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GABAERGIC CONTROL OF RAT SUBSTANTIA NIGRA DOPAMINERGIC NEURONS: ROLE OF GLOBUS PALLIDUS AND SUBSTANTIA NIGRA PARS RETICULATA

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Abstract—Dopaminergic neurons *in vivo* fire spontaneously in three distinct patterns or modes. It has previously been shown that the firing pattern of substantia nigra dopaminergic neurons can be differentially modulated by local application of $GABA_A$ and $GABA_B$ receptor antagonists. The $GABA_A$ antagonists, bicuculline or picrotoxin, greatly increase burst firing in dopaminergic neurons whereas $GABA_B$ antagonists cause a modest shift away from burst firing towards pacemaker-like firing. The three principal GABAergic inputs to nigral dopaminergic neurons arise from striatum, globus pallidus and from the axon collaterals of nigral pars reticulata projection neurons, each of which appear to act *in vivo* primarily on $GABA_A$ receptors (see preceding paper). In this study we attempted to determine on which afferent pathway(s) $GABA_A$ antagonists were acting to cause burst firing. Substantia nigra dopaminergic neurons were studied by single unit extracellular recordings in urethane anesthetized rats during pharmacologically induced inhibition and excitation of globus pallidus. Muscimol-induced inhibition of pallidal neurons produced an increase in the regularity of firing of nigral dopaminergic neurons together with a slight decrease in firing rate. Bicuculline-induced excitation of globus pallidus neurons produced a marked increase in burst firing together with a modest increase in firing rate. These changes in firing rate were in the opposite direction to what would be expected for a monosynaptic GABAergic pallidonigral input. Examination of the response of pars reticulata GABAergic neurons to similar manipulations of globus pallidus revealed that the firing rates of these neurons were much more sensitive to changes in globus pallidus neuron firing rate than dopaminergic neurons and that they responded in the opposite direction. Pallidal inhibition produced a dramatic increase in the firing rate of pars reticulata GABAergic neurons while pallidal excitation suppressed the spontaneous activity of pars reticulata GABAergic neurons.

These data suggest that globus pallidus exerts significant control over the firing rate and pattern of substantia nigra dopaminergic neurons through a disynaptic pathway involving nigral pars reticulata GABAergic neurons and that at least one important way in which local application of bicuculline induces burst firing of dopaminergic neurons is by disinhibition of this tonic inhibitory input. © 1999 IBRO. Published by Elsevier Science Ltd.

Key words: dopamine, GABA, firing pattern, bicuculline, muscimol, basal ganglia.

Substantia nigra dopaminergic neurons have well characterized projections throughout the forebrain where they play an essential role in motor functions and sensorimotor integration. Degeneration of these neurons is primary to the etiology of Parkinson's disease,[31] and dysfunction in dopaminergic neurotransmission has been implicated in the symptoms of neuropsychiatric illnesses such as schizophrenia.[11]

Substantia nigra dopaminergic neurons *in vivo* exhibit three different firing patterns or modes; a pacemaker-like regular firing pattern, a random pattern, and a burst firing pattern.[23,58,66] The random pattern is the most common pattern observed *in vivo* in anesthetized[58] or unanesthetized[66] rats although freely moving rats show burst firing more frequently than in either of the two *in vivo* immobilized preparations.[17] Pacemaker-like or random firing in dopamine neurons is thought to have a permissive role in initiating movement whereas burst firing is correlated with behavioral arousal and motivation.[48] In addition, burst firing has been inferred to result in more dopamine release per spike than firing in the random or pacemaker-like mode.[19,20,49] Thus, determining the origins of the different firing patterns in dopaminergic neurons is of great interest.

The pacemaker-like pattern is the only one that occurs spontaneously in mature dopaminergic neurons recorded *in vitro*,[21,35] suggesting that afferent inputs play an important role in the control of the firing pattern of nigrostriatal dopaminergic neurons. For this reason afferents to dopaminergic neurons have been studied in order to understand the regulation of dopaminergic neuron activity.

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Abbreviations: C.V., coefficient of variation; IPSP, inhibitory postsynaptic potential; NMDA, *N*-methyl-D-aspartate.

The three principal excitatory afferents to dopaminergic neurons arise from the subthalamic nucleus, the pedunculopontine nucleus and the cerebral cortex,[3,36,40,50,51] and these excitatory amino acid-containing inputs have received special attention with respect to afferent control of dopaminergic neuron firing pattern since pharmacological experiments have shown that *N*-methyl-D-aspartate (NMDA) receptor stimulation and blockade can alter burst firing in dopaminergic neurons.[8,10,32,41,52] (for recent review see Ref. 42) However, these afferents may not be the only relevant inputs for the control of the firing pattern *in vivo*, (e.g., see Refs 10, 14 and 58) and their physiological role in the production of burst firing is not yet clear. The predominant afferents to substantia nigra dopaminergic neurons are, in fact, GABAergic,[4,54] originating from the neostriatum,[25,56] globus pallidus[30,54] and substantia nigra pars reticulata.[12,29,58] Both $GABA_A$ and $GABA_B$ receptors have been identified on dopaminergic neurons,[38,57] and both spontaneous and stimulus-evoked $GABA_B$-mediated inhibitory postsynaptic potentials (IPSPs) have been observed *in vitro*.[6,37] GABAergic cells localized in the pars reticulata receive also GABAergic inputs from neostriatum and globus pallidus.[5,54]

Previous experiments have suggested that changes in $GABA_A$ and $GABA_B$ inputs can differentially affect the firing pattern of dopaminergic neurons *in vivo*. Local application of the $GABA_A$ antagonist, bicuculline, increases the number of neurons that fire in the burst mode[58] or shifts single neurons from a regular or random firing pattern to burst firing,[44] whereas local application of the $GABA_B$ antagonists, saclofen or CGP-55845A, did not increase burst firing but instead caused a modest shift towards the pacemaker-like mode,[44,58] as did lesion of globus pallidus[58] or hemisection posterior to globus pallidus.[15] It was suggested that the bicuculline-induced burst firing was modulated by disinhibition of $GABA_A$ inputs arising from the axon collaterals of the pars reticulata projection neurons whereas the saclofen-induced increase in pacemaker-like firing resulted from disinhibition of $GABA_B$ inputs arising from globus pallidus. In a companion paper[43] we showed that this idea was at least partially incorrect since the inhibition of dopaminergic neurons arising from stimulation of striatum, globus pallidus and pars reticulata *in vivo* all appeared to be mediated predominantly or exclusively by $GABA_A$ receptors, and that the effects of $GABA_B$ antagonists appeared to be presynaptic. In the present experiments we attempted to identify the pathway(s) that mediate the bicuculline-induced burst firing of nigral dopaminergic neurons by recording the activity of substantia nigra dopaminergic neurons during pharmacologically induced inhibition and excitation of globus pallidus. Portions of these results have been presented in abstract form.[7]

EXPERIMENTAL PROCEDURES

Animals and surgical procedures

Fifty-two male Sprague–Dawley rats (225–275 g; Zivic–Miller) were used. Animals were maintained in a controlled environment (22 ± 2°C, 12 h light/dark cycle) with food and water available *ad libitum*. All experiments were carried out in compliance with guidelines set forth in the PHS manual, "Guide for the Care and Use of Laboratory Animals". Rats were anesthetized with urethane (1.3 g/kg, i.p.) and placed in a stereotaxic frame. All wound margins and points of contact between the animal and the stereotaxic apparatus were infiltrated with lidocaine solution (2%) or ointment (5%). The animal's electrocardiogram was monitored continuously on an oscilloscope. In order to minimize pulsation, the atlanto-occipital membrane was punctured to release some cerebrospinal fluid. Body temperature was maintained at 37 ± 1°C with a thermostatically-controlled solid-state heating pad.

