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The Response of Subthalamic Nucleus Neurons to Dopamine Receptor Stimulation in a Rodent Model of Parkinson's Disease

Deborah S. Kreiss, Christopher W. Mastropietro, Saima S. Rawji, and Judith R. Walters

Experimental Therapeutics Branch, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland 20892-1406

Overactivity in the subthalamic nucleus (STN) is believed to contribute to the pathophysiology of Parkinson's disease. It is hypothesized that dopamine receptor agonists reduce neuronal output from the STN. The present study tests this hypothesis by using *in vivo* extracellular single unit recording techniques to measure neuronal activity in the STN of rats with 6-hydroxydopamine-induced lesions of the nigrostriatal pathway (a model of Parkinson's disease). As predicted, firing rates of STN neurons in lesioned rats were tonically elevated under basal conditions and were decreased by the nonselective dopamine receptor agonists apomorphine and L-3,4-dihydroxyphenylalanine (L-DOPA). STN firing rates were also decreased by the D2 receptor agonist quinpirole when administered after the D1 receptor agonist (±)-1-phenyl-2,3,4,5-tetrahydro-(1H)-3-benzazepine-7,8-diol (SKF 38393). Results of the present study challenge the prediction that dopaminergic agonists reduce STN activity predominantly through actions at striatal dopamine D2 receptors. Firing rates of STN neurons were not altered by selective stimulation of D2 receptors and were increased by selective stimulation of D1 receptors. Moreover, there was a striking difference between the responses of the STN to D1/D2 receptor stimulation in the lesioned and intact rat; apomorphine inhibited STN firing in the lesioned rat and increased STN firing in the intact rat. These findings support the premise that therapeutic efficacy in the treatment of Parkinson's disease is associated with a decrease in the activity of the STN, but challenge assumptions about the roles of D1 and D2 receptors in the regulation of neuronal activity of the STN in both the intact and dopamine-depleted states.

Key words: L-3,4-dihydroxyphenylalanine; apomorphine; 6-hydroxydopamine; (±)-1-phenyl-2,3,4,5-tetrahydro-(1H)-3-benzazepine-7,8-diol; quinpirole; haloperidol; dopamine; D1 receptor; D2 receptor; subthalamic nucleus; Parkinson's disease; basal ganglia; electrophysiology; burst analysis; firing pattern

In Parkinson's disease, the progressive loss of dopamine cells in the substantia nigra pars compacta leads to impaired information processing in the basal ganglia. Specifically, it is thought that parkinsonian pathophysiology results from over-inhibition of the thalamocortical pathway resulting from increased activity of the basal ganglia output structures, the internal globus pallidus and the substantia nigra pars reticulata. The enhanced activity of these output structures in the dopamine-depleted state may be due, in part, to an elevation of the excitatory drive from the subthalamic nucleus (STN) (Miller and DeLong, 1987). In accordance with this hypothesis, procedures thought to reduce subthalamic neuronal output have been found to reverse the behavioral effects of dopamine depletion in rats (Anderson et al., 1992; Blandini et al., 1995; Delfs et al., 1995), primates (Bergman et al., 1990; Aziz et al., 1991; Benazzouz et al., 1993), and humans (Benabid et al., 1994; Limousin et al., 1995; Pollak et al., 1996).

Although neurosurgical treatment has been found to be beneficial for some advanced parkinsonian patients, the prevailing strategy for the treatment of Parkinson's disease is pharmacological. Dopaminergic agonists are predicted to exert a therapeutic effect at the level of the subthalamus by indirectly reducing STN neuronal activity via stimulation of striatal dopamine D2 receptors (Albin et al., 1989; DeLong, 1990). Electrophysiological studies, however, found that dopamine and dopamine receptor agonists actually increased the activity of STN neurons in the intact rat (Mintz et al., 1986; Rouzaire-Dubois and Scarnati, 1987; Kreiss et al., 1996). One explanation for these unpredicted observations is that dopamine receptor stimulation at extrastriatal sites predominately influences the STN. For instance, the STN is modulated by afferents from cortical areas (Afsharpour, 1985; Campbell et al., 1985; Canteras et al., 1990) that are innervated by midbrain dopamine cells (Björklund and Lindvall, 1984; Descarries et al., 1987; Van Eden et al., 1987). In addition, the STN itself receives direct input from dopamine neurons (Meibach and Katzman, 1979; Campbell et al., 1985; Canteras et al., 1990; Hassani et al., 1997). There also is the possibility for a local dopaminergic effect because dopamine receptors exist within the STN (Martes et al., 1985; Bouthenet et al., 1987; Dawson et al., 1988) and local administration of dopamine receptor agonists alters STN neuronal firing rates (Mintz et al., 1986; Rouzaire-Dubois and Scarnati, 1987; Kreiss et al., 1996).

