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PALLIDAL CONTROL OF SUBSTANTIA NIGRA DOPAMINERGIC NEURON FIRING PATTERN AND ITS RELATION TO EXTRACELLULAR NEOSTRIATAL DOPAMINE LEVELS

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Abstract—The firing patterns of dopaminergic neurons *in vivo* are strongly modulated by afferent input. The principal GABAergic inputs to the dopaminergic neurons of the substantia nigra originate from neurons of the neostriatum, globus pallidus and substantia nigra pars reticulata. It has previously been shown that the firing pattern of nigral dopaminergic neurons can be manipulated by pharmacologically induced excitation or inhibition of the globus pallidus with relatively little effect on firing rate. We used this technique to explore the relation between the firing pattern of dopaminergic neurons and extracellular dopamine levels in the neostriatum *in vivo*. Specifically, we tested whether an increase in burst firing in dopaminergic neurons produced by increased pallidal activity led to increased extracellular dopamine levels in the neostriatum. Single unit extracellular recording combined with simultaneous microdialysis was used to measure the firing rates and patterns of dopaminergic neurons and extracellular striatal dopamine levels, respectively, during bicuculline-induced excitation of the globus pallidus. Pallidal excitation resulted in a marked increase in burst firing in dopaminergic neurons along with only a slight increase in firing rate, but produced a significant elevation (approximately 45%) in neostriatal dopamine levels. These data suggest that afferent-induced burst firing in dopaminergic neurons leads to an increase in extracellular dopamine levels in the neostriatum when compared with less bursty patterns with similar overall firing rates.

Key words: burst firing, microdialysis, globus pallidus, $GABA_A$ receptor, bicuculline, pars reticulata.

The spontaneous activity of midbrain dopaminergic neurons exists along a continuum of firing patterns *in vivo*. The continuum comprises a regular, pacemaker-like mode and a bursty mode at each end, and a random firing pattern in the middle, and is similar under general anesthesia, local anesthesia and in unanesthetized freely moving animals (Wilson et al., 1977; Grace and Bunney, 1984a, b; Tepper et al., 1995; Kitai et al., 1999; Hyland et al., 2002; see Diana and Tepper, 2002 for review). The factors responsible for initiating the bursty firing pattern are of particular interest as it has been demonstrated that electrical stimulation of the medial forebrain bundle with trains of stimuli resembling bursts leads to a greater increase in extracellular dopamine levels than regularly spaced stimuli with the same average frequency (Gonon and Buda, 1985; Gonon, 1988; Bean and Roth, 1991; Manley et al., 1992).

The majority of the afferents to substantia nigra dopaminergic neurons are GABAergic (Ribak et al., 1976; Smith et al., 1996). These arise from neurons of the striatum and globus pallidus (Grofová, 1975; Somogyi et al., 1981; Bolam and Smith, 1990; Smith and Bolam, 1990) as well as from axon collaterals of substantia nigra pars reticulata projection neurons (Grofová et al., 1982; Hajós and Greenfield, 1994; Tepper et al., 1995; Saitoh et al., 2004). Blockade of $GABA_A$ receptors on dopaminergic neurons leads to an increased proportion of neurons exhibiting the bursty firing pattern (Tepper et al., 1995) and causes neurons firing in the pacemaker or random firing patterns to shift to the bursty firing pattern (Paladini and Tepper, 1999).

Pharmacological excitation of globus pallidus neurons results in an increase in burst firing in dopaminergic neurons. This effect is particularly robust and results in the great majority of dopaminergic neurons within the substantia nigra firing in the bursty mode regardless of their initial firing pattern (Celada et al., 1999).

These results are opposite to those that would be expected if the globus pallidus were inhibiting the dopaminergic neurons monosynaptically (Paladini et al., 1999). Rather, pharmacological stimulation or inhibition of the globus pallidus appears to affect dopaminergic neurons principally by a disynaptic pathway through the substantia nigra pars reticulata from which inhibitory GABAergic axon collaterals project to dopaminergic neurons of the pars compacta (Tepper et al., 1995, 2002; Celada et al., 1999). Thus, activation of the globus pallidus leads to increased inhibition of substantia nigra pars reticulata projection neurons followed by disinhibition of dopaminergic neurons resulting in an increase in burst firing (Tepper et al., 1995; Celada et al., 1999).

Since in most of the previous studies examining the relation between pattern of firing and dopamine levels different firing patterns were mimicked by electrical stimulation of dopaminergic axons (Gonon and Buda, 1985; Gonon, 1988; Manley et al., 1992; Bean and Roth, 1991; Suaud-Chagny et al., 1991), these results were observed during synchronous discharge by multiple terminals. This sort of synchronous discharge among large numbers of dopaminergic neurons does not occur to any significant extent *in vivo* (Wilson et al., 1977), even in freely moving rats (Hyland et al., 2002). Therefore, the relationship be-

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Abbreviations: A, anterior; CSF, cerebrospinal fluid; CV, coefficient of variation; EDTA, ethylenediaminetetraacetic acid; L, lateral; VTA, ventral tegmental area.

