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_A,R)-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_A,R- and GABA_B,R-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_A,Rs) mediate a tonic current that profoundly influences postsynaptic excitability (Brickley et al., 1996; Semyanov et al., 2004). Although extrasynaptic GABA_A Rs 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, and are likely to be activated by the direct application of agonists; GABA_B,R antagonists have no impact on STN firing dynamics (Shen and Johnson, 2001; Urbain et al., 2002; Wilson et al., 1998).

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al., 2004). As such, it is unclear under what conditions postsynaptic GABAARs are activated by palidal input. At other synapses, the activation of extrasynaptic GABAARs 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 GABAARs 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 GABAARs. Given that the impact of extrasynaptic GABAAR and GABAAR activation may be as significant as that of synaptic GABAAR 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 GABAAR 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 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 cerebral spinal fluid (ACSF), which had been bubbled with 95% O2 and 5% CO2 and confused transcardially with 10 ml of ice-cold modified artificial CSF (M6,7-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 ± 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. 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 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 μl/site) within the GP of parasagittal slices held in an interface chamber (Fine Science Tools, Foster City, CA), using a 1.0 μl (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 ~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 OsO4 (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 Axiosvision software (Zeiss).

**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 MΩ, 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 ± 7.6 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 Linhas, 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. 1Bii). 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<sub>R</sub>-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 ± 25.7 pA, a 10–90% rise time of 0.84 ± 0.26 ms, and a weighted decay time constant of 8.4 ± 3.2 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, ~10 ms). The spontaneous events recorded from all 19 STN cells in the parasagittal slice occurred with a mean frequency of 15.5 ± 14.7 Hz and carried a mean charge of 6.9 ± 7.4 pC/s. The GABA<sub>R</sub> 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 ± 7.8 Hz and carried a mean charge of 4.0 ± 2.4 pC/s (Fig. 1Ci). In all 10 recordings, application of the antagonists significantly reduced both the frequency and size of these events (WSR tests; GABA<sub>R</sub>-blocked frequency, 0.7 ± 0.8 Hz; n = 10; p = 0.002; GABA<sub>A</sub>-blocked mean charge, 0.2 ± 0.1 pC/s; n = 10; p = 0.002). The nine other neurons were challenged with 500 nM TTX (Fig. 1Cii). The mean frequency of events recorded under these conditions was one-half of that recorded under control conditions (WSR test; control, 20.5 ± 19.1 Hz; TTX, 10.9 ± 9.2 Hz; n = 9; p = 0.004). Bath-applied TTX also significantly reduced the mean charge of spontaneously occurring events (WSR test; control, 10.0 ± 9.8 pC/s; TTX, 3.4 ± 3.2 pC/s; n = 9; p = 0.02). Thus, phasic currents recorded from STN neurons in the parasagittal preparation are reduced by GABA<sub>A</sub> and voltage-dependent sodium (Na<sub>V</sub>) 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 pallidodsubthalamic 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<sub>A</sub> IPSPs was −78.9 ± 2.5 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<sub>A</sub>R 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. 1Di). 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. 1Dii). This hyperpolarization was subsequently blocked by the application of 1 μM CGP (Fig. 1Diii). Thus, both GABA<sub>R</sub>- and GABA<sub>A</sub>R-mediated responses can be observed in individual STN neurons after stimulation of the GP.

**STN firing dynamics are shaped by spontaneous GABA<sub>A</sub>R- but not GABA<sub>A</sub>R-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<sub>A</sub>R-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 ± 7.5 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 ± 7.7 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.
GABAergic input from the GP may also influence STN firing rate and pattern through the activation of postsynaptic GABA_A Rs. 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_A 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_A Rs to influence STN activity.

**Postsynaptic GABA_A Rs are not tonically active**

Tonic current is thought to be mediated by GABA_A Rs 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_A Rs 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 R-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 (Kᵥ) 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 mimicking 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 Kᵥ 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 = 0.019), 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_A R antagonists was similar in both cases, data were pooled (WSR test; baseline in control, −57.8 ± 26.0 pA; baseline in GABA_A R antagonists, −55.7 ± 25.4 pA; n = 15; p = 0.135). To ensure that tonic current is indeed manifest under conditions for this neuron are shown to the right.

