

Globus Pallidus Neurons Dynamically Regulate the Activity Pattern of Subthalamic Nucleus Neurons through the Frequency-Dependent Activation of Postsynaptic GABA_A and GABA_B Receptors

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Reciprocally connected GABAergic neurons of the globus pallidus (GP) and glutamatergic neurons of the subthalamic nucleus (STN) are a putative generator of pathological rhythmic burst firing in Parkinson's disease (PD). Burst firing of STN neurons may be driven by rebound depolarization after barrages of GABA_A receptor (GABA_AR)-mediated IPSPs arising from pallidal fibers. To determine the conditions under which pallidosubthalamic transmission activates these and other postsynaptic GABARs, a parasagittal mouse brain slice preparation was developed in which pallidosubthalamic connections were preserved. Intact connectivity was first confirmed through the injection of a neuronal tracer into the GP. Voltage-clamp and gramicidin-based perforated-patch current-clamp recordings were then used to study the relative influences of GABA_AR- and GABA_BR-mediated pallidosubthalamic transmission on STN neurons. Spontaneous phasic, but not tonic, activation of postsynaptic GABA_ARs reduced the frequency and disrupted the rhythmicity of autonomous firing in STN neurons. However, postsynaptic GABA_BRs were only sufficiently activated to impact STN firing when pallidosubthalamic transmission was elevated or pallidal fibers were synchronously activated by electrical stimulation. In a subset of neurons, rebound burst depolarizations followed high-frequency, synchronous stimulation of pallidosubthalamic fibers. Although GABA_BR-mediated hyperpolarization was itself sufficient to generate rebound bursts, coincident activation of postsynaptic GABA_ARs produced longer and more intense burst firing. These findings elucidate a novel route through which burst activity can be generated in the STN, and suggest that GABARs on STN neurons could act in a synergistic manner to generate abnormal burst activity in PD.

Key words: basal ganglia; network; synchrony; burst; synaptic transmission; Parkinson's disease

Introduction

The frequency and pattern of action potentials generated by neurons within the subthalamic nucleus (STN) are important determinants of both the normal and pathological output of the basal ganglia. In resting animals, STN neurons are spontaneously active, with a firing rate between 10 and 30 Hz and an irregular firing pattern (Wichmann et al., 1994; Urbain et al., 2002). During movement, STN neurons display somatotopic, spatiotemporally related changes in activity. In parkinsonian animals, in which movement is impaired, precise somatotopy is lost, and there is an emergence of correlated, rhythmic burst firing (Bergman et al., 1994; Soares et al., 2004). Such activity, which is phase-related to resting tremor, is also a feature of STN recordings from Parkinson's disease (PD) patients (Hutchison et al., 1998; Levy et al., 2000; Amirnovin et al., 2004).

STN burst firing may be driven, in part, by reciprocally connected GABAergic neurons of the globus pallidus (GP) (Plenz and Kital, 1999; Gillies et al., 2002; Terman et al., 2002). *In vitro*, stimulation of barrages of phasic GABA_A IPSPs can sufficiently hyperpolarize STN neurons so that on termination of synaptic activity there is a rebound depolarization, which may be associated with a burst of high-frequency firing (Bevan et al., 2002a). This rebound burst activity may be generated in STN neurons *in vivo* if GABAergic inputs from the GP are correlated, as in idiopathic and experimental models of PD (Raz et al., 2000; Levy et al., 2002; Soares et al., 2004).

GABA released from pallidosubthalamic terminals may also bind other pathophysiologically relevant postsynaptic targets. In the hippocampus and cerebellum, extrasynaptic GABA_A receptors (GABA_ARs) mediate a tonic current that profoundly influences postsynaptic excitability (Brickley et al., 1996; Semyanov et al., 2004). Although extrasynaptic GABA_ARs have been identified on STN neurons (Galvan et al., 2004), tonic current has not been studied. In addition, STN neurons express relatively high levels of GABA_BRs, primarily at extrasynaptic sites (Galvan et al., 2004). Although GABA_BRs are activated by the direct application of agonists, GABA_BR antagonists have no impact on STN firing dynamics (Shen and Johnson, 2001; Urbain et al., 2002; Wilson et

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al., 2004). As such, it is unclear under what conditions postsynaptic GABA_BRs are activated by pallidal input. At other synapses, the activation of extrasynaptic GABA_BRs requires an elevated concentration of extracellular GABA (Mody et al., 1994; Isaacson, 2000), which arises during synchronous presynaptic activity, for example, during hippocampal or thalamic oscillations (Kim et al., 1997; Scanziani, 2000; Jacobsen et al., 2001). In these regions, activation of postsynaptic GABA_BRs modulates the frequency of the network rhythm. It appears then, that the synchronous firing of GP neurons, characteristic of PD, could lead to the activation of STN GABA_BRs. Given that the impact of extrasynaptic GABA_AR and GABA_BR activation may be as significant as that of synaptic GABA_AR activation, we developed a brain slice preparation, in which STN neurons received spontaneous GABAergic input from the GP, to study the relative contributions of postsynaptic GABA_AR activation on STN activity.

Materials and Methods

Slice preparation. Experimental procedures were performed using brain slices obtained from 62 13- to 21-d-old male C57BL/6 mice. All experiments were conducted in compliance with the rules set forth by the Northwestern University Animal Care and Use Committee and the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*. Animals were deeply anesthetized with ketamine–xylazine and perfused transcardially with 10 ml of ice-cold modified artificial CSF (ACSF), which had been bubbled with 95% O₂ and 5% CO₂ and contained the following (in mM): 230 sucrose, 2.5 KCl, 26 NaHCO₃, 1.25 NaH₂PO₄, 0.5 CaCl₂, 10 MgSO₄, and 10 glucose. After perfusion, the animal was decapitated, and the brain was rapidly removed from the skull. In an attempt to maintain the pallidum projection, 350- μ m-thick parasagittal slices were cut at an angle of 20° from the midline, measured from the caudal pole of the brain. To do this, each hemisphere of the brain was glued, medial face down, to a 20° Perspex ramp, fixed to the stage of a vibratome (Vibratome 3000; Vibratome, St. Louis, MO), and then immersed in ice-cold modified ACSF. In this manner, one or two slices that contained both STN and GP were obtained from each hemisphere. For experiments in which slices were bathed in 4-aminopyridine (4-AP), a small razor cut was made caudal to the GP to sever GP axons from their parent cell bodies, so that there was no confounding influence of this agent on the somatodendritic excitability of GP neurons. Slices were subsequently transferred to a holding chamber, where they were submerged in ACSF, which was bubbled continuously with 95% O₂ and 5% CO₂, maintained at room temperature, and contained the following (in mM): 126 NaCl, 2.5 KCl, 26 NaHCO₃, 1.25 NaH₂PO₄, 2 CaCl₂, 2 MgSO₄, and 10 glucose.

Visualized recording. Single slices were transferred to a submerged recording chamber and continuously perfused (2–3 ml/min) with a slightly modified oxygenated ACSF that contained the following (in mM): 126 NaCl, 3.5 KCl, 26 NaHCO₃, 1.25 NaH₂PO₄, 1 CaCl₂, 1 MgCl₂, and 10 glucose. This solution is believed to more accurately represent the ionic composition of brain interstitial fluid than traditional ACSF (Sanchez-Vives and McCormick, 2000). Except for recordings addressing tonic current, for which the bath solution was kept at room temperature to maintain long-term baseline stability (Brickley et al., 1996; Semyanov et al., 2003; Yeung et al., 2003), all recordings were made with the bath solution heated to between 35 and 37°C. A 40 \times water immersion objective was used to identify individual cells within the STN using infragradient contrast video microscopy (Infrapatch Workstation; Luigs and Neumann, Ratingen, Germany). Somatic recordings were made using patch pipettes pulled from nonfilamented borosilicate glass (Warner Instrument, Hamden, CT) on a P-97 Flaming/Brown micropipette puller (Sutter Instrument, Novato, CA). For voltage-clamp recordings, pipettes were filled with a solution containing the following (in mM): 135 CsCl, 3.6 NaCl, 1 MgCl₂, 10 HEPES, 0.1 Na₄EGTA, 0.4 Na₃GTP, and 2 Mg_{1.5}ATP. N-2(2,6-Dimethylphenylcarbamoylmethyl)triethylammonium bromide (QX314) (2.5 mM) was also included in the majority of pipettes used for voltage-clamp recordings to block postsynaptic

