to the spontaneous activity (Fig. 9).

**Firing patterns**

The firing pattern of most of GPe neurons in awake monkeys is of high frequency with pause type (Anderson and Horak 1985; DeLong 1971; Tremblay et al. 1989). In the neurons pretreated with NBQX or in neurons recorded after the blockade of the STN, firing became very regular after the application of gabazine. Recordings of rat GPe neurons in slice preparations indicated that both Str stimulation-induced IPSPs and local axon collaterals induced large spontaneous unitary IPSPs cause various lengthening of the interspike intervals, depending on the timing of the occurrence of IPSPs between spikes (Kita 1996; Nambu and Llinás 1997; Stanford 2003). These results are consistent with the idea that both Str and local axon collaterals can evoke IPSPs that are powerful enough to produce irregularities in the firing of GPe neurons. Another obvious reason for the increase in the regularity in high-frequency firing neurons is the strong activation of membrane properties that support the high-frequency firing (Bar-Gad et al. 2001). The application of NBQX also increased the regularity of the firing. Although the amplitude of unitary EPSPs evoked by STN-GPe axons is small when recorded at the soma, they are capable of inducing spikes at distal dendrites (Hanson and Jaeger 2002, Hanson et al. 2004). The NBQX effect may be due to blocking both the firing triggered by excitatory postsynaptic potentials (EPSPs) and the recurrent IPSPs evoked by that firing.

Two other observations may be worthy of further discussion. The first was that in some GPe neurons, the pauses of firing became more apparent after local gabazine injection, although these pauses disappeared in other neurons. A very similar observation was reported in a previous study using the local injection of bicuculline, a GABA_A antagonist, in the awake-monkey GPe (Matsumura et al. 1995). This raises the possibility that intrinsic mechanisms of GPe neurons may generate the pauses within an appropriate membrane potential range. The second interesting observation was that in some GPe neurons, the application of NBQX induced a few seconds of a strong active phase alternating with a few seconds of a complete silent phase that was very similar to those observed after the muscimol blockade of the STN (Hamada and Hasegawa 1994; Nambu et al. 2000). This suggests the possibility that a loss of the STN-GPe synaptic inputs that were mediated by AMPA/kainate receptors was responsible for the development of the alternately occurring long active and silent phases. Co-application of NBQX and gabazine eliminated the silent phases and made the GPe neurons fire very regularly and oscillate at a high rate. Thus it is conceivable that the membrane properties that support high-frequency oscillations become dominant in neurons that are capable of generating active and silent phases at a particular membrane voltage range (Stanford 2003). After muscimol blockade of the STN, gabazine could also abolish the long silent phases in most, but not all, GPe neurons. Therefore it is likely that GABAergic inputs contribute to the occurrence of the silent phase even though the inputs are not absolutely necessary. The precise mechanisms underlying these responses are still unknown at this time.

**Cortical stimulation-induced responses**

The common response pattern of GPe neurons to M1 or SMA stimulation was a sequence of early excitation, inhibition, and late excitation, as reported previously both in rats and monkeys (Kita 1992; Nambu et al. 2000; Ryan and Clark 1991). Several excitatory and inhibitory drives forming this response pattern can be considered based on the major synaptic connections between the cortex and the GPe and based on the cortical stimulation induced responses in the Str and the STN that have been reported in previous studies (Kita 1992, 1994; Kita and Kitai 1991; Nambu and Llinás 1997; Nambu et al. 2002, 2003; Ogura and Kita 2000; Tremblay et al. 1989). The early excitation has been considered to be due to the disynaptic cortico-STN-GPe connection with very fast conducting axons (Fig. 9, A and B) (Kita 1992; Nambu et al. 2000;
Ryan and Clark 1991). The present results indicated that the early excitation was mediated mainly by AMPA/kainate receptors and partially by NMDA receptors.

The present study confirmed that GABA<sub>A</sub> receptors mediated the inhibition following the early excitation. Two synaptic inputs can be considered for the inhibition (Fig. 8D). One is the local axon collaterals of GPe projection neurons. The early excitation in the GPe mentioned in the preceding text should evoke recurrent inhibition in the postsynaptic neurons. Another is the cortico-Str-GPe input. Because the conduction velocity of Str axons is slow (Tremblay et al. 1989; Yoshida et al., 1993), the inhibition should arrive a few milliseconds after the arrival of the early excitation. The relative strength of the GPe and Str inhibitions was not assessed in the present study. However, it was certain that the cortico-Str-GPe inhibition was strong enough to stop the spontaneous firing of GPe neurons as cortical stimulation effectively inhibited GPe activity after elimination of the excitation by local NBQX or muscimol blockade of the STN.