Information on the effects of dopaminergic agonists on STN neuronal activity in the dopamine-depleted state is limited. Dopamine D1 receptor stimulation was found to enhance the expression of c-*fos* in the STN of 6-hydroxydopamine-lesioned rats (Ruskin and Marshall, 1995), suggesting that the activity of STN neurons was increased. 2-Deoxyglucose uptake in the subthala-

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Correspondence should be addressed to Dr. Judith Walters, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Building 10/5C-103, 9000 Rockville Pike, Bethesda, MD 20892-1406.

Dr. Kreiss' present address: University of Central Arkansas, Department of Biology, Lewis Science Center, Conway, AR 72035.

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mus of dopamine-depleted monkeys (Mitchell et al., 1992) and rats (Trugman and Wooten, 1987; Engber et al., 1990) was also found to be increased after administration of dopamine receptor agonists; however, the interpretation of this result is unclear because it could reflect increased metabolism in inhibitory and/or excitatory afferents. Another study reported that the variability in response among STN neurons to the dopamine receptor antagonist haloperidol was increased in lesioned rats (Hollerman and Grace, 1992).

The present study investigated the regulation of STN neuronal activity in rats with 6-hydroxydopamine-induced lesions of the nigrostriatal pathway. We tested the hypotheses that firing rates of STN neurons in the parkinsonian state would be tonically elevated under basal conditions and would be decreased by administration of the nonselective dopamine receptor agonists apomorphine and L-3,4-dihydroxyphenylalanine (L-DOPA). Assumptions about the mechanisms mediating the effects of dopaminergic agonists in the STN were then examined by investigating the neuronal responses to agonists selective for dopamine D1 (D1 receptor family: D_1 and D_5) and D2 (D2 receptor family: D_2, D_3, and D_4) receptors in the STN of both the lesioned and intact rat.

MATERIALS AND METHODS

Materials. Benserazide hydrochloride, L-DOPA methyl ester hydrochloride, (±)-SKF 38393 [(±)-1-phenyl-2,3,4,5-tetrahydro-(1H)-3-benzazepine-7,8-diol] hydrochloride, (+)-SCH 23390 [(*R*)-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-(1H)-3-benzazepine] hydrochloride, and (−)-quinpirole hydrochloride (LY-171555) were obtained from Research Biochemicals, Natick, MA. Apomorphine hydrochloride and 6-hydroxydopamine hydrochloride were obtained from Sigma (St. Louis, MO). Haloperidol was obtained from McNeil Pharmaceutical (Spring House, PA). Gallamine triethiodide was obtained from American Cyanamid Co. (Pearl River, NY). Mepivacaine hydrochloride was obtained from Winthrop Pharmaceuticals (New York, NY). Drug doses refer to the weight of the salts.

Surgical procedures. Standard techniques (Bergstrom et al., 1982; Kreiss et al., 1996) were used to record from STN neurons in male Sprague Dawley rats (300–500 gm; Taconic, Germantown, NY) housed under environmentally controlled conditions and fed laboratory chow and water *ad libitum*. Because general anesthetics have been shown to block effects of dopamine receptor agonists in the STN (Kreiss et al., 1996), electrophysiological recordings in the present study were conducted in locally anesthetized animals. All experiments were conducted in strict accordance with *Guide for Care and Use of Laboratory Animals* from the National Institutes of Health (Cohen et al., 1985). In addition, experimental procedures were designed to minimize animal discomfort. At the beginning of an experiment, rats were anesthetized with halothane, a tracheotomy was performed, and the trachea was intubated with a cannula. Rats were maintained under halothane anesthesia during all surgical procedures. Incision sites and pressure points were thoroughly infiltrated with the long-acting local anesthetic mepivacaine hydrochloride. An ocular lubricant (Lacrilube; Allergan Pharmaceuticals, Irvine, CA) was applied to prevent discomfort caused by corneal drying. Rats were placed into a stereotaxic instrument, and a hydraulic microdrive was used to lower a recording electrode through a small burr hole drilled in the skull over the left STN using the following Paxinos and Watson (1986) coordinates: 5.1 mm anterior to the lambdoid suture, 2.2 mm lateral to lambda, and 6.8–8.0 mm ventral to the dura. Once the surgical procedures were completed, the animal was immobilized with gallamine triethiodide at 16 mg/kg, administered through a lateral tail vein, and artificially respired via the intubated cannula on room air at a rate adjusted to maintain an expired CO_2 of 3.5–4.2%, as measured by a CO_2 analyzer. Additional doses of gallamine were given as needed during the course of the recording session. Body temperature was maintained at 37–38°C using a heating pad and a rectal thermometer.