sodium, potassium, and hyperpolarization-activated cation currents. For current-clamp recordings, pipettes were filled with a solution containing the following (in mM): 110 K-MeSO₄, 25 KCl, 3.6 NaCl, 1 MgCl₂, 10 HEPES, 0.1 Na₄EGTA, 0.4 Na₃GTP, and 2 Mg_{1.5}ATP. All internal solutions were made up to a pH of 7.3 and an osmolarity of \sim 290 Osm. For current-clamp recordings, moments before seal formation was attempted, gramicidin was added to the pipette solution at a concentration of \sim 20 μ g/ml. Cell-attached patch recordings were made with pipettes filled with the same solution as that used in the bath. The resistance of filled pipettes was between 2 and 6 M Ω . Membrane activity was directly recorded after seal formation in the voltage-clamp mode at a holding potential of 0 mV for cell-attached experiments, after adequate perforation (with a series resistance between 30 and 70 M Ω) in current-clamp mode, and after seal rupture (whole-cell configuration) in the voltage-clamp mode using an EPC 9/2.C amplifier (HEKA Elektronik, Lambrecht, Germany), which was operated using Pulse 8.5 software (HEKA Elektronik). Signals were low-pass filtered at a frequency (1.7–17 kHz) that was one-third of the frequency of digitization (5–50 kHz). All current-clamp data and the majority of voltage-clamp data were sampled at 50 kHz. Very long periods (>5 min) of voltage-clamp data were sampled at lower frequencies to maintain manageable file sizes. Deliberate or accidental establishment of the whole-cell configuration from the perforated configuration was recognized as a sudden drop in series resistance, an increase in the amplitude of recorded action potentials, a depolarizing shift in the reversal potential of GABA_A IPSPs, and an average offset in membrane potential of 5.2 mV. The value of the offset was similar to the empirically calculated (Barry, 1994) junction potential between the electrode solution and the external media of 8.1 mV. Thus, the voltage reported for perforated patch current-clamp data in this study is therefore likely to be <3 mV more depolarized than the true voltage. The empirically calculated junction potential between the bath solution and the intracellular solution used for voltage-clamp experiments was found to be 5 mV; this potential has been applied to all relevant command potentials. Whereas fast capacitive transients of the pipette were nulled on-line, there was no compensation of series resistance or whole-cell capacitance. For voltage-clamp recordings, series resistance was measured from the membrane response to a 50-ms-long, 10 mV hyperpolarizing step from the holding potential. Voltage-clamp recordings with series resistances that fluctuated $>20\%$ within an experiment were not included in the analyses. For current-clamp data, the deviation in voltage response attributable to series resistance was subtracted off-line as described previously (Bevan et al., 2000).

In a subset of current-clamp experiments, an attempt was made to evoke IPSPs in individually recorded STN neurons through bipolar electrical stimulation of the GP or internal capsule. The poles of stimulation were selected from a custom-built matrix of four rows of five electrodes (FHC, Bowdoinham, ME). Electrodes within a row were 240 μ m apart, and rows were offset by 600 μ m. The two electrodes selected for stimulation were those that produced the largest IPSP in the absence of antidromic activation. Typically, stimulus intensity was 200–400 μ A. A single pulse of stimulation was 100 μ s long.

Reagents. Drugs were prepared as concentrated stock solutions and diluted in ACSF, before being, in most cases, bath applied. For the analysis of tonic current, GABA_AR antagonists were fast-applied directly to the STN using a gravity-driven sewer pipe perfusion system (Cell Micro-Controls, Norfolk, VA). Ionotropic glutamatergic transmission was blocked by the continuous application of 50 μ M D-(–)-2-amino-5-phosphonopentanoic acid (APV) and 20 μ M 6,7-dinitroquinoxaline-2,3-dione (DNQX). These agents, plus SR 95531 [2-(3-carboxypropyl)-3-amino-6-(4-methoxyphenyl)pyridazinum] hydrobromide [gabazine (GBZ)], (2S)-3-[[[(1S)-1-(3,4-dichlorophenyl)ethyl]amino-2-hydroxypropyl](phenylmethyl)phosphinic acid (CGP), QX314, and picrotoxin were obtained from Tocris (Ellisville, MO). Gramicidin, biocytin, and 4-AP were obtained from Sigma (St. Louis, MO).

Data analysis. The frequency of occurrence of synaptic events, as well as their peak amplitude, 10–90% rise time, area, and weighted decay were obtained using pClamp (Molecular Devices, Union City, CA). The total net inward charge was calculated by integrating all periods in which current was >2 SDs below the mean baseline holding current. Thus,

mean charge (pC/s) represents the total charge, calculated in this manner, divided by the length of the period analyzed. The mean baseline holding current was calculated using 20–50 100-ms-long segments during which no IPSCs were evident.

The time of the positive peak of single action potentials/currents recorded in current-clamp/cell-attached mode was obtained using Origin 5.0 (Microcal Software, Northampton, MA) and used to calculate the mean interspike interval (ISI). The precision of single-spike activity was quantified using the coefficient of variation (CV), which was obtained for a period of data (typically the first 101 events within a recorded sequence of activity) by dividing the SD of ISIs by the mean ISI.

Numerical data are presented as mean \pm SD, and the distribution of data is represented graphically in box plots. In these, the central line represents the median, the inner edges represent the interquartile range, and the outer edges represent the 1–99% range. The mean of the data is also represented with a filled square. Although data sets did not appear to deviate from a normal distribution, sample sizes were small. Therefore, nonparametric statistics were used because they are subject to fewer assumptions concerning the distribution of data. Paired and unpaired experimental data sets were compared using the Wilcoxon signed rank (WSR) test and the Mann–Whitney U (MWU) test, respectively, and values of $p < 0.05$ were considered significant. The Kolmogorov–Smirnov (K–S) test was used to compare the frequency distributions of the firing rate and CV of GP neurons with normal distributions of random numbers with similar means and SDs, generated using Statview (SAS Institute, Cary, NC). Statistical analyses were also performed using JMP (SAS Institute).

Histology. Injections of biocytin (3%, dissolved in ACSF) were made at two to three sites (10–150 μ l/site) within the GP of parasagittal slices held in an interface chamber (Fine Science Tools, Foster City, CA), using a 1.0 μ l (26 gauge) syringe that was connected to a glass micropipette to minimize disruption at the injection site. Injections were made over 5–20 min at each site. The syringe was retracted \sim 5 min after each injection. Slices were then held in the chamber for 5–7 h before overnight fixation at 4°C in 4% paraformaldehyde diluted in 0.1 M phosphate buffer, pH 7.4 (PB). Slices were then washed in PBS before incubation, for 4–6 h at room temperature, in avidin–biotin–peroxidase complex (Vectastain Elite ABC; Vector Laboratories, Burlingame, CA) diluted (1:50) in PBS containing 1% bovine serum albumin and 0.3% Triton X-100. Avidin–biotin–peroxidase bound to biocytin tracer was then visualized using diaminobenzidine tetrahydrochloride (DAB peroxidase kit; Vector Laboratories). After the reaction, slices were washed in 0.1 M PB and treated with OsO_4 (0.2% in 0.1 M PB) for 20–30 min. Slices were then dehydrated in a graded series of alcohol and propylene oxide before being embedded in Durcupan resin (Fluka, Seelze, Germany) on glass slides. The resin was then cured for 24 h at 60°C. Analysis and digital micrography of biocytin-labeled neuronal elements were performed using a Zeiss (Thornwood, NY) Axioskop microscope equipped with a digital CCD camera (AxioCam; Zeiss) that was operated using Axiovision software (Zeiss).