Electrical stimulation

Bipolar stimulating electrodes consisting of 100 μm diameter enamel-coated stainless steel wires with tips separated by 100–500 μm were placed in the substantia nigra pars reticulata (from bregma: AP −5.7, 2.0 lateral to the midline and −8.4 from the cortical surface), subthalamic nucleus (from bregma: AP −3.8, 2.4 lateral to the midline and −8.0 from the cortical surface) and neostriatum (from bregma,: AP 0.5, L 3.4 and −4.1 from the cortical surface, with lateral angle of 15° to allow the placement of the infusion cannulae in globus pallidus) in order to antidromically identify globus pallidus neurons (from substantia nigra and/or subthalamic nucleus) and dopaminergic neurons (from neostriatum). In a few cases, a stimulating electrode was implanted in the ipsilateral thalamus (from lambda: AP 5.1, 2.0 lateral to the midline and −6.1 from the cortical surface) for antidromic identification of pars reticulata projection neurons.

Constant current electrical stimuli were generated with a Winston A-65 timer/stimulator and SC-100 constant current stimulus isolator and consisted of single monophasic square wave pulses that ranged from 0.1–2.0 mA at a duration of 500 μs, delivered at 0.67 Hz.

Recordings

Recording electrodes were fabricated from 2.0 mm o.d. capillary tubing (WPI) on a Narishige vertical pipette puller and possessed *in vitro* impedances of approximately 20 MΩ when filled with 1 M NaCl. The electrode impedance was lowered to between 4 and 10 MΩ by passing 500 ms 150 V d.c. pulses (Grass stimulator, model S-48) through the electrode.

Single-unit extracellular recordings were amplified with a Neurodata IR183 preamplifier and displayed on a Tektronix 5113A storage oscilloscope. All data were recorded on magnetic tape for off-line analysis. Dopaminergic neurons were identified by their extracellular waveforms, often characterized by a prominent notch in the initial positive phase and having a duration of 2–5 ms, slow spontaneous activity and long latency antidromic responses evoked from neostriatum that consisted mostly of initial segment only spikes.[13,28,59] The antidromic nature of striatal-evoked responses was determined by collision extinction with spontaneously occurring spikes.[18]

Several non-dopaminergic pars reticulata neurons were tentatively identified as GABAergic output neurons on the basis of their high, regular firing rates (15–50 spikes/s), narrow action potential waveforms and/or antidromic activation from ipsilateral thalamus.[13,28] A small number of

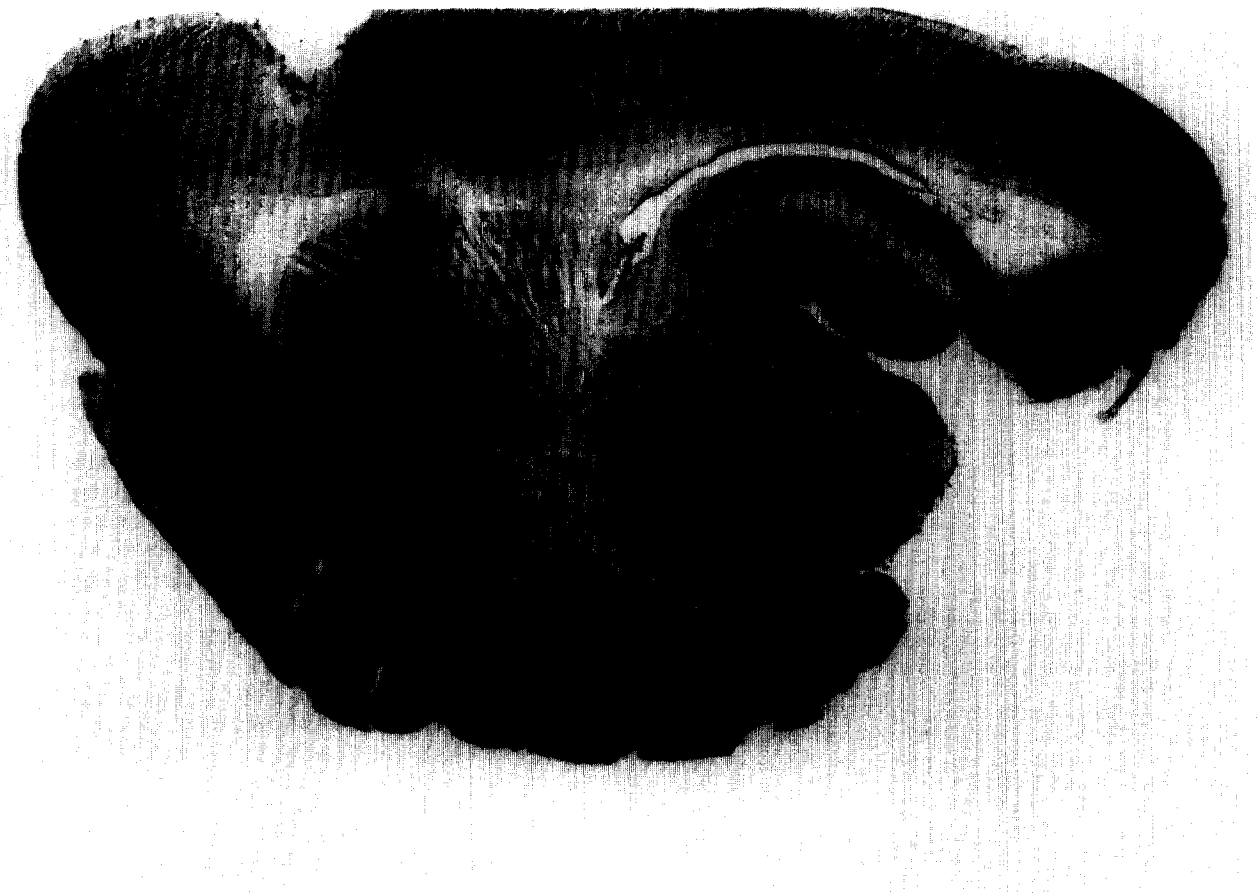

Fig. 1. Digital photomontage of a Nissl-stained parasagittal section illustrating the tract of infusion cannulae with the tip positioned in globus pallidus.