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tween endogenous, afferent-induced burst firing and extracellular dopamine accumulation in the neostriatum remains unclear. In addition, phasic increases in the firing of pallidal neurons occur *in vivo* both from disinhibition (Tremblay and Filion, 1989; Kita and Kitai, 1991) and monosynaptic excitation (Kita and Kitai, 1991; Kita, 1992), but there are no data on how these changes in the activity of neurons in the basal ganglia affect neostriatal dopamine levels. Here we confirm that excitation of globus pallidus leads to burst firing of substantia nigra dopaminergic neurons with only modest increases in overall firing rate and show that this afferent-induced burst firing leads to a significant increase in extracellular dopamine levels in neostriatum. Portions of these data were previously reported in abstract form (Lee et al., 2002).

EXPERIMENTAL PROCEDURES

Animals and surgery

All procedures were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Rutgers Animal Care and Facilities Committee. Every effort was made to minimize the number of animals used and their suffering. A total of 44 adult male Sprague–Dawley rats (Zivic-Miller Laboratories, Pittsburgh, PA, USA) weighing between 200 and 300 g at the time of the experiment were used. Animals were housed individually in plastic shoebox cages with food and water provided *ad libitum* under conditions of constant temperature (21 °C) and humidity (40%). A 12-h light/dark cycle was maintained.

Animals were anesthetized with a mixture of ketamine (80 mg/kg i.p.; Phoenix Pharmaceutical Inc., St. Joseph, MO, USA) and xylazine (12 mg/kg i.p.; Sigma, St. Louis, MO, USA) for microdialysis probe implantation and guide cannula placement. All wound margins were infiltrated with lidocaine solution (2%) and points of contact between the animal and the stereotaxic apparatus coated with Xylocaine ointment (5%). Animals were mounted in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA, USA) in a flat-skull position. For globus pallidus infusions, a 26 ga. guide cannula fitted with an obturator (Plastics One, Inc., Roanoke, VA, USA) was implanted at a frontal angle of 30° at coordinates (with respect to bregma) anterior (A) 2.0 mm and lateral (L) 3.2 mm and lowered 5.7 mm from the surface of the dura. When the internal cannula, which protruded 1 mm from the tip of the guide cannula, was inserted during the experiment, the lumen of the infusion cannula was located in the center of the globus pallidus at A: −1.3 mm, L: 3.2 mm and −5.8 mm ventral from the cortical surface. The guide cannula was held in place with cranioplastic cement (Plastics One, Inc.) that anchored it to a stainless steel skull screw (Plastics One, Inc.).

The microdialysis probe was placed A: 0.5 mm, L: 2.5 mm and lowered 6.5 mm from the dural surface (Paxinos and Watson, 1986). The probe assembly was then fixed to two skull screws and the skull using cranioplastic cement. An additional stainless steel screw was inserted into a burr hole that was positioned over the substantia nigra (from lambda A: 2.1 mm, L: 2.0 mm) for removal on the day of recording.

Microdialysis probe construction

Microdialysis probes of the vertical concentric design adapted from previously described methods (Abercrombie and Finlay, 1991) were constructed for all experiments. Briefly, fused silica capillary tubing (Polymicro Technologies, Phoenix, AZ, USA) was inserted through the wall of polyethylene tubing (PE-10 tubing; Clay Adams, Parsippany, NJ, USA) and into its lumen such that the fused silica capillary tubing extended 10 mm beyond the tip of the polyethylene tubing. A semipermeable microdialysis membrane (molecular weight cutoff=6 kDa; outside diameter=200 μm; Spectrum Laboratories, Rancho Dominguez, CA, USA) was placed over the tip of the fused silica capillary tubing and affixed to the polyethylene tubing with epoxy. The tip of the microdialysis membrane was also filled with epoxy to provide an impermeable seal. The fused silica capillary tubing was fixed in position with epoxy and an impermeable seal was created at the point of entry to the polyethylene tubing. Artificial cerebrospinal fluid (CSF) was perfused through the polyethylene tubing followed by the microdialysis membrane and exited through the fused silica capillary tubing. The exposed portion of the microdialysis membrane was coated with epoxy leaving a 2.0 mm active zone at the end of the probe. Artificial CSF was delivered through a single-channel fluid swivel (Instech Laboratories, Inc., Plymouth Meeting, PA, USA) allowing for free movement of the animal. Microdialysis probes were continuously perfused with artificial CSF (in mM: 147 NaCl, 2.5 KCl, 1.3 $CaCl_2$, 0.9 $MgCl_2$, pH=7.4) at a rate of 1.5 μl/min delivered by a syringe pump (Harvard Apparatus, Holliston, MA, USA). Prior to probe implantation, probes were calibrated to determine *in vitro* recovery rates and probes falling outside of the 10–15% range were excluded. Dialysate values are not corrected for *in vitro* probe recovery.

Extracellular recordings

Simultaneous single unit extracellular recording and *in vivo* microdialysis were conducted at least 18 h after probe implantation. Animals were anesthetized with urethane (1.3 g/kg, i.p.) and body temperature was maintained at 37 °C. Following induction of anesthesia, the screw occluding the craniotomy above the substantia nigra pars compacta was removed and the atlanto-occipital membrane was punctured to drain some CSF and reduce pulsation during recordings.