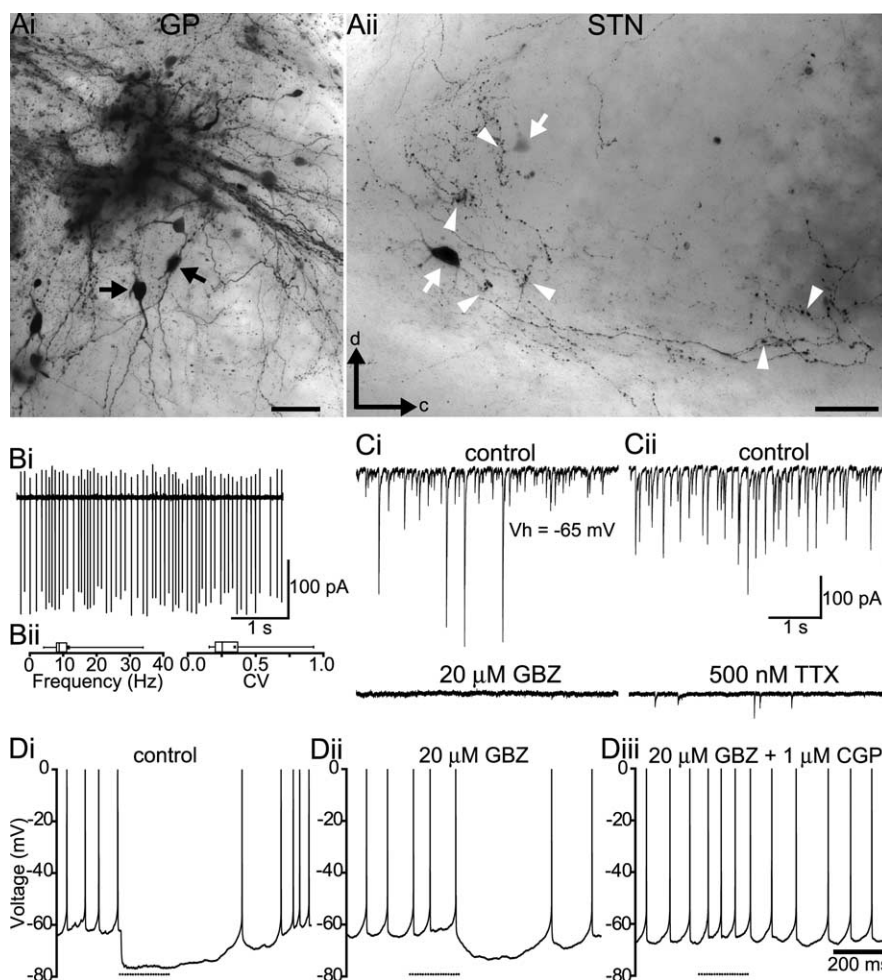


Figure 1. Evidence for intact pallidosubthalamic projections in parasagittal slices. **Ai, Aii**, Photomicrographs of a site of limited biocytin injection in the GP (**Ai**) and corresponding labeling in the STN of the same slice (**Aii**). **Ai**, Extracellular deposits of tracer were taken up by neurons within the GP (black arrows). **Aii**, Labeled fibers were seen in the STN. Some axons possessed multiple varicosities that appeared to contact the proximal regions of unlabeled STN neurons (white arrowheads). Retrogradely labeled STN neurons were also observed (white arrows). d, Dorsal; c, caudal. Scale bars, 50 μ m. **Bi, Bii**, GP cells are spontaneously active in the parasagittal preparation. **Bi**, Representative cell-attached recording from a GP neuron within a slice bathed in APV and DNQX. This neuron, held at 0 mV, had an average firing rate of 11.0 Hz and a CV of 0.33, both near the respective means of the population sample (**Bii**). **Ci, Cii**, GABA_A-mediated, TTX-sensitive currents recorded from STN neurons. **Ci**, Spontaneously occurring transient inward currents are seen in a voltage-clamp recording of an STN neuron held at -65 mV and bathed in APV and DNQX. These currents were sensitive to bath application of GABA_A antagonists. Vh, Holding potential. **Cii**, Spontaneously occurring transient inward currents were also reduced in frequency and mean charge by bath application of TTX. **Di–Diii**, Stimulation-evoked GABA_A- and GABA_B-mediated IPSPs in single STN neurons. **Di**, Representative current-clamp recording demonstrating that a 20 pulse train of 100 Hz bipolar electrical stimulation delivered to the GP evoked a powerful, sustained hyperpolarization in STN neurons within parasagittal slices. In this and subsequent figures, action potentials have been truncated at 0 mV and stimulus artifacts have been removed for clarity, and the time of individual stimuli are demarcated by filled circles. **Dii**, Although a component of the hyperpolarizing response was sensitive to the application of 20 μ M GBZ, a major portion of this response remained under these conditions. **Diii**, The second component of the response was removed by the application of 1 μ M CGP.

Results

Anatomical and functional connectivity is maintained in the parasagittal preparation

To determine whether pallidosubthalamic projections were maintained in our preparation, we performed extracellular injections of biocytin in the GP of eight parasagittal slices. Analysis of each slice confirmed that biocytin was injected solely ($n = 4$) or predominantly ($n = 4$) into the GP (Fig. 1Ai). Both anterogradely labeled axons/terminals and retrogradely labeled neurons were observed in the STN in six of eight slices (Fig. 1Aii). The appearance of many of the labeled axons and their termination pattern were similar to those described previously in studies of

labeled GP neurons: individual axons possessed several large varicosities, which apposed the soma and proximal regions of STN neurons (Bevan et al., 1997, 1998).

To ascertain the level of activity of GP neurons, cell-attached patch recordings were obtained from parasagittal slices bathed in 50 μM APV and 20 μM DNQX. Membrane seals always led to electrode-tip resistances that were $>300\text{ M}\Omega$, and data were accepted only if the signal-to-noise ratio was >4 . Although reasonable membrane-patch seals were obtained in 16 cases, four putative neurons remained silent throughout the recording period. As can be seen in Figure 1, *Bi* and *Bii*, the remaining 12 neurons were spontaneously active, firing in a single-spike manner with an average firing rate of $11.4 \pm 7.6\text{ Hz}$, which is within the range previously reported for spontaneously active GP neurons in mouse (Chan et al., 2004), rat (Cooper and Stanford, 2000), and guinea pig slice preparations (Nambu and Llinas, 1994), but well below the 25–100 Hz typically recorded from the GP in alert, resting rodents and primates (DeLong, 1971; Gardiner and Kitai, 1992; Raz et al., 2000; Urbain et al., 2000; Kita et al., 2004; Soares et al., 2004). Single-spike firing was irregular: the average CV across the 12 neurons was 0.35 ± 0.22 (Fig. 1 *Bii*). Neither the frequency distribution of mean firing rate ($n = 12$; K–S test; $p = 0.9447$) or mean CV ($n = 12$; K–S test; $p = 0.5272$) were significantly different from a normal distribution, suggesting that the data were derived from a single population of neurons with respect to their spontaneous firing activity.

To assess whether these spontaneously active GP neurons provided a GABA_A-mediated input to the STN, whole-cell recordings were performed using cesium chloride-containing pipettes, and STN neurons were voltage-clamped at a holding potential of -65 mV . As seen in Figure 1, *Ci* and *Cii*, spontaneous, irregular, transient inward currents were recorded from STN neurons under control conditions (slices bathed in 50 μM APV and 20 μM DNQX). The average spontaneous event recorded from 19 neurons under these conditions had an amplitude of $-64.0 \pm 25.7\text{ pA}$, a 10–90% rise time of $0.84 \pm 0.26\text{ ms}$, and a weighted decay time constant of $8.4 \pm 3.2\text{ ms}$. The kinetics of the events recorded in the parasagittal slice are comparable with those measured for spontaneous events recorded from STN neurons within rat coronal slices (Baufreton et al., 2001) (reported decay, $\sim 10\text{ ms}$). The spontaneous events recorded from all 19 STN cells in the parasagittal slice occurred with a mean frequency of $15.5 \pm 14.7\text{ Hz}$ and carried a mean charge of $6.9 \pm 7.4\text{ pC/s}$. The GABA_A antagonists GBZ (20 μM ; $n = 3$) or picrotoxin (100 μM ; $n = 7$) were bath applied after the acquisition of sufficient control data for 10 of these neurons, in which events occurred with a mean frequency of $11.0 \pm 7.8\text{ Hz}$ and carried a mean charge of $4.0 \pm 2.4\text{ pC/s}$ (Fig. 1 *Ci*). In all 10 recordings, application of the antagonists significantly reduced both the frequency and size of these events (WSR tests; GABA_A-blocked frequency, $0.7 \pm 0.8\text{ Hz}$; $n = 10$; $p = 0.002$; GABA_A-blocked mean charge, $0.2 \pm 0.1\text{ pC/s}$; $n = 10$; $p = 0.002$). The nine other neurons were challenged with 500 nM TTX (Fig. 1 *Cii*). The mean frequency of events recorded under these conditions was one-half of that recorded under control conditions (WSR test; control, $20.5 \pm 19.1\text{ Hz}$; TTX, $10.9 \pm 9.2\text{ Hz}$; $n = 9$; $p = 0.004$). Bath-applied TTX also significantly reduced the mean charge of spontaneously occurring events (WSR test; control, $10.0 \pm 9.8\text{ pC/s}$; TTX, $3.4 \pm 3.2\text{ pC/s}$; $n = 9$; $p = 0.02$). Thus, phasic currents recorded from STN neurons in the parasagittal preparation are reduced by GABA_A antagonists and voltage-dependent sodium (Na_v) channel blockade. Because the cell-attached recordings from GP neurons and voltage-clamp recordings from STN neurons determined the firing rate of GP

neurons and the frequency of spontaneous postsynaptic events, respectively, it is tempting to make inferences concerning the pattern of innervation of STN neurons by GP neurons. However, this was not attempted given that the probability of release of GABA from GP terminals is unknown and the number of GP–STN axons retained within the slice underrepresents the number in the intact brain.

To provide additional evidence that the transient inward currents observed in our voltage-clamp recordings represent GABAergic transmission at pallidosubthalamic synapses, bipolar electrical stimulation was applied to the GP in parasagittal slices bathed in 50 μM APV and 20 μM DNQX. IPSPs evoked in the STN were recorded using the gramicidin-based perforated-patch current-clamp technique. In 10 of 10 neurons in which recordings were obtained, single pulses of bipolar stimulation applied to the GP elicited single IPSPs (data not shown). These IPSPs ranged in size from 5 to 15 mV, and had similar kinetics to those observed by Bevan et al. (2002a). By evoking IPSPs at various levels of polarization in six of these cells, it was determined that the equilibrium potential of GABA_A IPSPs was $-78.9 \pm 2.5\text{ mV}$. In all 10 neurons examined, a single pulse of bipolar stimulation could no longer elicit any postsynaptic response after the bath application of 20 μM GBZ, indicating these IPSPs were GABA_AR-mediated. Paired with the anatomical evidence, the data obtained from these recordings suggest that the most likely source of spontaneous GABAergic input to the STN in parasagittal slices is the GP.