globus pallidus neurons were identified as pallidonigral projection neurons by antidromic activation from substantia nigra.[36]

Globus pallidus drug infusions

Rats were implanted with dual 32-g stainless steel cannulae (Small Parts Inc.) at a frontal angle of 30° in order to allow simultaneous placement of the cannulae and recording electrodes in globus pallidus. The burr hole for the cannulae was drilled at coordinates (with respect to bregma) A: 2.0, and L: 3.2 and the cannulae were advanced 6.7 mm from the surface at 30°. This resulted in the tip of the cannulae being aimed at the approximate center of globus pallidus at A: −1.3 mm, L: 3.2 mm and −5.8 mm from the cortical surface. Each cannula was attached to a 10 μl Hamilton syringe in a Harvard Apparatus syringe pump by a short length of teflon tubing. After recording baseline spontaneous activity, the $GABA_A$ receptor agonist, muscimol (800 μM; Sigma, St Louis, MO) or the $GABA_A$ receptor antagonist, bicuculline methiodide (1000 μM; RBI, Natick, MA), was infused into the globus pallidus over the course of 1 min. All drugs were dissolved in 0.9% sodium chloride and injected at a volume of 200 nl which has been reported to diffuse out to a maximum effective diameter of 0.4–0.6 mm.[39] In some cases a second infusion of drug was administered if the first one had no effect. In several experiments, the effects of sequential infusions of muscimol followed by bicuculline were studied. A maximum of two neurons were used from each rat, one on each side of the brain, in order to avoid residual effects from prior globus pallidus infusions.

Histology

At the end of each experiment rats were given a lethal overdose of urethane and perfused with 0.9% saline followed by 10% buffered formalin. Brains were removed, sectioned and stained with Neutral Red for histological verification of the recording, stimulating and drug infusion sites.

Data analysis

Only neurons from rats found to have the infusion cannulae tip located in globus pallidus (see Fig. 1) were used in the analysis. Changes in the firing rate and pattern were quantified by analysing a 2 min epoch centering around the maximal changes exhibited by a particular neuron following drug infusion and comparing them to a similar 2-min epoch taken within 5 min prior to the onset of the drug infusion. At least 5 min of baseline firing or 1000 spikes were recorded from each dopaminergic neuron before infusing drugs into the globus pallidus. In the case of substantia nigra pars reticulata GABAergic neurons, at least 4 min of baseline was recorded.

Methods for statistical analysis and classification of firing pattern have been previously described.[45,58,66] In brief, autocorrelograms were constructed from 2-min samples of

spontaneous activity. The method for the construction of the autocorrelograms has been described in detail previously.[27] Cells that exhibited three or more regularly occuring peaks in the autocorrelogram were defined as pacemakers, cells that exhibited an initial peak followed by a decay to a steady state were classified as bursty, and the those that displayed an initial trough that rose smoothly to a steady state value were classified as random.[45,58,66] The number of peaks in the autocorrelogram which occurred at integral multiples of the mean interspike interval represented an additional quantitative index of the regularity of firing, as did the coefficient of variation (C.V.), which was calculated as the ratio of the S.D. of the interspike interval divided by the mean interspike interval. To further analyse the structure of burst firing, a computer was programmed to detect bursts, defined as starting with the first interspike interval of 80 ms or less and terminating with the first interspike interval of 160 ms or greater as suggested by Grace and Bunney.[23] The fraction of all action potentials that occurred in bursts containing 2, 3, 4, 5, 6 or more than 6 spikes for each neuron was calculated by dividing the number of spikes within bursts of specified durations by the total number of spikes recorded for each neuron. The proportion of spikes which were fired in bursts that occurred in bursts containing 2, 3, 4, 5, 6 or more than 6 spikes for each neuron was similarly calculated by dividing the number of spikes within bursts of specified durations by the number of spikes that occurred in bursts of any size for each neuron.

Data were analysed using ANOVA for repeated measures or with paired t-tests with statistical significance set at $P<0.05$. Unless otherwise specified, all values are expressed as the mean ± S.E.M.

RESULTS

Pharmacological manipulation of globus pallidus neuron firing rates

In order to verify the ability of the pallidal infusions to modulate globus pallidus neuronal activity, a small number of neurons in globus pallidus was recorded from prior to and after local infusion of bicuculline (200 nl, 1000 μM) and/or muscimol (200 nl, 800 μM). Under control conditions globus pallidus neurons exhibited a mean firing rate of 51.7 ± 13 spikes/s ($n=6$). Local administration of muscimol produced essentially complete suppression of the spontaneous activity of pallidal neurons cells (mean firing rate after muscimol infusion= 3.2 ± 2.9 spikes/s, $n=6$), decreasing the firing rate by over 93%. In contrast, bicuculline infusion increased the firing rate to 80.6 ± 23.2 spikes/s ($n=2$), an increase of 55%. These effects are illustrated for one typical antidromically identified pallidonigral projection neuron in Fig. 2. The latency to reach 50% of the maximal effect was approximately 2.5 min for muscimol administration and 1.5 min for bicuculline administration. These results verified the ability of local infusion of muscimol or bicuculline to effectively modulate pallidal neuron firing rate.

Effects of pharmacological manipulation of globus pallidus on firing rate of dopaminergic neurons

The latency from the onset of pallidal infusion to maximal effects on firing rate of dopaminergic neurons was 3.3 ± 0.3 min for muscimol and 3.8 ± 0.3 min for bicuculline. All firing rate parameters were measured during the 2 min period of maximal effect. The mean spontaneous firing rate of dopaminergic neurons under control conditions was 4.58 ± 0.27 spikes/s ($n=45$). Pallidal infusions of muscimol or bicuculline significantly altered the mean firing rate ($F=22.85$, d.f.=62, $P<0.01$) (Table 1). Muscimol infusions produced a decrease in the firing rate of 20/34 neurons, an increase in firing rate in 1/34 neurons and no change in firing rate in 13/34 neurons. Summing over all neurons, muscimol produced a slight but significant decrease in the mean firing rate of dopaminergic neurons (83.3 ± 2.8% of basal values, $t=-3.94$, d.f.=33, $P<0.01$).

When administered alone, bicuculline produced an increase in firing of 12/12 dopaminergic neurons. When administered after muscimol, 18 of 19 neurons showed an increase in firing rate. Since there were no significant differences in the firing rate increases under these two conditions, the data were pooled. Bicuculline infusion produced a significant increase in dopaminergic neuron firing rate (140.7 ± 19.8% of basal values, $t=3.82$, d.f.=30, $P<0.01$). This is illustrated for one representative dopaminergic neuron in Fig. 3. These data are summarized in Table 1.