The late excitation was also due to mostly AMPA/kainate and some NMDA receptor activation. The major source of the late excitation might be the STN-GPe inputs for the following two reasons. First, stimulation of the cortex also induced a late excitation in STN neurons with a latency that was a few milliseconds shorter than that of the GPe. Second, muscimol blockade of the STN diminished or abolished the late response in the GPe (Nambu et al. 2000; present study). There are two possible driving forces for the late excitation in the STN. The major force is the activation of the Str-GPe-STN, indirect, pathway that should induce a disinhibition in the STN (Albin et al. 1989; Delong 1990). This possibility was confirmed by recent studies that a local injection of muscimol in the Str or of gabazine in the GPe diminished the late excitation without affecting the early excitation of STN neurons (Nambu et al. 2002, 2003) and also by the present observation that local injection of gabazine diminished or abolished the late excitation of the GPe. The other possible driving force of the late STN excitation is an anodal-brake rebound depolarization that follows the preceding inhibition induced by the cortico-STN-GPe-STN pathway (Fig. 9, A and B) as STN neurons in slice preparations evoke a strong anodal-brake depolarization (Bevan et al. 2002; Nakanishi et al. 1987; Plenz and Kitai 1999). These two driving forces would be dependent on the magnitudes of both the early excitation and the inhibition of the GPe (Fig. 9, B and D). Other excitory force contributing to the late excitation of the GPe might be the disinhibition of the collateral inputs with its strength dependent on the strength of the preceding inhibition.

Figure 9D shows a simple numerical simulation in which the amplitudes of the aforementioned excitatory and inhibitory inputs were adjusted to mimic actual unit recording shown in Fig. 9C (for details of the simulation, see the figure legend). Figure 9, E and F, simulates an 80% reduction of both the GABAergic and glutamatergic inputs by selective antagonists, respectively. An incomplete blockade was assumed because the local injection method might not assure the total blockade of the synaptic inputs. The results of the simulations were very similar to the actual data shown in Figs. 2 and 5.

In some GPe neurons, a slow inhibition and a slow excitation followed the late excitation. Similar slow inhibition and slow excitation were observed in the Str and the STN after stimulation of the cortex and were considered to be due to a disfacilitation of cortical inputs and to a rebound excitation, respectively (Fujimoto and Kita 1993; Wilson 1986). The slow inhibition and excitation in the GPe were diminished by both local injection of NBQX and muscimol blockade of the STN, while local gabazine injection was without effect. These observations suggest that the slow responses in the GPe reflect the slow responses in the STN.

**Long-lasting inhibition and pauses of GPe neurons observed after the blockade of glutamatergic inputs**

As discussed in the preceding text, after the muscimol blockade of the STN or after the local application of glutamate antagonists, some GPe neurons induced alternately occurring active and silent phases. Local application of gabazine eliminated the silent phases in most of the GPe neurons, suggesting that GABAergic inhibitory inputs play a role in inducing these silent phases. After the blockade of the STN, stimulation of the cortex should evoke inhibition through the cortico-Str-GPe disynaptic connections. The Str-GPe inhibition may be augmented after STN blockade because of the removal of the tonic presynaptic suppression of GABA release by group III metabotropic glutamate receptors (Matsui and Kita 2003). Cortical stimulation evoked inhibition that arrived during an active phase could terminate the active phase. It was also observed that stimulation applied during the silent phase could trigger the active phase with a 400- to 800-ms delay. These observations supplement the aforementioned suggestion that the alternately occurring active and silent phases may develop in neurons at a particular membrane potential range and that tonic GABAergic inputs contribute to bring the membrane into that range. The stimulation-induced IPSPs may flip the neurons from an active to a silent phase or vice versa by deactivating or deinactivating inward currents such as the persistent sodium current (Hashimoto and Kita 2003; Stanford 2003). However, the precise mechanisms underlying these responses are still unknown at this time.

**Functional implication**

The present results suggest that the level and pattern of unitary activity in the GPe are controlled by multiple factors including glutamatergic inputs, GABAergic inputs, membrane properties, and other inputs. These results may be useful in interpreting previous pathophysiological observations. Studies have shown that basal ganglia disorders are accompanied by changes in the level and pattern of GPe activity. The changes reported in the GPe of parkinsonian patients and of experimental parkinsonian animals include a modest decrease in the firing rate, an increase in the irregularity and bursting, and an increase in the sensory response such as to the movement of multiple joints (Beric et al. 1996; Filion and Tremblay 1991; Pan and Walters 1988; Sterio et al. 1994). A widely used basal ganglia model of Parkinson’s disease assumes an augmentation of the Str-GPe input (Albin et al. 1989; Delong 1990; Nini et al. 1995). The augmented Str-GPe inputs would increase the firing irregularity in an effect opposite to that of gabazine where the firing pattern was greatly regularized. The model used here also predicts a decrease in the level of the firing when Str-GPe inputs were augmented.
Dyskinesias can be experimentally induced by various treatments including a lesion or a chemical blockade of the STN, application of GABA antagonists in the GPi, or application of glutamate antagonists in the GPe (Crossman et al. 1988; Hamada and DeLong 1992; Hamada and Hasegawa 1994; Nambu et al. 2000; Robertson et al. 1989). Dyskinesias induced by blockade of the STN involve a decrease in the firing rate of GPi neurons, and in turn, decreased the activity of the GPe. Application of a glutamate antagonist in the GPi should also decrease the firing rate of GPi neurons. Therefore one of the common effects of these treatments that induce dyskinesias would be a decrease in the activity of the GPi. The strong active phases alternating with silent phases that developed in GPi neurons after the STN blockade also should contribute to the inhibition of GPi activity and promote the occurrence of dyskinesias, although the precise functional significance of the active and silent phases is unknown at this time. Another common aspect of these treatments is the blockade of the Str-GPe-STN, indirect, pathway. Our present observations are consistent with the idea that the indirect pathway provides significant control over the output of the basal ganglia and that the effectiveness of this pathway is critically dependent on both the early excitation and following inhibition of GPe neurons (Fig. 9).

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