Trains of bipolar stimulation were also applied to the GP while the membrane potential of single STN neurons was monitored with current-clamp recordings. Barrages of summing IPSPs coincident with the stimulating train were evident in recordings from the same group of 10 neurons described above, in response to sequences of 20 pulses delivered to the GP at 100 Hz (Fig. 1 *Di*). These IPSPs were blocked with 20 μM GBZ in 10 of 10 neurons. In two other neurons, a profound and prolonged hyperpolarization persisted after this treatment (Fig. 1 *Dii*). This hyperpolarization was subsequently blocked by the application of 1 μM CGP (Fig. 1 *Diii*). Thus, both GABA_AR- and GABA_BR-mediated responses can be observed in individual STN neurons after stimulation of the GP.

STN firing dynamics are shaped by spontaneous GABA_AR- but not GABA_BR-mediated input

The data obtained from whole-cell voltage-clamp recordings suggest that, in the parasagittal preparation, the neurons of the STN are subject to a consistent level of spontaneously occurring GABA_AR-mediated input. To examine the effect of this input on firing dynamics, gramicidin-based perforated-patch current clamp recordings were obtained from 22 STN neurons, bathed in 50 μM APV and 20 μM DNQX. Previous perforated-patch recordings have suggested that, in the absence of synaptic input, the neurons of the STN fire in a regular, single-spike manner (Bevan et al., 2002a; Hallworth et al., 2003). STN neurons in parasagittal slices also fired in a single-spike manner, with a mean firing rate of $9.6 \pm 7.5\text{ Hz}$ ($n = 22$), but as seen in Figure 2A, firing was irregular, producing an average CV of 0.30 ± 0.29 . In most cells, ongoing barrages of IPSPs were readily apparent. Application of GBZ (20 μM) led to a significant increase in the average firing rate of these cells (WSR test; GBZ frequency, $11.7 \pm 7.7\text{ Hz}$; $n = 22$; $p = 0.005$) (Fig. 2B,C). The antagonist also led to a significant decrease in the average CV (WSR test; GBZ CV, 0.17 ± 0.09 ; $n = 22$; $p = 0.004$) (Fig. 2B,C), as the occurrence of action potentials shifted to a more regular, precise pattern.

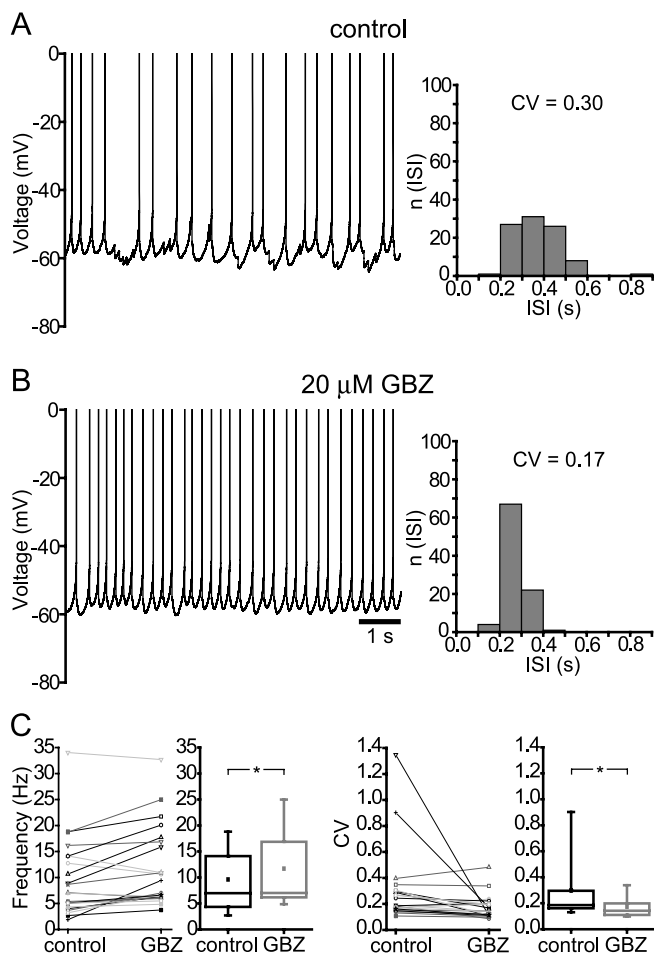


Figure 2. Spontaneous GABA_A-mediated IPSPs disrupt the pace and precision of firing of STN neurons. **A, B.** Activity of a representative neuron under control conditions and after bath application of GBZ. The ISI histograms generated from 100 ISIs occurring during the respective conditions for this neuron are shown to the right. **A.** Single-spike firing was slow and irregular before drug application, and IPSPs were evident during the ISIs (frequency, 2.7 Hz; CV, 0.30). **B.** Blockade of GABA_ARs with 20 μM GBZ increased the frequency and precision of activity (frequency, 3.7 Hz; CV, 0.17). **C.** Population firing rate and CV data. Nonparametric paired comparisons revealed that the mean firing rate in control was significantly lower than the mean firing rate in GBZ and that the mean CV in control was significantly higher than the mean CV in GBZ. * $p < 0.05$. Calibration in **B** also applies to **A**.

GABAergic input from the GP may also influence STN firing rate and pattern through the activation of postsynaptic GABA_BRs. To test this hypothesis, current-clamp recordings were obtained from 10 STN neurons in parasagittal slices in which fast glutamatergic and GABAergic synaptic transmission was blocked. Under these conditions, STN neurons fired in a regular, single-spike manner (CV, 0.16 ± 0.04) with a frequency of 9.8 ± 5.6 Hz (data not shown). The pace and precision of firing was not significantly changed by the subsequent application of 1 μM CGP, a selective GABA_B antagonist (WSR tests; CGP frequency, 11.6 ± 5.4 Hz; $n = 10$; $p = 0.064$; CGP CV, 0.14 ± 0.03 ; $n = 10$; $p = 0.16$), suggesting that GABA released spontaneously in parasagittal slices does not sufficiently activate postsynaptic GABA_BRs to influence STN activity.

Postsynaptic GABA_ARs are not tonically active

Tonic current is thought to be mediated by GABA_ARs primarily located at extrasynaptic sites, but different receptor subtypes are involved in specific neuronal populations (Mody, 2001; Stell et

al., 2003; Caraiscos et al., 2004; Semyanov et al., 2004). A significant proportion of α_1 subunit-containing GABA_ARs expressed on STN neurons are extrasynaptic (Galvan et al., 2004), and *in situ* hybridization has demonstrated that STN neurons express mRNA for γ and δ subunits (Wisden et al., 1992; Fritschy and Mohler, 1995; Kultas-Ilinsky et al., 1998), both thought to mediate tonic current in other neurons. To address whether any of the GABA_A-mediated effects observed in our current-clamp recordings were attributable to the activation of a tonic current, we performed whole-cell voltage-clamp recordings under conditions in which this current is typically identified, that is, when local levels of ambient GABA are elevated (Nusser and Mody, 2002; Stell and Mody, 2002; Semyanov et al., 2003; Stell et al., 2003; Wu et al., 2003). Using cesium chloride-containing intracellular pipettes, recordings were made at a holding potential of -65 mV from STN neurons within parasagittal slices in which presynaptic transmitter release was elevated with bath-applied 4-AP. This agent has been used to enhance activity at other central synapses, including those in the hippocampus (Segal, 1987; Otis and Mody, 1992), neostriatum (Flores-Hernandez et al., 1994), and dorsal column (Smith et al., 2000). Although typically considered a voltage-dependent potassium (K_v) channel antagonist, 4-AP is also known to have less specific effects, particularly on intracellular calcium dynamics (Grimaldi et al., 2001). Used at concentrations >1 mM, 4-AP has been shown to elevate intracellular inositol triphosphate levels in cortical neurons, leading to calcium release from intracellular stores. With the aim of minimizing such interactions, 4-AP was used at a concentration of 25–50 μM in this study. 4-AP is proposed to increase transmission at the pallidosubthalamic synapse through an inhibition of K_v channels suggested to be present in the axon and terminals of GP neurons (Rudy et al., 1999).