Extracellular single unit recordings. Extracellular single unit activity of spontaneously firing STN neurons was recorded with single barrel glass microelectrodes filled with 2% Pontamine sky blue dye in 2 M NaCl (Bergstrom et al., 1982). The electrode tips were broken back to a diameter of 1–2 μm. Electrode *in vitro* impedances ranged between 3.0 and 6.0 MΩ (at 135 Hz). Extracellularly recorded action potentials were passed through a high input impedance amplifier and monitored on an oscilloscope and an audiomonitor. All STN neurons recorded exhibited a biphasic (±) waveform. Discriminated signals were stored both on computer disk and chart paper and analyzed using the Rate/Interspike Interval Data Acquisition and Analysis Program for personal computers (Symbolic Logic, Dallas, TX). Only one cell per animal was studied. Electrophysiological recordings in lesioned animals were performed ipsilateral to the site of lesion and conducted 8–16 weeks after lesion. Neurons were identified by their stereotaxic location and by the histological location of the electrode tip after iontophoresis of Pontamine sky blue from the recording electrode (−18 μA for 20 min) at the completion of an experiment.

6-Hydroxydopamine lesion procedure. Rats (250–275 gm) were anesthetized with chloral hydrate (400 mg/kg, i.p.) and mounted in a stereotaxic apparatus. Unilateral 6-hydroxydopamine lesions were placed in the left nigrostriatal pathway at a site just anterior to the substantia nigra and dorsal to the median forebrain bundle through an injection cannula positioned using the following Paxinos and Watson (1986) stereotaxic coordinates: 4.4 mm anterior to the lamboid suture, 1.2 mm lateral to the midline suture, and 8.2 mm ventral to the surface of the skull. 6-Hydroxydopamine (6 μg/3 μl of 0.1% w/v ascorbic acid in 0.9% saline) was injected slowly over a 3 min period. The cannula was left in place for 2 min and then slowly removed. Approximately 4 hr after recovery from anesthesia, the animals were observed for postural deviation and turning behavior; animals with poor responses were eliminated from the study. Rats with successful lesions were identified 4–8 weeks after surgery by demonstrating apomorphine-induced contralateral rotation (Hudson et al., 1993) at a rate of >6 turns/min after a subcutaneous injection of apomorphine at 0.05 mg/kg. Rats included in this study showed an average rotation rate of 9.4 ± 0.3 turns/min (mean ± SEM; $n = 57$). Previous studies from this laboratory have used HPLC to demonstrate that the striatal dopamine level on the lesioned side in these rotation-screened animals was 1–3% of that on the unlesioned side (Pan and Walters, 1988; Carlson et al., 1990; Huang and Walters, 1994, 1996). Electrophysiological studies were conducted in lesioned rats no earlier than 2 weeks after the apomorphine-induced rotation screening.

Drug administration. The effects of systemic administration of drugs was investigated by establishing a basal firing rate over a 4–5 min period and then administering drugs or saline intravenously as a bolus through the tail. The peripheral decarboxylase inhibitor benserazide (50 mg/kg) was administered intraperitoneally at least 40 min before administration of L-DOPA to prevent conversion of L-DOPA to dopamine in the peripheral nervous system. Except for apomorphine that was dissolved in saline (0.9% NaCl), drugs were dissolved in deionized water at varying concentrations such that a volume of 1 ml was injected per kg of rat body weight. SCH 23390 was dissolved in a small volume (50–80 μl) of 0.001 M HCl and then brought up to a final volume of 0.5 mg/ml with deionized water.