**Figure 2.** Spontaneous GABA_A R-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, 0.17). B, Blockade of GABA_A Rs 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.
line in control, $-45.6 \pm 25.9 \text{ pA}$; baseline in GBZ, $-25.2 \pm 13.5 \text{ pA}$; $n = 5$; $p = 0.0431$) and hippocampal granule cells (WSR test; baseline in control, $-55.4 \pm 7.7 \text{ pA}$; baseline in GBZ, $-46.3 \pm 8.1 \text{ pA}$; $n = 6$; $p = 0.031$) voltage-clamped at $-65 \text{ mV}$ (data not shown). Together, these data suggest that GABA$_{A}$R-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$_{A}$R and GABA$_{B}$R activation during conditions of elevated GABA release

The 4-AP-mediated increase in pallidosubthalamic transmission also permitted us to test the hypothesis that subthalamic GABA$_{A}$Rs are preferentially activated when the level of pallidal input is elevated. Cesium chloride-based whole-cell voltage-clamp recordings from STN neurons within parasagittal slices bathed in 50 $\mu$M 4-AP and held at 37°C revealed large, frequent inward currents ($n = 6$; control mean frequency, 43.9 $\pm$ 24.4 Hz; control mean charge, 54.5 $\pm$ 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$_{A}$R antagonists (WSR tests; GBZ mean frequency, 1.6 $\pm$ 2.5 Hz; $n = 6$; $p = 0.031$; GBZ mean charge, 1.0 $\pm$ 1.5 pC/s; $n = 6$; $p = 0.031$), representing a $\sim$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 $\pm$ 6.6 Hz. The single-spike activity recorded was irregular, with an average CV of 0.38 $\pm$ 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 KV3 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 $\mu$M GBZ (Fig. 4B, D), the average frequency of spontaneous activity was nearly doubled (WSR test; 4-AP frequency, 6.8 $\pm$ 7.8 Hz; GBZ frequency, 12.0 $\pm$ 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 $\pm$ 0.18; GBZ CV, 0.11 $\pm$ 0.05; $n = 5$; $p = 0.0431$).

Additional application of CGP (1 $\mu$M) was subsequently attempted, and recordings were obtained during conditions of both GABA$_{A}$R and GABA$_{B}$R 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 $\pm$ 0.05; CGP CV, 0.13 $\pm$ 0.06; $n = 5$; $p = 0.6858$), it led to an increase in the firing rate of every cell examined (WSR test; 4A-P and GBZ frequency, 11.7 $\pm$ 7.2 Hz; CGP frequency, 12.9 $\pm$ 7.4 Hz; $n = 5$; $p = 0.0431$). Together, these data suggest that, under conditions in which synaptic GABA is elevated, activation of GABA$_{A}$Rs and GABA$_{B}$Rs influences the frequency and precision of single-spike firing of individual STN neurons. This influence is weighted toward GABA$_{A}$Rs, however, because their blockade more profoundly changes the manner in which STN neurons discharge, whereas subsequent blockade of GABA$_{B}$Rs 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 $\pm$ 5.4 Hz; 4-AP, GBZ, and CGP, 12.9 $\pm$ 7.4 Hz; $n = 5$; $p = 0.905$), nor was the average CV (MWU test; GBZ and CGP, 0.14 $\pm$ 0.03; 4-AP, GBZ, and CGP, 0.13 $\pm$ 0.06; $n = 5$; $p = 0.358$), suggesting that 25–50 $\mu$M 4-AP had no confounding influence on the intrinsic excitability of STN neurons.