Effects of pharmacological manipulation of globus pallidus on firing pattern of dopaminergic neurons

Pallidal infusions of muscimol or bicuculline consistently altered the C.V. of dopaminergic neurons ($F=32.57$; d.f.=2, 61; $P<0.01$). Muscimol-induced inhibition of globus pallidus neurons produced a significant decrease in the C.V. of dopaminergic neurons ($t=-5.16$, $P<0.01$, $n=32$; see Table 1). In contrast, bicuculline-induced excitation of globus pallidus neurons (either alone or after muscimol infusion) produced a significant increase in the C.V. of dopaminergic neurons ($t=5.26$, $P<0.01$, $n=30$). When bicuculline was administered after muscimol, 17 neurons showed an increase in C.V. while two showed no change ($t=4.29$, $P<0.01$, $n=19$). Infusion of bicuculline alone into globus pallidus produced a significant increase ($t=5.86$, $P<0.01$; $n=12$) of the C.V. in all neurons tested.

The number of peaks in the autocorrelograms was also counted. Muscimol and subsequent bicuculline infusions into globus pallidus consistently changed the number of peaks in the autocorrelogram ($\chi^2=13.2$, $n=18$, $P<0.01$) as shown for one typical dopaminergic neuron in Fig. 4. Under control conditions the autocorrelation histogram exhibited an initial trough followed by a steady increase in firing probability that stabilized at a steady state level, typical of neurons firing in the random mode (Fig. 4A). Inhibition of pallidal neurons by infusion of muscimol resulted in the appearance of regularly spaced peaks in the autocorrelation histogram, typifying the regular pacemaker-like mode (Fig. 4B).

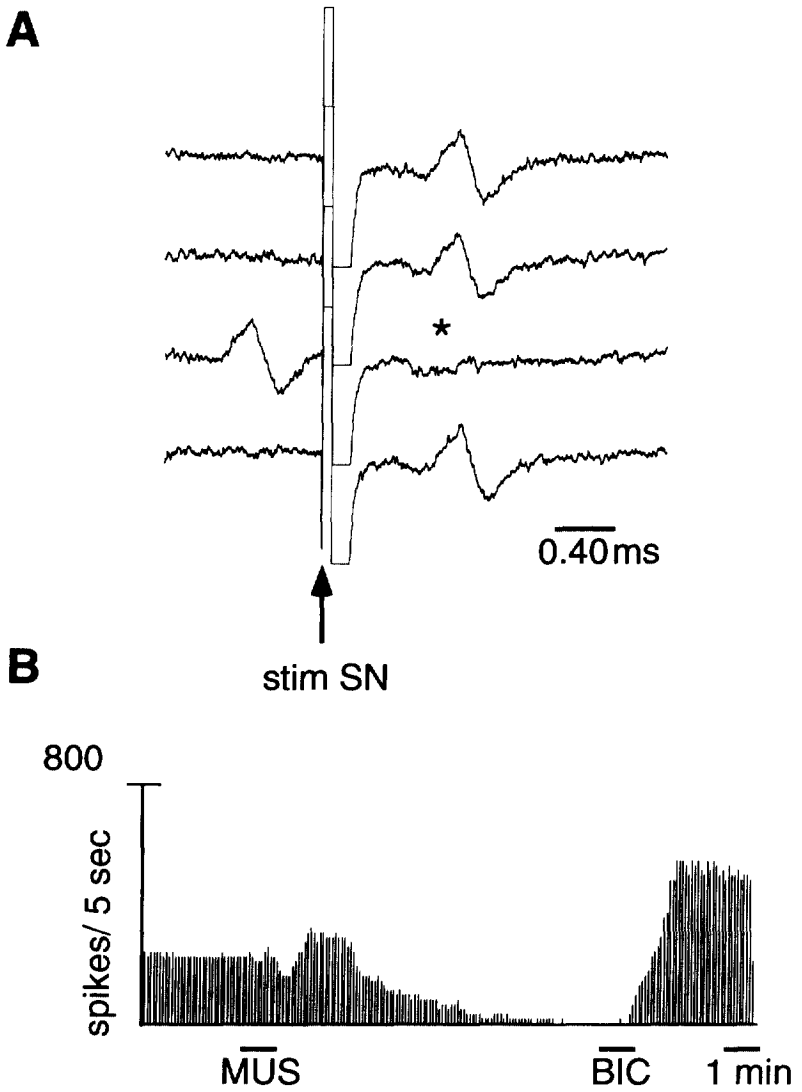

Fig. 2. Effects of pallidal infusion of muscimol (800 μM, 200 nl) and bicuculline (1000 μM, 200 nl) on the spontaneous firing of a pallidonigral neuron. (A) Electrical stimulation of substantia nigra pars reticulata (arrow) evokes a short latency antidromic response. Asterisk denotes antidromic spike missing due to collision with spontaneous action potential. (B) Ratemeter from the same neuron before and during local administration of muscimol (800 μM, 200 nl) and bicuculline (1 mM, 200 nl). Muscimol infusion produced complete cessation of spontaneous activity within several minutes. The inhibitory effects of muscimol were reversed by bicuculline which led to a doubling of firing rate over the pre-drug baseline values.

Subsequent pallidal infusion of bicuculline eradicated the regular firing and caused the autocorrelogram to exhibit an initial trough, followed by a peak which decayed to steady state, typical of the burst firing pattern of dopaminergic neurons (Fig. 4C). A summary of the changes in firing patterns based on analysis of the autocorrelograms is illustrated in Fig. 5.

The effects of manipulation of the firing rates of pallidal neurons on the firing pattern of dopaminergic neurons could also be seen by analysis of the internal structure of the burst firing. Muscimol and subsequent bicuculline infusions into globus pallidus consistently changed the overall number of spikes fired in bursts ($F=17.92$, d.f.$=61$, $P<0.01$). Post hoc analyses showed that the overall proportion of spikes fired in bursts differed significantly between control ($4.6\pm2.0\%$, $n=45$) and bicuculline ($20.7\pm3.7\%$, $n=31$). Although the number of spikes per burst tended to be lower after muscimol ($0.6\pm0.3\%$, $n=34$), this did not reach statistical significance. Figure 6A illustrates a detailed breakdown of the effects of inhibition and excitation of globus pallidus on the proportion of spikes fired in bursts consisting of 2, 3, 4, 5, 6 and more than 6 spikes per burst. Bicuculline produced a significant increase in the proportion of bursts consisting of more than two spikes ($P<0.05$ for all comparisons). When only spikes that occurred within bursts were considered (Fig. 6B), bicuculline was seen to

Table 1. Spontaneous activity of dopaminergic neurons after manipulation of globus pallidus

	Pre-Drug Control	Muscimol in GP	Bicuculline in GP
Firing rate (Hz)	4.58 ± 0.27 (45)	3.91 ± 0.27 (34)*	5.39 ± 0.25 (31)*
% of basal firing rate	100 (45)	83.3 ± 2.84 (34)*	140.7 ± 19.8 (31)*
Coefficient of variation of ISI	0.31 ± 0.02 (45)	0.27 ± 0.02 (34)*	0.48 ± 0.22 (31)*
Number of peaks in autocorrelogram	2.3 ± 0.4 (45)	3.7 ± 0.5 (34)*	1.1 ± 0.2 (31)*
% spikes in burst	4.6 ± 2.0 (45)	0.6 ± 0.3 (34)*	20.9 ± 3.7 (31)*
Number of spikes per burst	3.5 ± 0.6 (24)	3.1 ± 0.7 (9)	4.3 ± 0.5 (29)

Firing rate and pattern of substantia nigra dopamine neurons after pharmacological inhibition and excitation of globus pallidus.
*Significantly different from pre-drug control values. Numbers in parentheses refer to the number of cells measured.
GP, globus pallidus; ISI, interspike interval.