Data analysis. Firing rates were expressed as a percent of basal firing and were averaged over 4–10 min after administration of drug or saline, except for the effect of haloperidol that was measured 1–5 min after injection. A change of >30% of basal firing rate was considered a significant alteration for an individual cell, whether it was a drug-induced effect or an antagonist-induced reversal of a drug effect. Differences between experimental groups were determined by calculating the means ± SEM and by analyzing these either using ANOVA at $p < 0.05$ followed by Dunnett's or Newman–Keuls *post hoc* test as noted or using Student's t test (InStat, version 2.04; GraphPad, San Diego, CA).

Analysis of firing pattern of each STN cell under basal conditions was conducted on the 1000 (±10) spike events occurring immediately before drug or saline administration. The coefficient of variation for interspike intervals associated with the 1000 spikes [which provides a measure of the regularity of spike events (see Johnson, 1996)] was calculated by dividing the SD by the mean interspike interval value. The method of Kaneoke and Vitek (1996) was used to compare the "burstiness" of the cells in the intact and lesioned groups. This burst detection method determines the amount of bursting in a spike train by examining the distribution pattern of the discharge of a cell and identifying a burst period as one in which there is a statistically greater number of spikes in comparison with other intervals in the spike train. The spike train is divided into a series of intervals. The number of intervals containing 0, 1, 2, etc. spikes is determined, and a discharge density histogram is constructed showing interval frequency versus discharge density. The histogram is then examined to determine whether its distribution pattern is significantly different from that of a Poisson distribution with a mean of 1 and is positively skewed (χ^2 test set at a significance level 0.05); if so, the

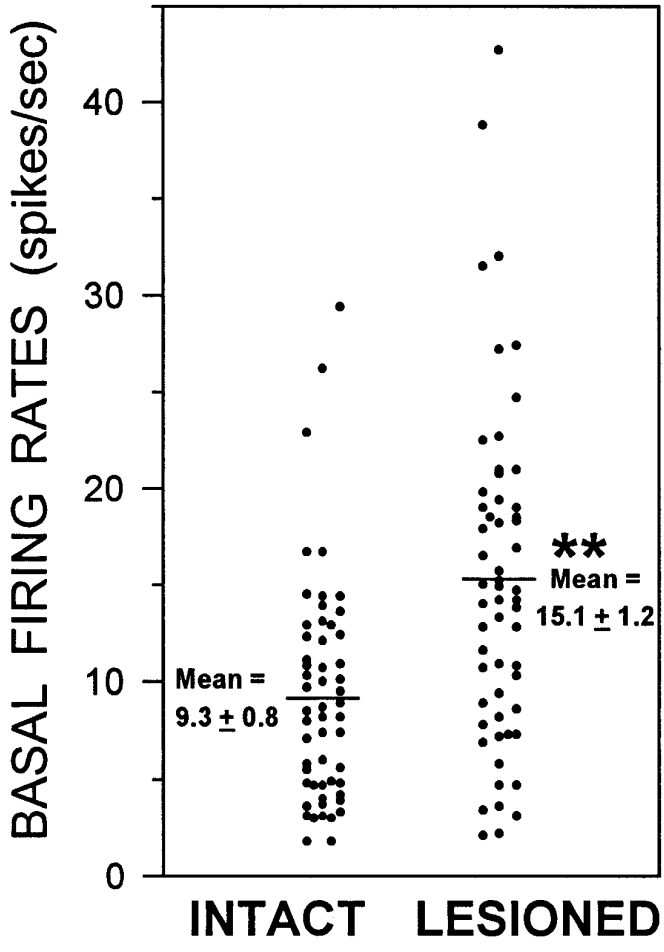

Figure 1. Distribution of basal neuronal firing rates of STN neurons in the intact rat (*left*; $n = 55$) and in the lesioned rat (*right*; $n = 57$). *Solid symbols* illustrate the spontaneous firing rate of individual neurons expressed as spikes per sec. The *asterisks* indicate that the overall mean basal firing rate for STN neurons in lesioned rats was significantly higher than the mean basal firing rate in intact rats ($p < 0.01$).

threshold interspike interval for identifying a burst period is determined by analysis of the slope of the discharge density histogram of each individual cell. The amount of bursting detected is a relative measure dependent on the interval length used to analyze the spike train. For the present study, an interval length equal to the mean interspike interval was used to divide up the spike train of 1000 events. A burst period was identified when more than two interspike intervals that are equal to or less than the threshold interspike interval occur in succession.