Voltage-clamp recordings from eight STN neurons in slices bathed in 25 μM 4-AP, 50 μM APV, and 20 μM DNQX, performed at room temperature to maintain a stable baseline over long periods, revealed large, frequent inward currents when cells were held at -65 mV (control mean frequency, 14.8 ± 7.8 Hz; control mean charge, 51.8 ± 52.4 pC/s). Figure 3 shows that subsequent fast application of 20 μM GBZ significantly reduced the size and number of these phasic events (WSR tests; GBZ mean frequency, 0.2 ± 0.4 Hz; $n = 8$; $p = 0.008$; GBZ mean charge, 0.2 ± 0.2 pC/s; $n = 8$; $p = 0.008$), with no accompanying change in baseline current (WSR test; baseline in control, -53.0 ± 28.2 pA; baseline in GBZ, -50.6 ± 28.4 pA; $n = 8$; $p = 0.109$). Because there is some uncertainty about the effectiveness of this concentration of GBZ in blocking tonic current (Yeung et al., 2003; Bieda and MacIver, 2004), we also fast-applied a mixture of a higher concentration (100 μM) of GBZ in combination with 100 μM picrotoxin to a subset of STN neurons. This treatment was also found to have no significant impact on baseline holding current (WSR test; baseline in control, -63.2 ± 9.2 pA; baseline in 100 μM GBZ and picrotoxin, -61.5 ± 8.4 pA; $n = 7$; $p = 0.688$). Because the impact of GABA_AR antagonists was similar in both cases, data were pooled (WSR test; baseline in control, -57.8 ± 26.0 pA; baseline in GABA_AR antagonists, -55.7 ± 25.4 pA; $n = 15$; $p = 0.135$). To ensure that tonic current is indeed manifest under conditions of bath-applied 4-AP in neurons in which it has been identified previously, we recorded from cerebellar and dentate gyrus granule cells (Brickley et al., 1996; Stell and Mody, 2002; Stell et al., 2003). Under conditions of continually applied 25 μM 4-AP, 50 μM APV, and 20 μM DNQX, subsequent rapid application of 20 μM GBZ resulted in a significant shift in the baseline holding current of both cerebellar granule cells (WSR test; base-

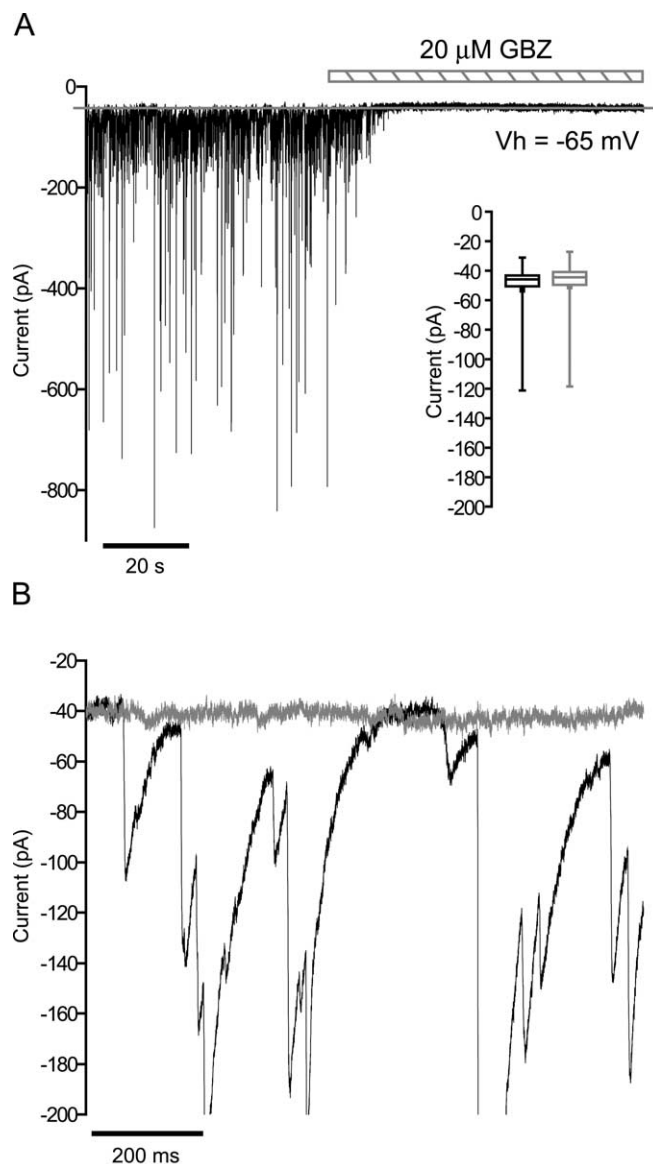


Figure 3. GABA_A-mediated tonic current is not observed in STN neurons. **A**, Representative example in which large, frequent inward currents recorded from an STN neuron in the presence of 4-AP were abolished by the fast application of GBZ, with no accompanying change in baseline holding current. The thin gray line represents average baseline holding current for predrug period. The inset indicates population data of average baseline current. Vh, Holding potential. **B**, Overlay of the first (black) and last (gray) second of data shown in **A** confirms that GBZ application does not influence baseline holding current.

line in control, -45.6 ± 25.9 pA; baseline in GBZ, -25.2 ± 13.5 pA; $n = 5$; $p = 0.0431$) and hippocampal granule cells (WSR test; baseline in control, -55.4 ± 7.7 pA; baseline in GBZ, -46.3 ± 8.1 pA; $n = 6$; $p = 0.031$) voltage-clamped at -65 mV (data not shown). Together, these data suggest that GABA_A-mediated tonic current is not present in STN neurons, even when presynaptic GABA release, which leads to the powerful, phasic activation of postsynaptic GABA_A receptors, is elevated.

GABA_AR and GABA_BR activation during conditions of elevated GABA release

The 4-AP-mediated increase in pallidum subthalamic transmission also permitted us to test the hypothesis that subthalamic GABA_BRs are preferentially activated when the level of pallidal input is elevated. Cesium chloride-based whole-cell voltage-clamp re-

cordings from STN neurons within parasagittal slices bathed in $50 \mu\text{M}$ 4-AP and held at 37°C revealed large, frequent inward currents ($n = 6$; control mean frequency, 43.9 ± 24.4 Hz; control mean charge, 54.5 ± 35.8 pC/s) (data not shown). Although these currents were recorded under conditions in which ionotropic glutamatergic transmission was blocked, they were highly sensitive to GABA_AR antagonists (WSR tests; GBZ mean frequency, 1.6 ± 2.5 Hz; $n = 6$; $p = 0.031$; GBZ mean charge, 1.0 ± 1.5 pC/s; $n = 6$; $p = 0.031$), representing a ~ 14 -fold greater mean GBZ-sensitive charge when compared with parasagittal slices maintained in APV and DNQX only (53.5 pC/s in slices bathed in 4-AP vs 3.8 pC/s under control conditions). This was manifest in current-clamp recordings as barrages of IPSPs evident throughout the ISI (Fig. 4A). The eight STN neurons from which perforated-patch current-clamp recordings were obtained under conditions of bath-applied 4-AP, APV, and DNQX had an average firing rate of 7.2 ± 6.6 Hz. The single-spike activity recorded was irregular, with an average CV of 0.38 ± 0.15 . Although it might be suggested that the relatively low firing rate observed is the product of a direct 4-AP-mediated blockade of K_v3 channels expressed in STN neurons (Rudy et al., 1999; Wigmore and Lacey, 2000; Baranauskas et al., 2003), phasic GABAergic events contribute to the disruption in pace. Indeed, in recordings obtained from six neurons after the application of $20 \mu\text{M}$ GBZ (Fig. 4B,D), the average frequency of spontaneous activity was nearly doubled (WSR test; 4-AP frequency, 6.8 ± 7.8 Hz; GBZ frequency, 12.0 ± 6.5 Hz; $n = 6$; $p = 0.031$). The precision of spontaneous activity was also significantly increased by this treatment (WSR test; 4-AP CV, 0.39 ± 0.18 ; GBZ CV, 0.11 ± 0.05 ; $n = 5$; $p = 0.0431$).

Additional application of CGP ($1 \mu\text{M}$) was subsequently attempted, and recordings were obtained during conditions of both GABA_AR and GABA_BR blockade for five of the six neurons (Fig. 4C,E). Although this treatment had no effect on the precision of single-spike activity (WSR test; 4-AP and GBZ CV, 0.11 ± 0.05 ; CGP CV, 0.13 ± 0.06 ; $n = 5$; $p = 0.6858$), it led to an increase in the firing rate of every cell examined (WSR test; 4-AP and GBZ frequency, 11.7 ± 7.2 Hz; CGP frequency, 12.9 ± 7.4 Hz; $n = 5$; $p = 0.0431$). Together, these data suggest that, under conditions in which synaptic GABA is elevated, activation of GABA_ARs and GABA_BRs influences the frequency and precision of single-spike firing of individual STN neurons. This influence is weighted toward GABA_ARs, however, because their blockade more profoundly changes the manner in which STN neurons discharge, whereas subsequent blockade of GABA_BRs had relatively minimal impact.

It should be noted that the average firing rate was not significantly different between spontaneous and 4-AP conditions when all GABAergic activity was blocked (MWU test; GBZ and CGP, 11.6 ± 5.4 Hz; 4-AP, GBZ, and CGP, 12.9 ± 7.4 Hz; $n = 5$; $p = 0.905$), nor was the average CV (MWU test; GBZ and CGP, 0.14 ± 0.03 ; 4-AP, GBZ, and CGP, 0.13 ± 0.06 ; $n = 5$; $p = 0.358$), suggesting that 25 – $50 \mu\text{M}$ 4-AP had no confounding influence on the intrinsic excitability of STN neurons.