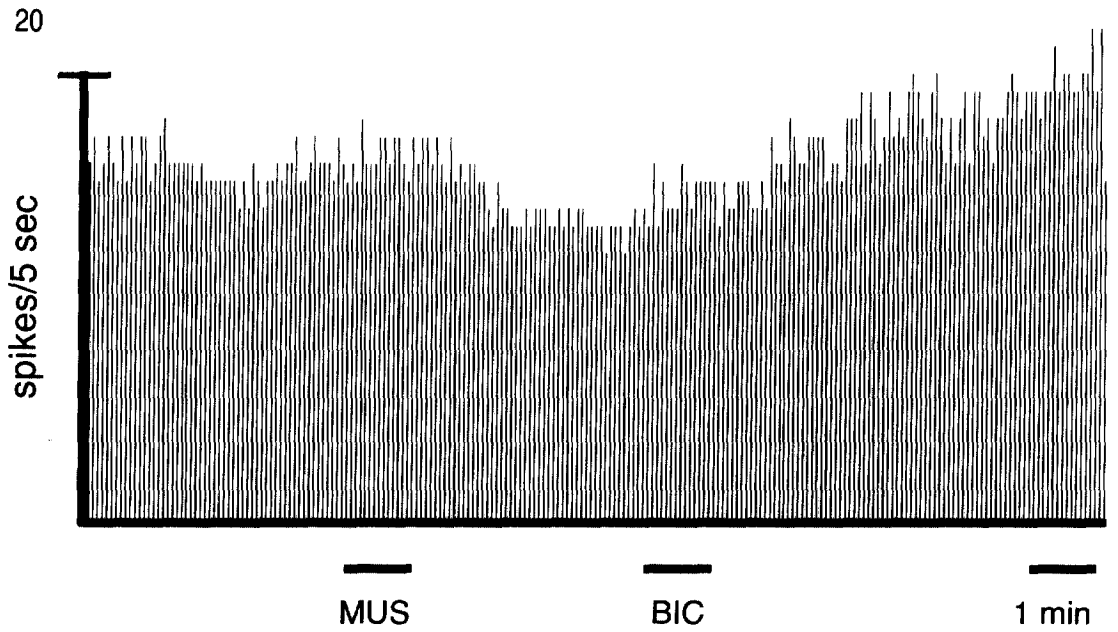

Fig. 3. Effects of pallidal infusion of muscimol followed by bicuculline on the firing rate of a representative dopaminergic neuron. Inhibition of globus pallidus produces a modest but statistically significant reduction in the firing rate of the dopaminergic neuron. Excitation of pallidal neurons by a subsequent local infusion of bicuculline reverses the effect of the prior muscimol infusion leading to an increase in firing rate of the dopaminergic neuron.

significantly decrease the proportion of burst spikes that occurred in two spike bursts while increasing the proportion of burst spikes occurring in bursts consisting of four and six spikes, thus demonstrating that bicuculline-induced bursts tended to consist of more spikes than spontaneously occurring bursts.

Effects of pallidal inhibition and excitation on pars reticulata GABAergic neuron firing

Although there are direct GABAergic projections from globus pallidus to nigral dopaminergic neurons, the fact that inhibition of pallidal neurons produced a slight decrease in the firing rate of dopaminergic neurons rather than the increase that would be expected consequent to inhibition of a monosynaptic GABAergic projection, whereas excitation of pallidal neurons led to a slight increase in dopamine neuron firing rate suggested that these effects may be mediated indirectly. In order to determine if these effects could be mediated through pars reticulata GABAergic neurons, recordings were made from pars reticulata GABAergic neurons while infusing muscimol or bicuculline into globus pallidus.

Under pre-drug control conditions, pars reticulata GABAergic neurons fired at a rate of 29.3 ± 3 spikes/s (n=19). After pallidal drug infusion the delay to reach 50% of the maximum effect in the firing rate was 3.0 ± 0.4 min in the case of muscimol administration and 1.8 ± 0.4 min for bicuculline. Pharmacologically-induced inhibition of globus pallidus neurons caused a large increase in the firing rate of pars reticulata GABAergic neurons to 312.3 ± 29.3% of control values (n=15). This is

illustrated for two representative neurons, one of them antidromically identified as a nigrothalamic projection neuron, in Fig. 7. This effect was antagonized by subsequent excitation of globus pallidus neurons by local infusion of bicuculline, leading to a reduction in firing rate of pars reticulata neurons to 25.5 ± 9.6% of basal values.