Recording electrodes were made from 2.0 mm outside diameter borosilicate glass capillary tubing (World Precision Instruments, Inc., Sarasota, FL, USA) and pulled on a Narishige PE-2 vertical pipette puller (Narishige Co., Tokyo, Japan). *In vitro* impedance was lowered to between 5 and 10 MΩ by passing 500 ms 150 V d.c. pulses (Grass stimulator, model S-48; Grass-Telefactor, West Warwick, RI, USA) through the electrode. Single unit extracellular recordings were amplified with a Neurodata IR183 preamplifier (Neuro Data Instruments Corporation, New York, NY, USA) and displayed on a Tektronix 5113A storage oscilloscope (Tektronix, Inc., Beaverton, OR, USA). All data were recorded on magnetic tape for off-line analysis.

Dopaminergic neurons were identified based on well-established electrophysiological criteria (Guyenet and Aghajanian, 1978; Deniau et al., 1978). Only one cell was recorded from most animals, but in some cases (n=6) a second cell was used following recovery of striatal dopamine levels to predrug baseline.

Dialysate collection and neurochemical analysis

Dialysis samples were collected every 5 min after induction of anesthesia on the day of recording throughout the experiment yielding volumes of 7.5 μl/sample. Post-infusion sample collection began 1 min after the end of the infusion to more accurately align the period of neurochemical analysis with the period of maximal change in burst firing. Samples were placed onto dry ice immediately after collection and stored at −80 °C until analyzed. Samples of 5 μl were analyzed for dopamine content by HPLC coupled with electrochemical detection. A Velosep RP-18 column (100×3.2 mm; Applied Biosystems, Inc., Foster City, CA, USA) was used. The mobile phase consisted of 0.1 M sodium acetate buffer, pH 4.1, 0.1 mM EDTA, 1.2 mM sodium octyl sulfate, and 6.5% (v/v) methanol. An electrochemical detector (Waters model 460; Millipore Corporation, Bedford, MA, USA) with an amperometric electrode set at an applied potential of +0.6 mV

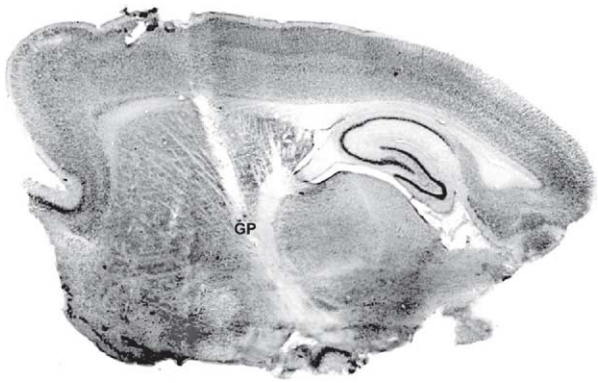

Fig. 1. Cresyl Violet-stained parasagittal section showing typical cannula tract terminating within the globus pallidus (GP).

was used to detect dopamine and metabolites. Mobile phase was delivered at a flow rate of 0.7 ml/min by a solvent delivery pump (model LC-10AD; Shimadzu Corporation, Columbia, MD, USA). Twenty microliters of 10 nM dopamine standard in 0.1 M perchloric acid was used to calibrate the system daily. Retention time was used to identify dopamine which was quantified on the basis of peak height. The limit of detection for dopamine was approximately 0.1 pg/5 μl sample.

Drug preparation and delivery

A 33 g. cannula (Small Parts, Inc., Roanoke, VA, USA) was filled with bicuculline methiodide (1 mM; Sigma, St. Louis, MO, USA) dissolved in 0.9% saline and attached by a length of teflon tubing to a 10 μl Hamilton syringe mounted in a syringe pump. Drug infusions were performed for 1 min following acquisition of baseline electrophysiological activity delivering a volume of 200 nl which has been reported to diffuse to a maximum effective diameter of 0.4–0.6 mm (Myers, 1971). This volume and concentration of bicuculline has been shown to be effective at eliciting excitation of pallidal neurons (Celada et al., 1999). In some cases a second infusion of drug was made if the first had no effect. In control experiments, saline was infused into the globus pallidus.

Histology

At the end of each recording session, rats were given a lethal overdose of urethane and transcardially perfused with 0.9% saline followed by 10% buffered formalin. Brains were removed and stored in 10% buffered formalin overnight, after which 50 μm parasagittal sections were obtained on a freezing microtome and stained with Neutral Red to verify placement of the guide and infusion cannulae as well as the microdialysis probe. Data were used only from animals in which the microdialysis probe was located within the neostriatum and the infusion cannula tip was located in the globus pallidus. Twelve animals were excluded by these criteria. Fig. 1 is a parasagittal section of a representative brain stained with Neutral Red showing the infusion cannula tract terminating in the globus pallidus.

Electrophysiological data analysis

Electrophysiological data were collected on a Macintosh computer outfitted with a National Instruments MIO16L multifunction board (National Instruments, Austin, TX, USA) and analyzed using custom-made software (SpikeTrain). At least 1000 spikes or 5 min of baseline data were collected from each neuron prior to infusion of bicuculline into the globus pallidus. A 2 min epoch of baseline data were used to determine the maximal changes in firing pattern and rate by comparing it to a 2 min epoch centered around the maximal deviation from baseline following drug infusion.