Postsynaptic GABA_BR activation leads to a transient reduction in STN activity

To examine whether synchronous high-frequency activity of pallidum subthalamic fibers leads to the activation of postsynaptic GABA_BRs, perforated-patch current-clamp recordings were obtained from STN neurons within parasagittal slices while pallidal fibers were driven synchronously with high-frequency (20 pulses at 100 Hz) electrical stimulation, before and after bath applica-

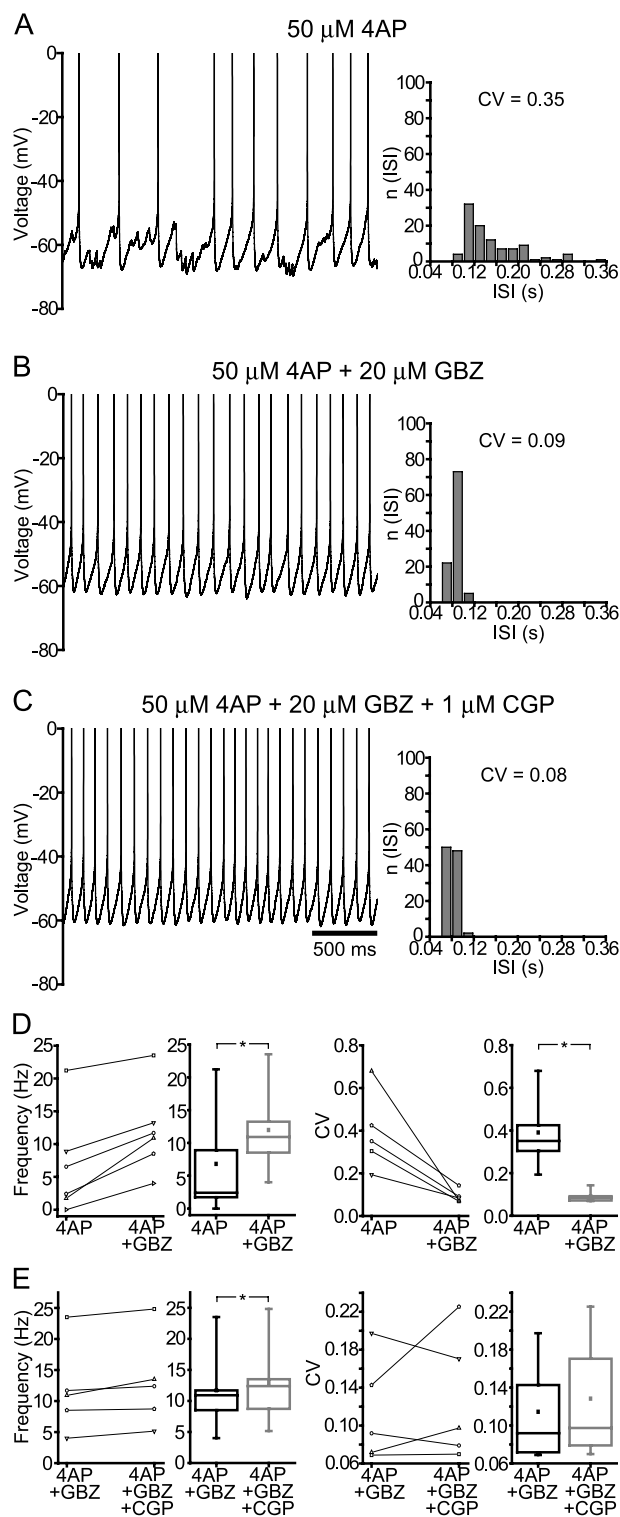


Figure 4. GABA_A and GABA_B receptors are both activated during conditions of elevated GABA release, but GABA_A activation influences the firing of STN neurons to a greater extent. **A–C**, Activity of a representative neuron under conditions in which presynaptic activity was elevated with 4-AP before and after subsequent block of ionotropic and metabotropic GABA_B receptors. The ISI histograms generated from 100 ISIs occurring during the respective conditions for this neuron are shown to the right. **A**, Both the pace and precision of single-spike firing were low (frequency, 6.6 Hz; CV, 0.35) under conditions of bath-applied 50 μ M 4-AP, which was associated with barrages of IPSPs. **B**, Blockade of GABA_A receptors with 20 μ M GBZ increased the frequency and precision of activity (frequency, 11.7 Hz; CV, 0.09). **C**, Additional blockade of GABA_B receptors with 1 μ M CGP had no additional influence on single-spike rhythmicity but slightly increased the frequency of activity (frequency, 12.4 Hz; CV, 0.08). **D**, **E**, Population firing rate and CV data under each condition. * $p < 0.05$. Calibration in **C** also applies to **A** and **B**.

tion of a GABA_B antagonist. Across the population of STN neurons from which recordings were obtained, in the presence of APV, DNQX, and GBZ, stimulation of the GP ($n = 3$) or internal capsule rostral to the STN ($n = 9$) resulted in a transient reduction in firing rate. This was evident as a trough in the peristimulus time histogram immediately after the stimulation period, during which firing was 2 SDs below the prestimulation level (Fig. 5*Ai*). The cumulative sum of the peristimulus time histogram also fell below 2 SDs of the mean prestimulation score within this period (Ellaway, 1978; Magill et al., 2004). The subsequent high-frequency response apparent in the raster displays of a subset of STN neurons will be the focus of the following section (see below). A range of stimulation-evoked reductions in firing were observed, with some neurons displaying less evident IPSPs and more modest suppressions in firing (Fig. 5*Aii*, sweep 1b), whereas others displayed larger IPSPs and more pronounced disruptions (Fig. 5*Aii*, sweep 2a). In the subset of neurons in which a pronounced, prolonged hyperpolarizing response was observed ($n = 7$), the average latency of this response was 135.7 ± 85.4 ms, and the average time to the peak of the response was 310.9 ± 105.3 ms. The average reversal potential of the response was found to be -93.3 ± 6.6 mV in the three neurons in which stimulation-evoked responses were obtained from a range of membrane potentials (predicted E_K , -97.0 mV). Subsequent application of 1 μ M CGP abolished the stimulation-evoked reduction in STN firing rate (Fig. 5*Bi*, *Bii*). These data demonstrate that synchronous, high-frequency activation of pallidosubthalamic fibers generates both subtle and overt postsynaptic GABA_B-mediated responses, which in each case lead to a transient reduction in the single-spike firing of STN neurons.

It should be noted that, under conditions of ionotropic glutamate, ionotropic GABA, and metabotropic GABA receptor blockade, high-frequency stimulation of the caudal internal capsule occasionally generated a transient increase in STN activity. Although we did not examine this further, it may be attributable to an activation of postsynaptic metabotropic glutamate receptors (Awad et al., 2000).

Postsynaptic GABA_B activation can contribute to rebound excitation in STN neurons

Although high-frequency electrical stimulation of pallidal fibers was found to consistently lead to a GABA_B-mediated suppression of STN activity, it was also found to generate a subsequent period of elevated firing in a subset of the same neurons. Four of 20 STN neurons within parasagittal slices bathed in APV and DNQX in which tetanic stimulation was applied to the caudal GP ($n = 10$) or the rostral internal capsule ($n = 10$) responded with a rebound depolarization, associated with a burst of high-frequency firing (Fig. 6*A*). Notably, in all four cases, stimulation was applied to the internal capsule immediately rostral to the STN, presumably leading to the synchronization of a higher proportion of pallidal fibers. Such responses have previously been observed in STN neurons after bipolar electrical stimulation of the rostral internal capsule in sagittal preparations, and were attributed to the GABA_A IPSP-mediated de-inactivation of class 3 voltage-dependent calcium (Ca_v3) channels (Bevan et al., 2002a). However, in that study, the putative contribution of metabotropic GABA_B receptors to rebound burst firing was not examined because these receptors were blocked a priori. With GABA_B receptors intact, we found that, after the application of 20 μ M GBZ, three of the four STN neurons still generated rebound bursts after the same tetanic stimulation protocols (Fig. 6*B*). In the three neurons in which rebound bursts persisted after GABA_A receptor block-