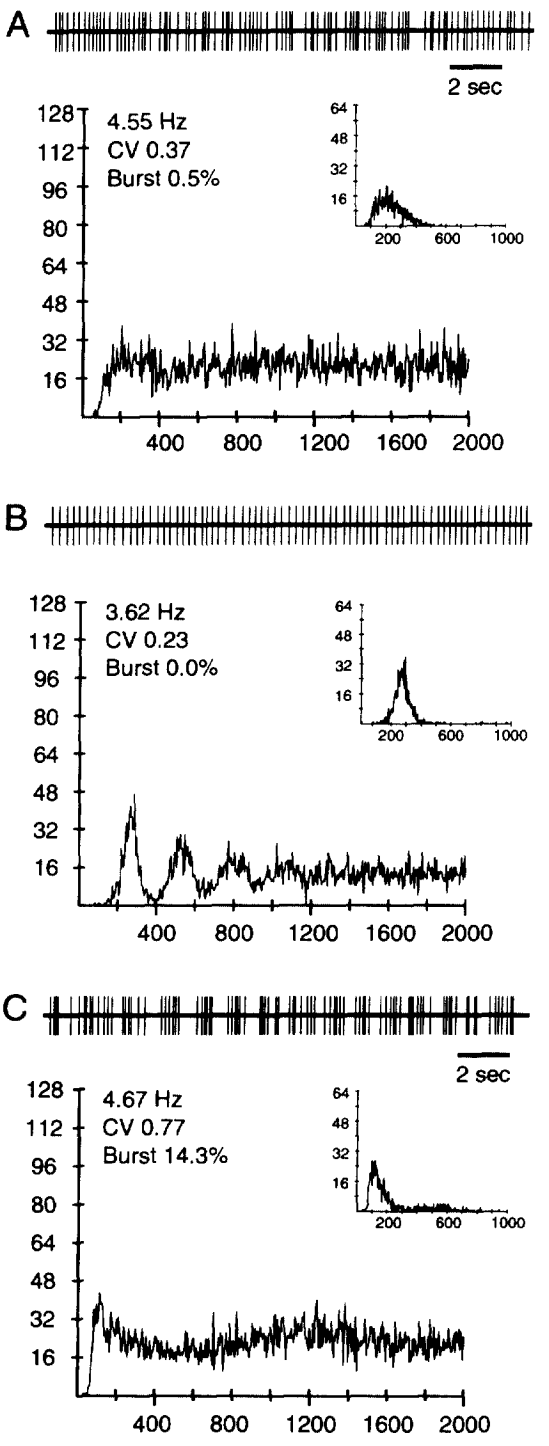

Fig. 4. Effects of decreasing and increasing pallidal activity on the firing pattern of one representative dopaminergic neuron. (A) Spike train (upper panel), autocorrelogram and first order interval histogram (inset) of a typical dopaminergic neuron showing random firing under control conditions. (B) After muscimol-induced inhibition of globus pallidus the firing rate decreased and the firing pattern shifted to the regular, pacemaker-like mode, as can be seen in the spike train (upper panels), the autocorrelogram which now exhibits several peaks at integral multiples of the mean interspike interval and the decreased C.V. (C) A subsequent infusion of bicuculline into the globus pallidus increased the firing rate of the neuron and not only reversed the pacemaker effect of the prior muscimol infusion but shifted the dopaminergic neuron into the burst firing mode, characterized by the single initial peak in the autocorrelogram, the greatly increased C.V., and increased proportion of spikes fired within bursts. Each autocorrelogram and first order interval histogram was constructed from 1000 spikes. Bin width for autocorrelograms was 5 ms, for first order interval histograms, 3 ms. Text insets show mean firing rate, C.V. and percent of spikes fired in bursts for the 1000 spike sample used for the histograms.

DISCUSSION

Globus pallidus output and dopaminergic neuron firing pattern

Inhibition of globus pallidus neurons shifted the firing pattern of dopaminergic neurons towards a regular, pacemaker-like mode whereas excitation of globus pallidus neurons caused dopaminergic neurons to fire in the burst mode. These observations are in agreement with previous findings that kainic acid lesion of the ipsilateral globus pallidus[58] or hemisection just posterior to globus pallidus[15] increased the proportion of dopaminergic neurons that fired in a pacemaker-like mode.

Although there is always the concern in experiments of this kind that anesthesia may have influenced the results, in this case that appears unlikely. Previous studies of pars reticulata and globus pallidus GABAergic neurons revealed no differences in the response to systemic administration of $GABA_A$ agonists or antagonists among chloral hydrate-anesthetized rats or unanesthetized gallamine-paralysed rats. (e.g., see Ref. 63). Similarly, the response of pars reticulata GABAergic neurons to systemically administered apomorphine did not differ among chloral hydrate-anesthetized and unanesthetized rats.[65] Finally, the spontaneous activity of pars reticulata neurons does not appear to be markedly different between anesthetized[13,28] and unanesthetized[33,66] rats. Thus there is no reason *a priori* to think that the relations among globus pallidus, substantia nigra pars reticulata and nigrostriatal dopaminergic neurons in the present study result from an artifact of anesthesia.

The effects of manipulating the activity of globus pallidus on the firing pattern of dopaminergic neurons were accompanied by changes in firing rate that were inconsistent with their mediation by a monosynaptic GABAergic input from globus

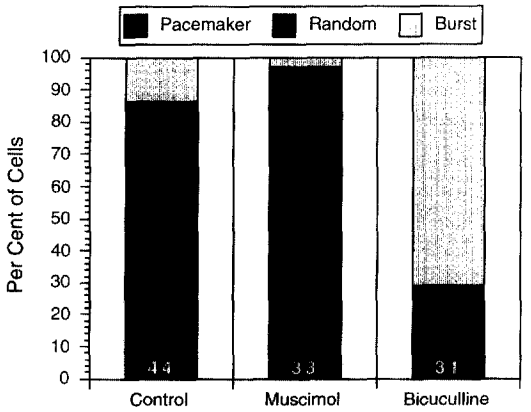

Fig. 5. Distribution of firing patterns of dopaminergic neurons after manipulation of pallidal firing rate by local infusion of muscimol (800 μM, 200 nl) or bicuculline (1 mM, 200 nl). Firing patterns were classified on the basis of the autocorrelation histograms as described in the Experimental Procedures section. Numbers in bars refer to the number of neurons in each condition.

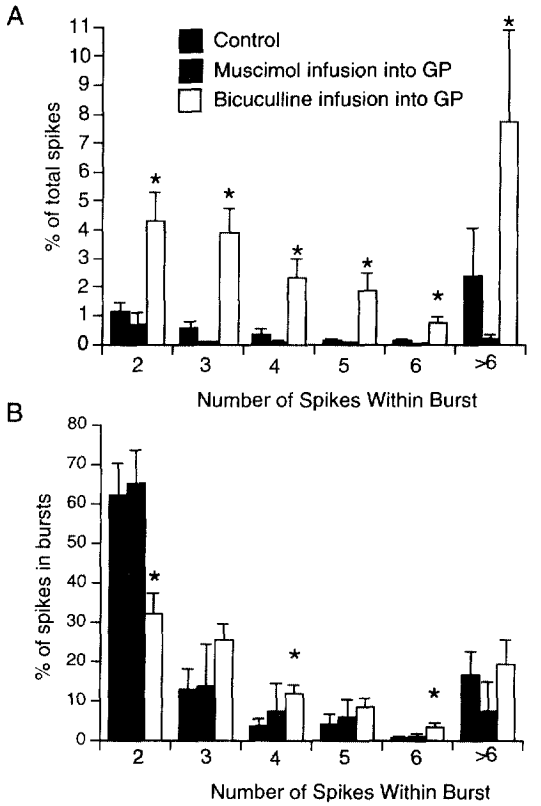

Fig. 6. Detailed description of the structure of bursts in dopaminergic neurons. (A) Bar graph illustrating the proportion of all spikes fired that occurred in bursts of 2, 3, 4, 5, 6 or more than 6 spikes. Note that bicuculline significantly increases the proportion of spikes fired in bursts of all durations, signifying an overall increase in burst firing. (B) Bar graph illustrating proportion of spikes fired within bursts that occurred in bursts of 2, 3, 4, 5, 6 or more than 6 spikes. Note that bicuculline significantly decreased proportion of spikes fired within bursts consisting of 2 spikes while significantly increasing the proportion of spikes fired within bursts consisting of 4 or 6 or more spikes, indicating that bicuculline tended shift the distribution of bursts away from 2 spike doublets and towards longer bursts. Asterisk denotes significantly different from control at $P<0.05$.

pallidus. The firing rate of dopaminergic neurons was reduced slightly by inhibition of globus pallidus and increased slightly by excitation of globus pallidus. Since globus pallidus provides a GABAergic input to dopaminergic neurons[16,30,43] one would expect inhibition of globus pallidus neurons to lead to an *increase* of the firing rate of these dopaminergic neurons and excitation of globus pallidus neurons to lead to a *decrease* in their firing rate. The opposite effects observed suggest that the changes in firing rate and pattern of substantia nigra dopamine neurons after the manipulation of globus pallidus activity were mediated, at least in part, indirectly through another inhibitory input which also receives input from globus pallidus.