Methods for statistical analysis and classification of firing pattern have been described previously (Perkel et al., 1967; Tepper et al., 1995; Wilson et al., 1977). Autocorrelograms were constructed from the 2 min epochs both prior to and following drug infusion using a bin width of 4 ms for intervals up to 2000 ms and were used to qualitatively classify neurons as firing in the pacemaker, random, or bursty firing pattern. Three or more regularly occurring peaks in the autocorrelogram was characteristic of the pacemaker firing pattern, an initial trough that rose smoothly to a steady state was classified as the random firing pattern and an initial peak followed by a decay to a steady state was characteristic of the bursty firing pattern.

The regularity of the firing patterns was indexed using the coefficient of variation (CV), calculated as the standard deviation of the interspike interval divided by the mean interspike interval. The number of spikes fired in bursts was also counted. Bursts were defined as beginning with the presence of an interspike interval of 80 ms or less and terminating with an interspike interval of 160 ms or greater (Grace and Bunney, 1984b). The percentage of total spikes fired in bursts was calculated by adding the individual fractions of all action potentials occurring in bursts containing two, three, four, five, six, or greater than six spikes which was itself calculated by dividing the number of spikes within bursts of the respective number of spikes by the total number of spikes recorded for that epoch. Additionally, the firing rate was measured for each pre and post-drug epoch. The time of onset of changes in firing pattern and their duration were estimated by examining sequential autocorrelograms which were constructed from 120 s epochs beginning at the onset of bicuculline infusion.

Statistical procedures

All data are presented as mean±S.E.M. Statistical analyses were performed using SAS (SAS Institute, Inc., Cary, NC, USA). Firing rate, CV, and percentage of total spikes fired in bursts were analyzed using paired *t*-tests with the criterion for significance set to $P<0.05$. Dopamine levels were analyzed using one way repeated measures analyses of variance with time as the repeated factor followed by Dunn's multiple comparison test ($P<0.05$).

RESULTS

Effects of pallidal excitation on the firing rate of dopaminergic neurons

Infusion of bicuculline into the globus pallidus produced a slight but significant increase in the firing rate of dopaminergic neurons in the substantia nigra. Dopaminergic neurons exhibited a mean spontaneous firing rate of 4.29±0.26 spikes/s. Following infusion of bicuculline into the globus pallidus, the firing rate decreased in 10/29 neurons and increased in the remaining 19/29 neurons. Overall, the mean firing rate after bicuculline infusion increased to 4.63±0.26 spikes/s, corresponding to an 11.5±4.0% increase in firing rate from baseline ($t=2.13$, $P=0.04$; $n=29$).

Infusion of saline into the globus pallidus at the same volume and rate as used for the infusion of bicuculline had no effect on the firing rate of dopaminergic neurons (4.17±0.32 spikes/s to 4.14±0.33 spikes/s; $t=-0.53$, $P=0.63$, $n=4$) nor did it exert any detectable effect on the firing pattern.

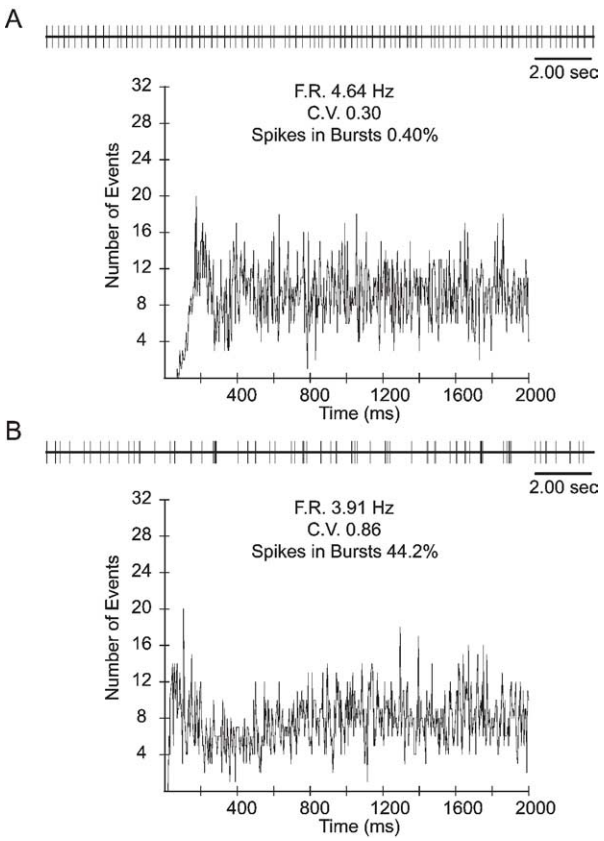

Fig. 2. Pallidal excitation induces burst firing in dopaminergic neurons. (A) Dopaminergic neuron firing in a random pattern under baseline conditions. (B) The same neuron recorded 2 min after infusion of bicuculline into the ipsilateral globus pallidus shows a marked shift to a bursty firing pattern, indicated by the initial peak in the autocorrelogram. Top insets show 20 s samples of the raw spike trains from which each autocorrelogram was constructed. Each autocorrelogram was constructed from 500 consecutive spikes with a bin width of 4 ms.