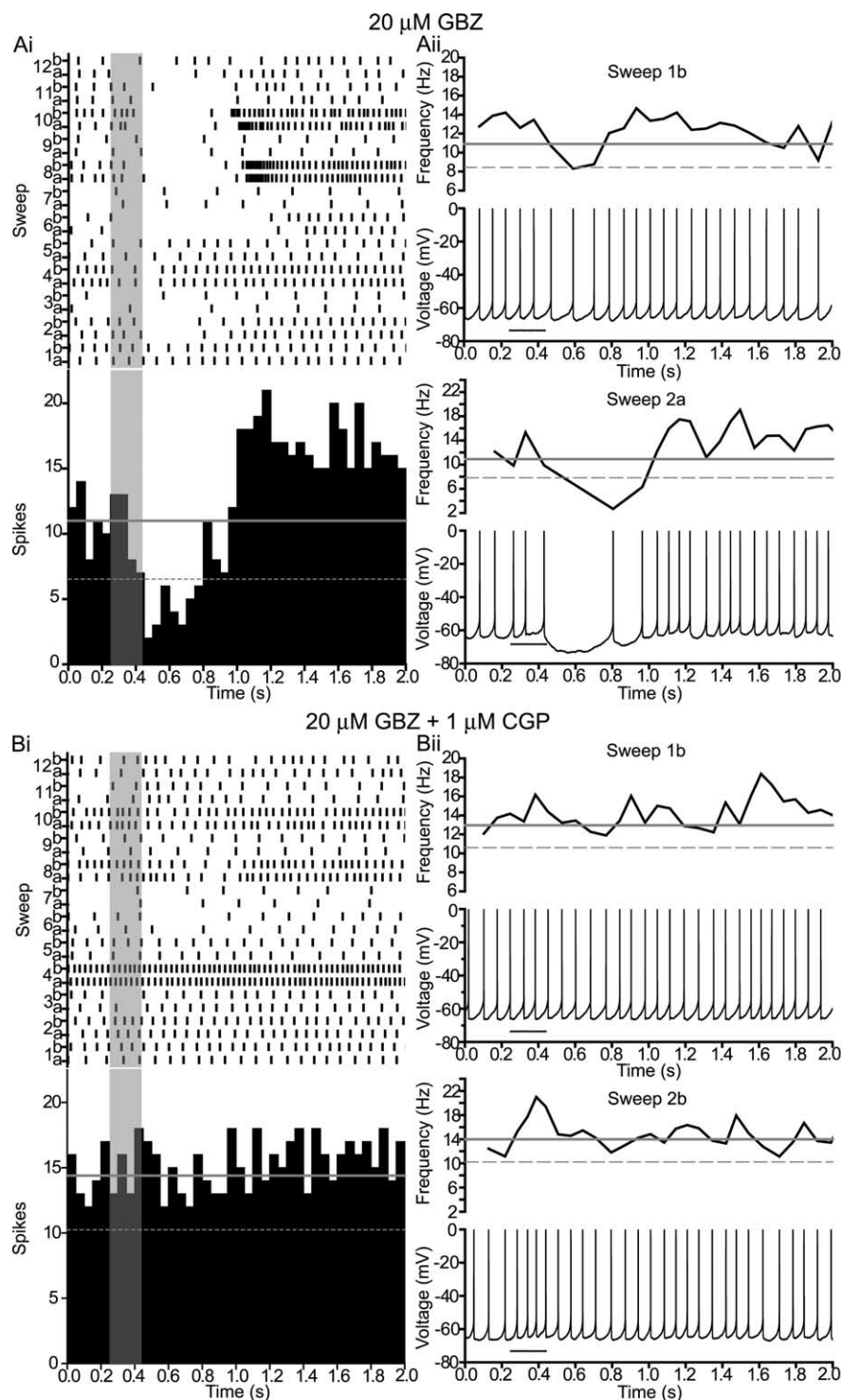


Figure 5. Stimulation of globus pallidus fibers leads to a transient GABA_B-mediated reduction in the firing rate of STN neurons. **Ai, Aii.** With 20 μ M GBZ bath applied, tetanic stimulation of pallidal fibers consistently resulted in a transient reduction in STN firing. **Ai.** The stimulus-evoked pause in firing was apparent in peristimulus raster plots (12 neurons, 2 trials per neuron) and was manifest as a trough in the peristimulus time histogram after the period of applied stimulation (gray box). The solid gray line represents the average counts per bin before stimulation. The dashed gray line represents 2 SDs below this average. The high-frequency activity subsequent to the pause in firing apparent in sweeps 8, a and b, and 10, a and b, is addressed in Results. Postsynaptic GABA_B activation can contribute to rebound excitation in STN neurons. **Aii.** Stimulus-evoked reductions in instantaneous firing rate in two separate neurons, illustrating the limits of the inhibitory effect observed. The solid gray line represents the average spontaneous firing rate. The dashed gray line represents the firing rate 2 SDs below this average. **Bi, Bii.** After the application of 1 μ M CGP, tetanic stimulation of pallidal fibers no longer produced a discernible reduction in firing. **Bi.** Raster plots and the accompanying peristimulus time histogram illustrate that ongoing activity was relatively unchanged by pallidal stimulation. **Bii.** Current-clamp recordings and accompanying analyses for the same neurons as in **Aii**, demonstrating the impact of high-frequency stimulation after CGP application.

ade, the average length of high-frequency firing (defined as that three times above spontaneous activity) (Bevan et al., 2000, 2002a) was reduced by 38.5% (from 194.2 ± 98.5 to 119.4 ± 73.7 ms), whereas the peak frequency attained during the burst period was reduced by 24.4% (from 98.4 ± 42.9 to 74.3 ± 43.9 Hz). Note that the depth of hyperpolarization and resulting intensity and duration of high-frequency firing were also related to the number of pulses in the stimulation train (Fig. 6). On subsequent application of 1 μ M CGP, high-frequency stimulation of the rostral internal capsule no longer evoked an IPSP or consequent rebound depolarization, indicating that the activation of postsynaptic GABA_BRs is sufficient to generate such responses in STN neurons (Fig. 6C). Importantly, the application of a brief hyperpolarizing current pulse was still able to generate rebound burst responses in STN neurons bathed in CGP, indicating that the failure to generate rebounds with synaptic stimulation was not the consequence of a confounding action of this agent on rebound generating mechanisms intrinsic to STN neurons.

Discussion

Pallidosubthalamic connections are preserved in the parasagittal slice

Our first objective was to maintain pallidosubthalamic connections in a brain slice. To achieve this, we obtained slices from mice, in which we may have retained up to one-fourth and one-half of the native GP and STN, respectively (Paxinos and Franklin, 2001). Together, anatomical and electrophysiological observations suggest that intact pallidosubthalamic projections were maintained. Deposits of biocytin in the GP of these slices consistently labeled fibers and somata in the STN. GP neurons were also spontaneously active, and action potential-dependent GABAergic IPSCs were recorded from STN neurons. Finally, electrical stimulation of the GP produced GABA_A-mediated responses in STN neurons.

Spontaneous pallidosubthalamic activity influences STN firing through the phasic activation of postsynaptic GABA_ARs

By performing current-clamp recordings of STN neurons within parasagittal slices, we were able to determine the impact of spontaneously occurring pallidosubthalamic transmission on postsynaptic activity. We found that GABA released under these conditions was sufficient to activate postsynaptic GABA_ARs, leading to a 20%

reduction in firing rate and a 40% reduction in the precision of firing. Tonic activation of GABA_ARs was never observed in STN neurons. Therefore, the change in single-spike activity observed on GABA_AR blockade was entirely attributable to the blockade of phasic events. Spontaneous pallidal input did not sufficiently activate postsynaptic GABA_BRs to influence STN firing. Similar observations have been made in other *in vitro* preparations in which spontaneous GABAergic transmission has been preserved (Hausser and Clark, 1997; Kononenko and Dudek, 2004).

Elevated/synchronous pallidosubthalamic transmission influences STN firing through actions at postsynaptic GABA_ARs and GABA_BRs

Applications of selective agonists have suggested that there are functional GABA_BRs on STN neurons (Shen and Johnson, 2001; Urbain et al., 2002), but the conditions under which these receptors are activated by the synaptic release of GABA have not been addressed. Under conditions of elevated GABA release (4-AP), pallidal input primarily influenced STN firing through barrages of GABA_AR-mediated IPSPs, but postsynaptic GABA_BR activation also reduced the firing of STN neurons by 10%. Postsynaptic GABA_BRs were also activated by tetanic, high-frequency stimulation of the caudal GP/internal capsule. Because the minimum distance between two poles of stimulation was 240 μ m, a large proportion of pallidal axons were presumably activated simultaneously. Stimulation-evoked GABA_BR activation led to a transient reduction in postsynaptic firing, but could also lead to a subsequent increase in firing by generating sufficient hyperpolarization to produce rebound bursts.

Together, these data suggest that postsynaptic GABA_BRs are activated only during periods of elevated/synchronized GP activity that lead to the spillover of GABA at pallidosubthalamic synapses. This proposition is consistent with anatomical assessments that suggest that high levels of various GABA_AR subunits are expressed in the STN (Wisden et al., 1992; Fritschy and Mohler, 1995; Kultas-Ilinsky et al., 1998; Smith et al., 2001), whereas only moderate immunoreactivity has been observed for GABA_BR subunits (Billinton et al., 2000; Charara et al., 2000; Smith et al., 2001). Moreover, whereas the majority of GABA_ARs are in the main body of symmetrical synapses, 77% of membrane-bound GABA_BRs are extrasynaptic (Galvan et al., 2004). Our observations are similar to those made in other brain regions in which a local increase in GABA concentration is necessary for the activation of extrasynaptic GABA_BRs (Isaacson et al., 1993; Mody et al., 1994; Mitchell and Silver, 2000; Scanziani, 2000). Although GABA is typically elevated to levels sufficient for the activation of postsynaptic GABA_BRs through the blockade of GABA uptake or