Role of subthalamic nucleus

It is conceivable that the effects of manipulating pallidal activity on dopaminergic neuron firing involved the subthalamic nucleus which receives a major input from globus pallidus.[2,34] Several reports have shown potent effects of NMDA receptor stimulation on burst firing of dopaminergic neurons[8,10,32,41,52] and it has been suggested that the subthalamic nucleus may be an important source of NMDA-induced burst firing of dopaminergic neurons. (e.g., see Refs 9 and 53). These data would predict that an increase in subthalamic nucleus activity would be correlated with an increase in burst firing while a decrease would lead to reduced burst firing. Although antidromic activation of subthalamic neurons by pallidal stimulation resulting in an axon reflex activation of substantia nigra remains a possibility,[62] stimulation of the globus pallidus has been shown to inhibit the firing of subthalamic neurons,[34] and thus if the subthalamic nucleus were involved in the pallidal modulation of dopaminergic neuron firing pattern, bicuculline infusions into globus pallidus should reduce burst firing whereas muscimol infusions should increase burst firing. Precisely the opposite effects were seen, suggesting that the observed effects were not mediated through a pallido–subthalamo–nigral pathway.

Role of substantia nigra pars reticulata

Another major target of pallidal output consists of the GABAergic projection neurons in substantia nigra pars reticulata.[54,55] In the present study, substantia nigra pars reticulata GABAergic neurons were strongly excited by inhibition of globus pallidus neurons, whereas excitation of globus pallidus neurons led to a powerful inhibition, in some cases a complete suppression, of spontaneous activity of pars

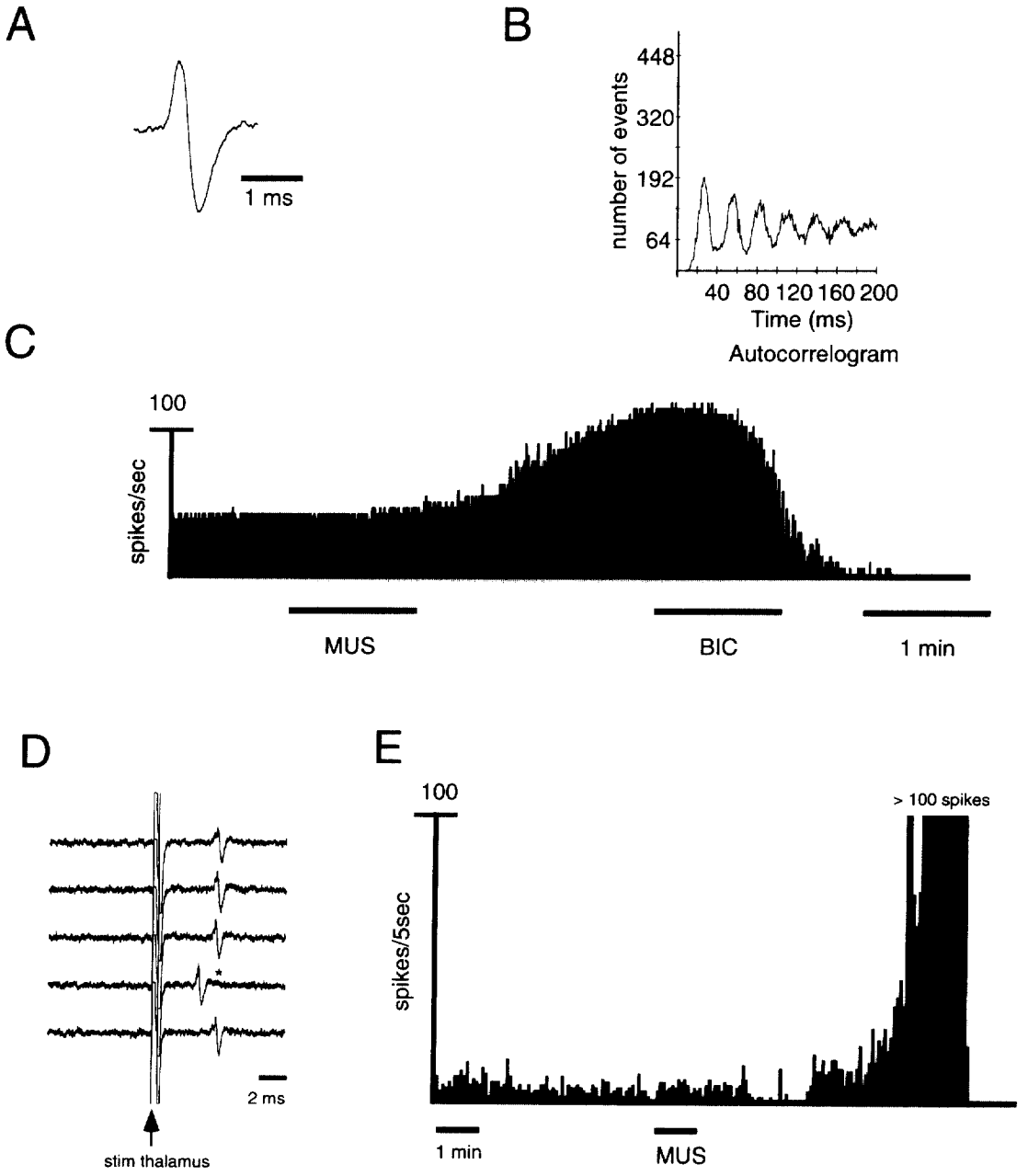

Fig. 7. Effects of manipulating pallidal activity on the spontaneous firing of two representative pars reticulata GABAergic neurons. (A) Extracellularly recorded waveform of a non-dopaminergic pars reticulata neuron. (B) Autocorrelogram showing repeating peaks every 30 ms signifying a regular, pacemaker-like firing pattern typical of pars reticulata GABAergic projection neurons. (C) Ratemeter showing the response of the neuron to inhibition of globus pallidus by infusion of muscimol (800 μM, 200 nl) followed by excitation by bicuculline (1000 μM, 200 nl). (D) Antidromic activation of a nigrothalamic neuron from thalamic stimulation. Note the collision extinction with a spontaneously occurring spike in the fourth trace (asterisk). (E) Ratemeter showing the response of the slowly firing nigrothalamic neuron in D to muscimol infusion (800 μM, 200 nl) into globus pallidus. The graph is truncated at 100 spikes/5 s. Note the extreme sensitivity of the neurons to manipulations of pallidal activity compared with that of the dopaminergic neuron shown in Fig. 3. Bars indicate the timing of the pallidal drug infusion.

reticulata GABAergic neurons. Compared to dopaminergic neurons, pars reticulata GABAergic neurons were far more sensitive to changes in firing rate of pallidonigral neurons. This finding is consistent with previous reports showing that pars reticulata GABAergic neurons are far more sensitive than dopaminergic neurons both to the inhibitory effects of locally and systemically administered GABA

agonists, (e.g., see Refs 22, 58, 63 and 64) as well as the inhibitory effects of striatal stimulation.[24] This inhibition appears to be principally $GABA_A$-mediated.[24,46] Together these data suggest that the effects of the pharmacologically-induced changes in globus pallidus neuron activity on the firing pattern of nigral dopaminergic neurons are mediated indirectly, principally through substantia nigra pars reticulata GABAergic neurons via a decrease in $GABA_A$ mediated inhibition, rather than through the monosynaptic pathway from globus pallidus to nigral dopaminergic neurons.