Effect of pallidal excitation on the firing pattern of dopaminergic neurons

Despite its modest effect on firing rate, bicuculline-induced excitation of the globus pallidus exerted profound effects on the firing pattern of dopaminergic neurons. Fig. 2 shows an autocorrelogram of a representative dopaminergic neuron firing in the random firing pattern under baseline conditions. Following administration of bicuculline into the globus pallidus, the neuron became markedly bursty, showing a large increase in the percentage of spikes fired in bursts and in the CV.

Overall, bicuculline caused a significant shift in the distribution of the firing patterns, with a marked increase in the proportion of neurons exhibiting a bursty pattern (58.6%) as indicated by their autocorrelograms compared with control (13.8%; $\chi^2=13.11$, $df=2$, $P<0.01$). Fig. 3A shows the percentage of cells exhibiting each of the firing patterns under control conditions and following infusion of bicuculline into the globus pallidus. The switch to bursty firing took place with a latency to onset of 3.3±0.3 min from the beginning of drug infusion and had an average duration of 4.3±0.5 min. The CV increased in 25/29 neurons, decreased in 3/29 neurons, and showed no change in one neuron. Overall, the mean CV increased from 0.44±0.05–0.71±0.06 ($t=4.41$, $P<0.01$, $n=29$) as shown in Fig. 3B corresponding to an 83.6±17.95% increase from baseline. The CV was not affected by infusion of saline into the globus pallidus (0.43±0.01–0.41±0.02; $t=-0.99$, $P=0.40$, $n=4$).

The overall percentage of total spikes fired in bursts increased in 26/29 neurons and decreased in 3/29 neurons following pallidal infusions of bicuculline. Dopaminergic neurons under baseline conditions fired 10.5±3.2% of their spikes in bursts compared with 27.3±4.5% following pallidal infusions of bicuculline ($t=4.50$, $P<0.01$, $n=29$). Fig. 3C illustrates the effect of bicuculline infusion on the structure of the bursts. Bicuculline caused an increase in the percentage of total spikes occurring in bursts of two ($t=2.93$, $P<0.01$, $n=29$), three ($t=4.39$, $P<0.01$, $n=29$), four ($t=4.10$, $P<0.01$, $n=29$), five ($t=2.57$, $P=0.02$, $n=29$), and greater than six ($t=3.12$, $P<0.01$, $n=29$) spikes. The percentage of total spikes fired in bursts was not affected by infusion of saline into the globus pallidus (4.26±1.44–3.29±1.50; $t=-0.66$, $P=0.55$, $n=4$).

Of spikes fired in bursts, bicuculline produced an increase in the percentage of spikes fired in bursts composed of three ($t=3.38$, $P<0.01$, $n=29$), four ($t=2.65$, $P=0.01$, $n=29$), and greater than six ($t=3.52$, $P<0.01$, $n=29$) spikes and a decrease in the percentage of spikes fired in two-spike bursts ($t=-3.85$, $P<0.01$; $n=29$) indicating that the bursts got longer following pallidal excitation as shown in Fig. 3D.

Effect of pallidal excitation on striatal dopamine levels

Bicuculline-induced excitation of the globus pallidus resulted in an increase in striatal dopamine levels. Absolute peak levels of dopamine increased from 2.21±0.18 pg/5 μl to 3.15±0.25 pg/5 μl corresponding to a 45.9±7.5% increase ($F_{(7,112)}=8.03$, $P<0.01$, $n=17$). The increase was statistically significant in the first postdrug sample and persisted into the second postdrug sample; however, the greatest increase occurred within the first 5 min sample. Fig. 4 shows mean striatal dopamine levels immediately before and following infusion of bicuculline into the globus pallidus.

The increase in extracellular neostriatal dopamine levels following pallidal bicuculline appeared to depend more on the increase in burst firing than on the modest overall increase in mean firing rate as neostriatal dopamine levels were consistently increased (1.86±0.37 pg/5 μl to 2.81±0.58 pg/5 μl, $n=5$, $F_{(7,28)}=2.83$, $P=0.02$) in experiments in which pallidal excitation led to a decrease in firing rate of dopaminergic neurons (5.27±0.47 Hz to 4.42±0.56 Hz, $n=5$, $t=-3.29$, $P=0.03$) as well as in experiments in which the increased bursting was accompanied by an increase in firing rate (2.24±0.22 pg/5 μl to 3.12±0.30 pg/5 μl, $F_{(7,63)}=5.41$, $P<0.01$, $n=10$ and 4.02±0.56 Hz to 4.83±0.57 Hz, $n=10$, $t=4.01$, $P<0.01$).

Infusion of saline into the globus pallidus had no effect on absolute peak levels of striatal dopamine (2.78±0.10 pg/5 μl to 2.77±0.07 pg/5 μl; $F_{(7,21)}=2.06$, $P=0.20$, $n=4$).