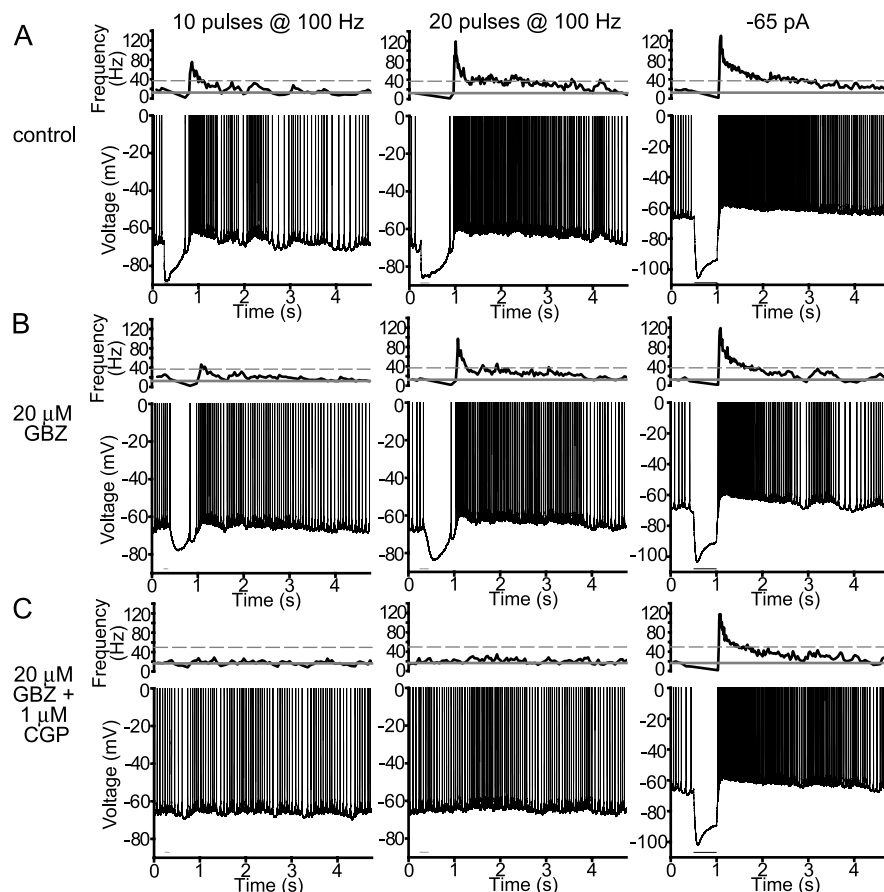


Figure 6. Synaptic activation of GABA_BRs generates rebound burst firing in STN neurons. **A**, Activity of a representative neuron in which tetanic stimulation applied to the caudal internal capsule evoked a transient hyperpolarization followed by a rebound depolarization and an associated period of high-frequency firing. Longer trains of stimulation generated more intense rebound responses. Rebound burst firing was also observed after the termination of a -65 pA current injection (thin black bar). The solid gray line represents average spontaneous firing rate. The dashed gray line represents three times the average firing rate. **B**, High-frequency rebound burst firing persisted in response to both stimulation of the internal capsule and negative current injection after the application of 20μ M GBZ. **C**, Additional application of 1μ M CGP abolished both the initial hyperpolarization and accompanying rebound burst response to tetanic stimulation. Rebound burst firing was still evoked on the removal of a -65 pA current step under these conditions.

tetanic electrical stimulation, *in vitro* recordings from hippocampal and thalamic synapses demonstrate that this also occurs when presynaptic neurons are synchronously active during network rhythms (Kim et al., 1997; Scanziani, 2000; Jacobsen et al., 2001).

Functional implications of postsynaptic GABA_BR activation

Mammalian STN neurons fire between 10 and 50 Hz in an irregular pattern (Wichmann et al., 1994; Levy et al., 2000; Urbain et al., 2002; Soares et al., 2004). Under these conditions, local application of GABA_AR antagonists increases STN activity (Rouzaire-Dubois et al., 1980; Urbain et al., 2002). In contrast, GABA_BR antagonists have no effect. However, interpretation of *in vivo* pharmacological manipulations can be difficult: drugs are applied at a distance from the recorded cell; the effective drug concentration is unknown; and multisynaptic effects are possible. By manipulating pallidosubthalamic transmission *in vitro*, we confirmed that, under spontaneous conditions, GABAergic input from the GP regulates STN activity primarily through the phasic activation of postsynaptic GABA_ARs, and not GABA_BRs.

Corticosubthalamic fibers provide a major excitatory input to the STN, but after the removal of this input, STN neurons discharge in an irregular single-spike manner, with an average CV of

0.58 (Magill et al., 2001). Our data suggest that inhibitory input from the GP accounts for much of the irregular nature of STN firing *in vivo*. *In vitro* and *in vivo* data indicate that STN neurons are active despite receiving continuous input from the GP (even in the absence of glutamatergic input) (Bevan and Wilson, 1999; Magill et al., 2000, 2001). Incomplete silencing of postsynaptic neurons is a common feature of information transfer at pallidal synapses, because other GP efferents maintain high firing rates in awake animals (Wichmann et al., 1999; Raz et al., 2000; Wichmann and Klemm, 2004). Pallidal inhibition may be less effective than predicted for a variety of reasons. Normally, GP firing is not synchronized or correlated (Nini et al., 1995; Raz et al., 2000; Soares et al., 2004). As such, GABA_AR-mediated IPSPs are less likely to summate and postsynaptic GABA_BRs may not be activated sufficiently to suppress firing. Presynaptic inhibition of GABA release (Shen and Johnson, 2000, 2001; Chen and Yung, 2005) and postsynaptic receptor saturation/desensitization (Nusser et al., 1997; Overstreet et al., 2000) are also possible. Finally, postsynaptic GABAR-mediated hyperpolarization could engage hyperpolarization-activated cyclic-nucleotide-gated channels, Ca_v3 channels, and/or Na_v channels that mediate inward currents that could counteract inhibition (Mainen and Sejnowski, 1995; Huguenard, 1996; McCormick and Bal, 1997). Because persistent inhibitory input from the GP does not silence STN neurons, their firing can be both upregulated and downregulated, making the nucleus a dynamic controller of basal ganglia network activity.

In vitro manipulations demonstrated that GABA_BRs can be sufficiently activated to impact firing when pallidosubthalamic activity is elevated. *In vivo*, subsets of GP neurons momentarily increase their firing after cortical stimulation meant to simulate a descending motor command. This excitation results in feedback inhibition (Ryan and Clark, 1992; Nambu et al., 2000; Kolomiets et al., 2001; Kita et al., 2004) and brief synchronization of the STN (Magill et al., 2004). Although *in vivo* recordings suggest that this inhibition is mediated primarily by GABA_ARs (Rouzaire-Dubois et al., 1980; Kita et al., 1983), the contribution of GABA_BRs has not been examined. Computer simulations suggest that cortically driven elevated firing in GP neurons may lead to GABA_BR-mediated hyperpolarization and subsequent rebound burst firing in STN neurons, which is necessary for switching between competing motor programs (Rubchinsky et al., 2003). Our *in vitro* results demonstrate that such a mechanism is plausible and provide motivation for *in vivo* study.

Implications for pathological activity

In idiopathic and experimental models of PD, populations of STN and GP neurons display correlated, rhythmic burst firing which may underlie, in part, motor symptoms (Bevan et al., 2002b; Amironov et al., 2004; Dostrovsky and Bergman, 2004). Computer modeling (Terman et al., 2002), *in vitro* (Plez and Kital, 1999; Bevan et al., 2002a), and *in vivo* (Paz et al., 2005) studies suggest that burst firing in STN neurons is driven, in part, by rebound depolarization that can be produced after barrages of GABA_AR-mediated IPSPs arising from pallidal fibers. These barrages were produced *in vitro* through synchronous, high-frequency activation of these fibers. We consistently found that synchronous, high-frequency activation of pallidal fibers could also activate postsynaptic metabotropic GABA_BRs. This had a considerable synergistic effect: the duration and intensity of high-frequency rebound burst firing in STN neurons were consistently greater when both GABA_ARs and GABA_BRs were activated. The recruitment of postsynaptic GABA_BR-mediated activity may

therefore be an important additional mechanism through which burst firing in the parkinsonian STN–GP network is generated. Given the relative time courses of ionotropic and metabotropic GABAR-mediated currents, GABA_BR-mediated rebound bursts may reduce the frequency of GABA_AR-mediated rhythms within the STN–GP network (as in other systems) (Kim et al., 1997; Scanziani, 2000; Sohal and Huguenard, 2003), which may in turn contribute to the range of oscillatory frequencies observed in parkinsonian animals and human patients (Bevan et al., 2002b; Brown, 2003; Dostrovsky and Bergman, 2004; Hutchison et al., 2004).

Electron microscopic analyses performed by Galvan et al. (2004) indicate that 64% of GABA_BRs are cytoplasmic and not expressed in the plasmalemma. As these authors suggest, the insertion of these receptors into the cell membrane may be regulated dynamically. Indeed, GABA_BR mRNA expression increases in the STN in an animal model of PD (Johnston and Duty, 2003). Therefore, the impact of postsynaptic GABA_BR activation in the parkinsonian basal ganglia may be of even greater significance than that established in our study of unlesioned animals. The findings described here should provide a foundation on which the principles of pallidosubthalamic transmission in parkinsonian animals can be addressed.

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