There is considerable evidence to support this proposal. Early studies suggested a reciprocal relation between the firing of substantia nigra dopaminergic neurons and non-dopaminergic pars reticulata neurons.[22] More recently, monosynaptic inhibition of the dopaminergic neurons by pars reticulata GABAergic projection neurons has been demonstrated. Stimulation of thalamus or tectum *in vivo* produces a short latency $GABA_A$-mediated inhibition of dopamine neurons by activating the axon collaterals of pars reticulata GABAergic projection neurons.[43,58] An inhibitory synaptic connection between pars reticulata GABAergic projection neurons and pars compacta dopaminergic neurons has been shown with *in vitro* intracellular recordings,[29] axon collaterals of intracellularly labeled pars reticulata projection neurons extend throughout pars reticulata and into pars compacta[26] and electrophysiologically identified pars reticulata GABAergic projection neurons have been shown to synapse on dopaminergic neurons in pars compacta.[12]

In the present study the pars reticulata neurons that responded so dramatically to changes in pallidal firing rate were not conclusively identified. However, they appear to be projection neurons based on the ease and frequency with which they were recorded, their high, tonic and regular firing pattern, and their apparent great sensitivity to GABAergic manipulation, characteristics that were shared by the two antidromically nigrothalamic neurons in the present study and by antidromically identified pars reticulata GABAergic projection neurons in other studies.[13,28,67]

Although it is conceivable that the pallidal-induced increases in bursting of dopaminergic neurons involving pars reticulata GABAergic projection neurons are mediated through a polysynaptic nigro–thalamo–cortico–nigral pathway resulting in increased glutamatergic input to dopaminergic neurons, (e.g., see Refs 1, 60 and 61) this seems unlikely given that local application of bicuculline or picrotoxin to single pars compacta dopaminergic neurons, a procedure unlikely to affect a large population of reticulata neurons, exerts exactly the same effects on bursting as does increasing pallidal activity.[43,44,58]

Thus, these data suggest that the modulation of the rate and firing pattern of substantia nigra dopaminergic neurons by pharmacologically-induced inhibition or excitation of globus pallidus is mediated primarily through an indirect pathway involving pallidal inputs to substantia nigra pars reticulata GABAergic neurons. Increases in the activity of pallidonigral neurons disinhibits dopaminergic neurons from a relatively potent and tonic $GABA_A$ inhibition exerted by substantia nigra pars reticulata GABAergic neurons leading to burst firing.[43,44,58] Conversely, decreases in the activity of pallidonigral neurons increases the firing rate of substantia nigra pars reticulata GABAergic neurons which increases $GABA_A$ inhibition on dopaminergic neurons, thereby slowing their firing rate, inhibiting burst firing and promoting pacemaker-like firing.

Pallidonigral circuitry

Although at first it seems difficult to explain why chemical and electrical stimulation of globus pallidus produce opposite effects on the firing or nigrostriatal neurons, this may be explained by differences in the sensitivity of dopaminergic and non-dopaminergic nigral neurons to GABA receptor stimulation. GABAergic pars reticulata neurons appear to be far more sensitive to GABAergic inhibition than dopaminergic neurons.[64] It may be that the chemical stimulation of globus pallidus produces an asynchronous increase in nigral GABA that affects predominantly the more sensitive pars reticulata GABAergic neurons, whereas electrical stimulation produces a large, synchronous burst of GABA release that is strong enough to directly inhibit the dopaminergic neurons. A similar phenomenon has been reported for the response of dopaminergic neurons to striatal stimulation. When striatum is stimulated electrically with single pulse electrical stimulation at moderate or strong intensity (>0.3 mA), the predominant response of dopaminergic neurons is also inhibition.[24,43] However, when trains of stimuli (20–50 μA, 20 Hz) are applied to striatum at very low intensity, there is an excitation of dopaminergic neurons, believed to be mediated through pars reticulata neurons.[24]

Thus, dopaminergic neurons are affected not only by the direct monosynaptic GABAergic pathway from globus pallidus, (e.g., see Refs 43 and 55) but also by a disynaptic pathway which has a pars reticulata GABAergic neuron as an intermediary. Whether the monosynaptic pathway from globus pallidus to nigrostriatal dopaminergic neurons or the disynaptic pathway from globus pallidus to GABAergic pars reticulata neurons to nigrostriatal neurons predominates appears to depend on the timing and/or amount of GABA release in substantia nigra.

The present data may also help to explain the complex responses of dopaminergic neurons to stimulation of the subthalamic nucleus. Although it has been reported that activation of subthalamic nucleus promotes or produces burst firing in

dopaminergic neurons, (e.g., see Refs 9, 47 and 53) careful examination of these data show that the effects of subthalamic stimulation on dopaminergic neurons are mixed, with initial inhibitory effects commonly reported or even predominant. The initial inhibition may result from a polysynaptic loop involving pars reticulata GABAergic neurons, as originally suggested by Feger and colleagues.[47] In addition, since the subthalamic nucleus sends a prominent projection to globus pallidus, and stimulation of subthalamic nucleus exerts mixed effects on pallidal neurons via a monosynaptic excitatory postsynaptic potential and the antidromic activation of a near simultaneous recurrent IPSP,[47] activation of the subthalamo–pallido–nigral loop may account for some of the mixed effects of subthalamic stimulation on dopaminergic neuron firing rate and pattern. In particular, in light of the present results it is conceivable that subthalamic nucleus evoked burst firing in dopaminergic neurons could result, at least in part, through excitation of pallidal output neurons.

CONCLUSION

In summary, changes in the activity of globus pallidus neurons *in vivo* leads to marked changes in the activity of dopaminergic neurons. Increased pallidal activity produces a slight increase in the activity of dopaminergic neurons with marked increases in burst firing. Decreased pallidal activity leads to modest decreases in the firing rate of dopaminergic neurons and a regularization of the firing pattern. These effects appear to be indirect, mediated through pars reticulata GABAergic neurons. and likely involve disinhibition of a tonic $GABA_A$ mediated tone, as discussed elsewhere.[44,58]

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