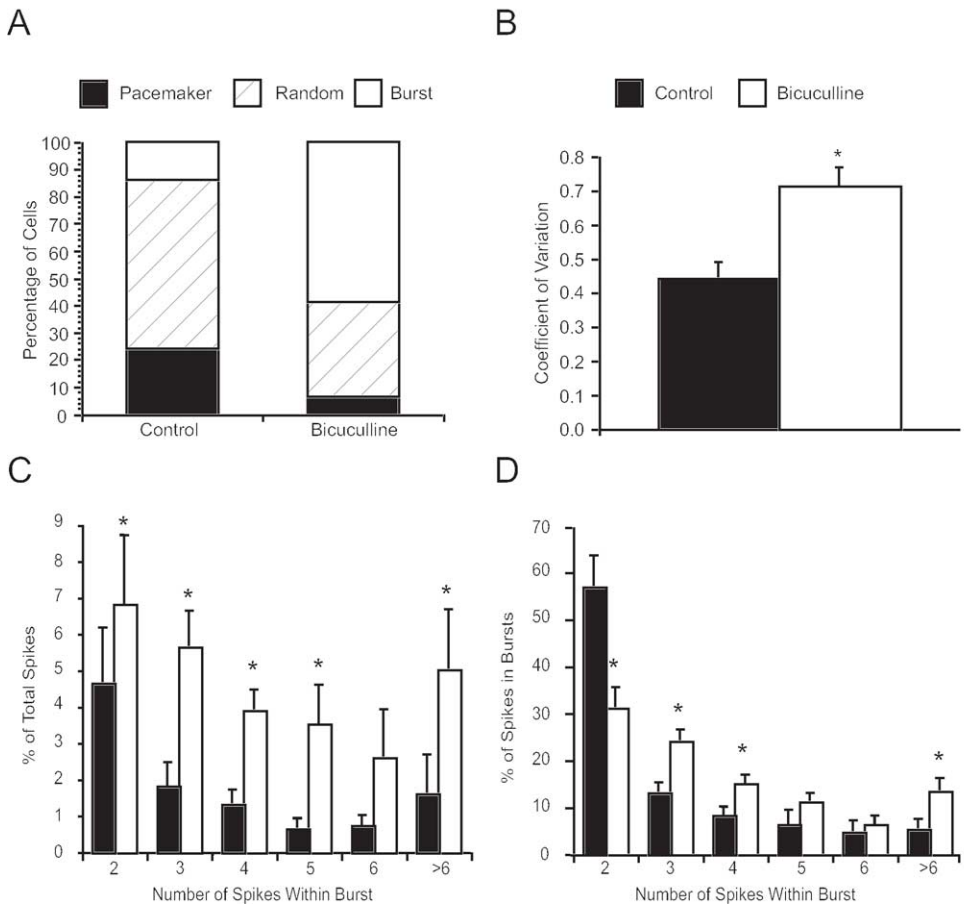

Fig. 3. (A) Infusion of bicuculline into the globus pallidus shifted the overall distribution of dopaminergic neuron firing patterns from one which consisted mostly of the random firing pattern to one where the bursty firing pattern was most prominent. (B) The shift toward the bursty firing pattern was accompanied by a significant increase in the CV. In contrast, bicuculline infusion caused only a modest increase (11.5±4%) in firing rate (not shown). (C) Bicuculline significantly increased the total number of spikes fired in bursts of two, three, four, five, and more than six spikes. (D) Considering only spikes fired in bursts, bicuculline significantly decreased the percentage of spikes fired within bursts comprised of two spikes, while increasing the percentage of spikes fired within bursts of three, four, and more than six spikes indicating that pallidal excitation makes the bursts get longer.

DISCUSSION

The present study confirms that pallidal excitation leads to increased burst firing of substantia nigra dopaminergic neurons as first reported by Celada et al. (1999). The new data extend these findings to show that this endogenously induced burst firing is associated with only a modest increase in firing rate, but nevertheless elicits a significant increase in extracellular neostriatal dopamine levels.

Local application of GABAergic antagonists causes dopaminergic neurons to fire in the bursty pattern independent of the baseline firing pattern (Tepper et al., 1995; Paladini and Tepper, 1999). The switch to burst firing was independent of change in firing rate and sometimes occurred in the presence of a decrease in firing rate (Paladini et al., 1999). Similar local blockade of $GABA_A$ receptors in substantia nigra or ventral tegmental area (VTA) has been shown to result in an increase in terminal area dopamine levels (Santiago and Westerink, 1992; Westerink et al., 1996; Ikemoto et al., 1997). Thus, interruption of GABAergic inputs to nigral dopaminergic neurons leads to burst firing and increases in dopamine release *in vivo*.

Nigrostriatal dopaminergic neuron activity is controlled by pallidal input through monosynaptic and disynaptic pathways

The globus pallidus provides one of the three principal GABAergic inputs to substantia nigra dopaminergic neurons (Smith and Bolam, 1990), the others being the neostriatum (Grofová, 1975; Somogyi et al., 1981) and the axon collaterals of the GABAergic pars reticulata projection neurons (Grofová et al., 1982; Hajós and Greenfield, 1994; Tepper et al., 2002; Saitoh et al., 2004) which have been shown to modulate burst firing in dopaminergic neurons *in vivo* (Tepper et al., 1995; Celada et al., 1999; Paladini et al., 1999). Electrical stimulation of globus pallidus leads to monosynaptic inhibition of nigrostriatal neurons that is mediated predominantly or exclusively by $GABA_A$ receptors *in vivo* (Paladini et al., 1999). However,