Postsynaptic GABA$_{A}$R activation leads to a transient reduction in STN activity

To examine whether synchronous high-frequency activity of pallidosubthalamic fibers leads to the activation of postsynaptic GABA$_{A}$Rs, 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 appli-
tion of a GABA_A 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. 5Ai). 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. 5Ai, sweep 1b), whereas others displayed larger IPSPs and more pronounced disruptions (Fig. 5Ai, 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 μM CGP abolished the stimulation-evoked reduction in STN firing rate (Fig. 5Bi, Bi). 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. 6A). 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_A receptors intact, we found that, after the application of 20 μM GBZ, three of the four STN neurons still generated rebound bursts after the same tetanic stimulation protocols (Fig. 6B). In the three neurons in which rebound bursts persisted after GABA_A block-
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 \pm 98.5 to 119.4 \pm 73.7 ms), whereas the peak frequency attained during the burst period was reduced by 24.4% (from 98.4 \pm 42.9 to 74.3 \pm 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 \mu 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\(_B\)Rs 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.

**Spontaneous pallidosubthalamic activity influences STN firing through the phasic activation of postsynaptic GABA\(_B\)Rs**

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\(_B\)Rs, leading to a 20%
reduction in firing rate and a 40% reduction in the precision of firing. Tonic activation of GABA$_A$R were never observed in STN neurons. Therefore, the change in single-spike activity observed on GABA$_A$R blockade was entirely attributable to the blockade of phasic events. Spontaneous pallidal input did not sufficiently activate postsynaptic GABA$_B$R 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$_A$R and GABA$_B$R

Applications of selective agonists have suggested that there are functional GABA$_A$R 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$_A$R-mediated IPSPs, but postsynaptic GABA$_B$R activation also reduced the firing of STN neurons by 10%. Postsynaptic GABA$_B$R 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 µm, a large proportion of pallidal axons were presumably activated simultaneously. Stimulation-evoked GABA$_B$R 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$_B$R 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$_A$R subunits are expressed in the STN (Wisden et al., 1992; Fritschy and Mohler, 1995; Kultas-Illinsky et al., 1998; Smith et al., 2001), whereas only moderate immunoreactivity has been observed for GABA$_B$R subunits (Billinton et al., 2000; Charara et al., 2000; Smith et al., 2001). Moreover, whereas the majority of GABA$_A$R are in the main body of symmetrical synapses, 77% of membrane-bound GABA$_B$R 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$_B$R (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$_B$R through the blockade of GABA uptake or 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 GABAR 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$_A$R antagonists increases STN activity (Rouzaire-Dubois et al., 1980; Urbain et al., 2002). In contrast, GABA$_B$R 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$_B$R, and not GABA$_A$R.

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 Kliem, 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<sub>A</sub>-mediated IPSPs are less likely to summate and postsynaptic GABA<sub>A</sub>Rs 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, C<sub>H</sub>3, channels, and/or N<sub>A</sub> 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 down-regulated, making the nucleus a dynamic controller of basal ganglia network activity.

In vitro manipulations demonstrated that GABA<sub>A</sub>Rs 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<sub>A</sub>Rs (Rouziere-Dubois et al., 1980; Kita et al., 1983), the contribution of GABA<sub>B</sub>Rs has not been examined. Computer simulations suggest that cortically driven elevated firing in GP neurons may lead to GABA<sub>B</sub>R-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; Amirnovin et al., 2004; Dostrovsky and Bergman, 2004). Computer modeling (Terman et al., 2002), in vitro (Plenz 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<sub>A</sub>-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<sub>B</sub>Rs. This had a considerable synergistic effect: the duration and intensity of high-frequency rebound burst firing in STN neurons were more robust with both GABA<sub>A</sub>Rs and GABA<sub>B</sub>Rs activated. The recruitment of postsynaptic GABA<sub>B</sub>-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 ionotrophic and metabotropic GABA-mediated currents, GABA<sub>B</sub>R-mediated rebound bursts may reduce the frequency of GABA<sub>A</sub>R-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<sub>B</sub>Rs are cytoplasmic and not expressed in the plasma membrane. As these authors suggest, the insertion of these receptors into the cell membrane may be regulated dynamically. Indeed, GABA<sub>B</sub>R mRNA expression increases in the STN in an animal model of PD (Johnston and Duty, 2003). Therefore, the impact of postsynaptic GABA<sub>B</sub>R 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.

**References**