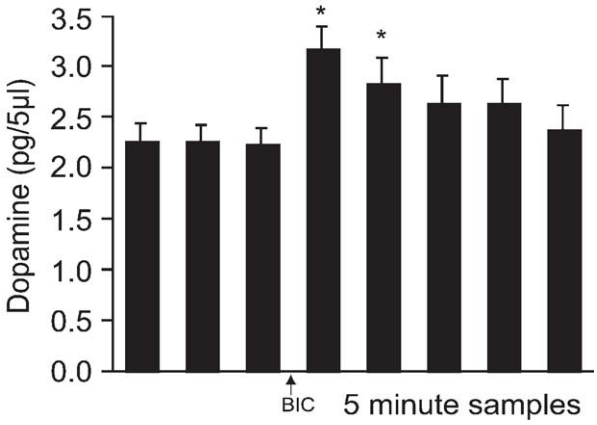

Fig. 4. Striatal dopamine levels after injection of bicuculline into the globus pallidus. The sample collection time was 5 min and a delay of 1 min was introduced following bicuculline infusion to better align the sampling period with the period of maximal increase in burst firing. The first three samples were obtained under baseline conditions during the 15 min immediately preceding a 1 min infusion of bicuculline (BIC) into the globus pallidus as indicated by the arrow. Striatal dopamine levels increased significantly following disinhibition of the globus pallidus in the two 5 min samples immediately following drug infusion and delay (n=17).

chemical manipulation of pallidal activity has a more complex effect on dopaminergic neuron activity. Kainate lesions of globus pallidus did not cause an increase in firing rate or an increase in burst firing of nigral neurons, as would be expected from the destruction of an inhibitory input, but in fact produced a significant decrease in burst firing without affecting mean firing rate (Tepper et al., 1995). Consistent with this, hemisection just posterior to globus pallidus produced essentially identical results, and led to a slight regularization of firing pattern with no significant change in mean firing rate (Engberg et al., 1997).

Subsequent experiments revealed that acutely silencing pallidal neurons by local infusion of muscimol led to a small but statistically significant decrease in firing rate and significant decreases in CV, percentage of spikes fired in bursts and an increase in regularity of firing. Local infusion of bicuculline which led to a 55% increase in pallidal firing rate produced the opposite effects, significantly increasing firing rate, CV and the percentage of spikes fired in bursts (Celada et al., 1999). Exactly the same results were obtained in the present study. In contrast, these same pallidal manipulations produced opposite, and much larger effects on substantia nigra pars reticulata GABAergic neurons (Celada et al., 1999). Together, these data were interpreted to mean that the effects of altering pallidal output on nigrostriatal dopaminergic neuron activity by lesion or drug infusion were mediated principally by a disynaptic loop through pars reticulata, whereas electrical stimulation produced principally monosynaptic inhibitory effects (Celada et al., 1999; Paladini et al., 1999).

The differential effects of chemical and electrical stimulation were explained by the greater sensitivity of GABAergic pars reticulata neurons to $GABA_A$-mediated inhibition than dopaminergic nigrostriatal neurons (e.g. Grace and Bunney, 1979, 1985; Waszczak et al., 1980). At least part of the difference is likely due to different chloride regulatory mechanisms which allow $GABA_A$ receptor stimulation to produce a greater hyperpolarization in the GABAergic neurons than in the dopaminergic neurons (Gulácsi et al., 2003). Other factors contributing to the greater sensitivity of the GABAergic neurons may be a different $GABA_A$ receptor subunit composition (e.g. Rodriguez-Pallares et al., 2000; Schwarzer et al., 2001) and/or a different density of receptors or GABAergic innervation. Regardless, it was hypothesized that electrical stimulation of the globus pallidus led to a large, synchronous release of GABA in substantia nigra that was sufficient to inhibit the dopaminergic neurons monosynaptically whereas chemical stimulation of the globus pallidus led to an asynchronous release that acted principally on the more sensitive pars reticulata neurons to inhibit them and disinhibit the dopaminergic neurons, leading to a bursting firing pattern (Celada et al., 1999).

Endogenous changes in firing pattern of dopaminergic neurons produce changes in extracellular striatal dopamine levels

The present data are consistent with these prior findings, and extend them by showing that pallidal excitation led to a significant increase in extracellular levels of dopamine in the neostriatum under conditions that produced only a very small (11.5%) mean increase in the firing rate of dopaminergic neurons. Almost all of the neurons recorded exhibited an increase in burst firing following excitation of the globus pallidus while only two thirds of the neurons exhibited an increase in firing rate. Thus the increase in extracellular dopamine levels is likely due principally to the change in firing pattern, rather than an increase in firing rate. That the firing pattern of dopaminergic neurons rather than the firing rate is the critical mediator of striatal dopamine levels is further supported by the observation that systemic administration of the $GABA_B$ receptor agonist, γ-hydroxybutyrate, produced a decrease in striatal dopamine levels which was accompanied by a regularization of the firing pattern of dopaminergic neurons without a significant decrease in firing rate (Nissbrandt et al., 1994).

Previous studies have shown supralinear dopamine release in terminal fields following electrical stimulation of the medial forebrain bundle designed to mimic endogenous bursts of dopaminergic neurons (Gonon and Buda, 1985; Gonon, 1988; Bean and Roth, 1991; Manley et al., 1992; Suaud-Chagny et al., 1992; Chergui et al., 1994), and Suaud-Chagny et al. (1992) have shown that local infusions of glutamate agonists into the VTA which increased both firing rate and bursting produced similar supralinear increases in extracellular dopamine levels in the nucleus accumbens. Similar results were reported by Murase et al. (1993) who showed that excitation or inhibition of prefrontal cortex by local injections of glutamate or lidocaine respectively, led to increases or decreases in nucleus accumbens dopamine levels measured with *in vivo* voltammetry. However, the present data are the first to show that manipulation of intrabasal ganglia afferents to substantia nigra which produce transient, endogenous

burst firing without significant alteration of firing rate also lead to increased extracellular levels of neostriatal dopamine.

In a recent study (Floresco et al., 2003), ventral pallidal infusions of bicuculline or muscimol were found to have no effect on mean firing rate or burst firing of dopaminergic neurons in the VTA, although muscimol significantly elevated the number of cells per track, a putative index of the proportion of spontaneously active neurons (for differing views on the interpretation of this measure, see Dai and Tepper, 1998 and West and Grace, 2000). Muscimol infusion led to a significant elevation of dopamine in the nucleus accumbens. In contrast, activation or inhibition of the pedunculopontine nucleus, a source of excitatory afferents to midbrain dopaminergic neurons, produced no change in the cells per track measure or mean firing rate but did significantly increase and decrease respectively burst firing in VTA dopaminergic neurons which was not associated with altered nucleus accumbens dopamine levels. However, when a dopamine uptake inhibitor was used which led to much larger levels of basal dopamine overflow, the effects of pallidal infusions on nucleus accumbens dopamine were unaltered, but the pedunculopontine stimulation now produced a large increase in extracellular dopamine levels. These data were interpreted to mean that *in vivo*, extracellular levels of dopamine are largely insensitive to changes in the firing rate or pattern of dopaminergic neurons and are instead regulated mostly by the number of dopamine neurons that are active at any one time (Floresco et al., 2003).

Insofar as the manipulation of ventral pallidum failed to alter the firing rate or pattern of VTA dopaminergic neurons, the findings of Floresco et al. (2003) are substantially different from ours and those of Celada et al. (1999). However, it should be noted that there are significant differences between the anatomical organization of the VTA and that of the substantia nigra, as well as differences in the organization of afferents. For example, whereas a significant majority of the afferents to nigral dopaminergic neurons is GABAergic, the majority of synapses onto VTA dopaminergic neurons is glutamatergic (Smith et al., 1996). Although a projection from the axon collaterals of GABAergic substantia nigra pars reticulata neurons to nigrostriatal dopaminergic neurons has been well established electrophysiologically (Hajós and Greenfield, 1994; Tepper et al., 1995; Paladini et al., 1999) and anatomically (Grofová et al., 1982; Tepper et al., 2002; Mailly et al., 2003), a similar relationship between the GABAergic and dopaminergic neurons of the VTA is suspected (e.g. Laviolette et al., 2004) but not so well established or characterized. If the GABAergic and dopaminergic neurons of the VTA lack the strong inhibitory relation that exists between the GABAergic and dopaminergic neurons in the substantia nigra (Tepper et al., 1995; Paladini et al., 1999), or express it to a lesser degree, one would expect the monosynaptic inhibitory ventral pallidal–dopaminergic VTA neuron pathway to predominate, as shown by Floresco et al. (2003). However, in the nigrostriatal system, with its preponderance of GABAergic inputs and the strong relation between the GABAergic neurons and the nigral dopaminergic neurons, the disynaptic effect predominates.

Although our data clearly show a relation between firing pattern and extracellular dopamine levels, we cannot rule out the possibility that the pallidal manipulations that produce burst firing in dopaminergic neurons also increase what Floresco et al. (2003) term population activity (the population of neurons that are spontaneously active at any one time). However, evidence against the existence of a large population of silent dopaminergic neurons that underlie the concept of population activity has been presented previously (Dai and Tepper, 1998) which raises issues with a strict interpretation of the cells per track measurement as a reliable index of "population activity." Furthermore, the type of afferent circuitry that could lead to excitation and recruitment of silent dopaminergic neurons without also affecting the firing rate or pattern of spontaneously active neurons as hypothesized by Grace and colleagues (e.g. West and Grace, 2000; Floresco et al., 2003) is not obvious. Regardless, the observation remains that endogenous burst firing, disassociated from increases in firing rate, leads to significant increases in neostriatal dopamine levels.

CONCLUSIONS

We have demonstrated that modulation of the intrinsic circuitry of the basal ganglia by disinhibition of the globus pallidus results in increased dopamine accumulation in the neostriatum. This is the result of increased burst firing in dopaminergic neurons rather than an increase in firing rate per se. Given the synaptic organization and physiological activity of the basal ganglia, disinhibition of the globus pallidus is likely to be an important mechanism mediating nigrostriatal activity and striatal dopamine levels *in vivo*.

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