RESULTS

STN neuronal firing under basal conditions

STN neurons recorded in intact rats exhibited spontaneous firing under basal conditions. As shown in Figure 1, basal STN neuronal firing rates (averaged over the 4–5 min preceding administration of drug or saline) in intact rats ranged from 1.8 to 29.4 Hz. The overall mean basal firing rate was 9.3 ± 0.8 Hz (mean ± SEM; $n = 55$). Mean basal firing rates of experimental subgroups of intact rats did not differ from one another, as indicated by ANOVA [$F_{(6,54)} = 1.63$; not significant (NS)].

An analysis of firing pattern was conducted on the 1000 spikes preceding administration of drug or saline. Interspike intervals of STN cells from intact rats had a mean value of 164 ± 15 msec ($n = 53$) and a mean coefficient of variation of 1.3 ± 0.1. Figure 2 illustrates representative firing patterns of cells from intact rats with low (*a*), median (*b*), and high (*c*) interspike interval coefficient of variation values. Sixty-two percent of the cells ($n = 33$) from the STN of intact rats were identified as bursting (e.g., Fig. 2*b*,*c*), using the method of Kaneoke and Vitek (1996). For these bursting cells, the mean interspike interval coefficient of variation was 1.5 ± 0.1 and the mean number of bursts per 10 sec was 1.6 ± 0.2. The mean number of bursts per 1000 spikes was 28.8 ± 3.2, the mean number of spikes occurring within a burst was 6.6 ± 0.3, and the mean value of interspike intervals occurring within a burst was 15.6 ± 1.6 msec. For the nonbursting cells ($n = 20$; e.g., Fig. 2*a*), the mean interspike interval coefficient of variation was 0.8 ± 0.1, which was significantly lower than the coefficient of variation for bursting cells ($p < 0.01$), reflecting the fact that nonbursting cells in intact rats had a more regular pattern of firing than did the bursting cells in intact rats.

STN neurons recorded in lesioned rats also exhibited spontaneous firing under basal conditions. In general, basal firing rates of STN neurons were faster in lesioned rats than those observed in the STN of intact rats (see Fig. 1). STN neuronal firing rates in lesioned rats ranged from 2.1 to 42.7 Hz. The overall mean basal firing rate (averaged over the 4–5 min preceding administration of drug or saline) for STN neurons in lesioned rats, 15.1 ± 1.2 Hz ($n = 57$), was significantly faster ($p < 0.01$) than the mean basal firing rate in intact rats (by 62%). Mean basal firing rates of experimental subgroups of lesioned rats did not differ from one another, as indicated by ANOVA [$F_{(5,56)} = 0.21$; NS].

Interspike intervals of STN cells from lesioned rats (as analyzed over the 1000 spikes preceding administration of drug or saline) had a mean value of 114 ± 13 msec ($n = 55$), which is significantly smaller than the value in intact rats ($p < 0.05$). The mean interspike interval coefficient of variation, 1.0 ± 0.05, was also significantly lower than the value in intact rats ($p < 0.05$), indicating that STN cells in lesioned rats have a more regular pattern of firing than do cells in intact rats (see Fig. 2*d–f*). Thirty-four percent of the cells ($n = 19$) from the STN of lesioned rats were identified as bursting (e.g., Fig. 2*f*), which is a smaller percent than was identified from the STN of intact animals. For these bursting cells in the lesioned rat, values of the mean interspike interval coefficient of variation (1.3 ± 0.1), the mean number of bursts per 1000 spikes (21.3 ± 5.5), the mean number of bursts per 10 sec (1.8 ± 0.4), the mean number of spikes occurring within a burst (5.8 ± 0.2), and the mean value of interspike intervals occurring within a burst (17.9 ± 5.6 msec) did not differ from corresponding values for bursting STN cells in intact rats. These results indicate that although lesion of dopamine cells reduced the burstiness of STN cells, dopaminergic lesion did not alter bursting rate or bursting characteristics. Nonbursting STN cells in lesioned rats ($n = 36$; e.g., Fig. 2*d*,*e*) had a mean interspike interval coefficient of variation of 0.9 ± 0.04, which was significantly smaller than the coefficient of variation for bursting cells in lesioned rats ($p < 0.01$).

Response to administration of apomorphine

Administration of the nonselective dopamine D1/D2 receptor agonist apomorphine consistently increased STN neuronal firing rates in the intact rat, as exemplified in Figure 3 (*left top*). Figure 3 (*left bottom*) shows that all of the eight cells examined in the STN of intact rats significantly increased their firing rates (>30% change from basal rate) after administration of apomorphine at

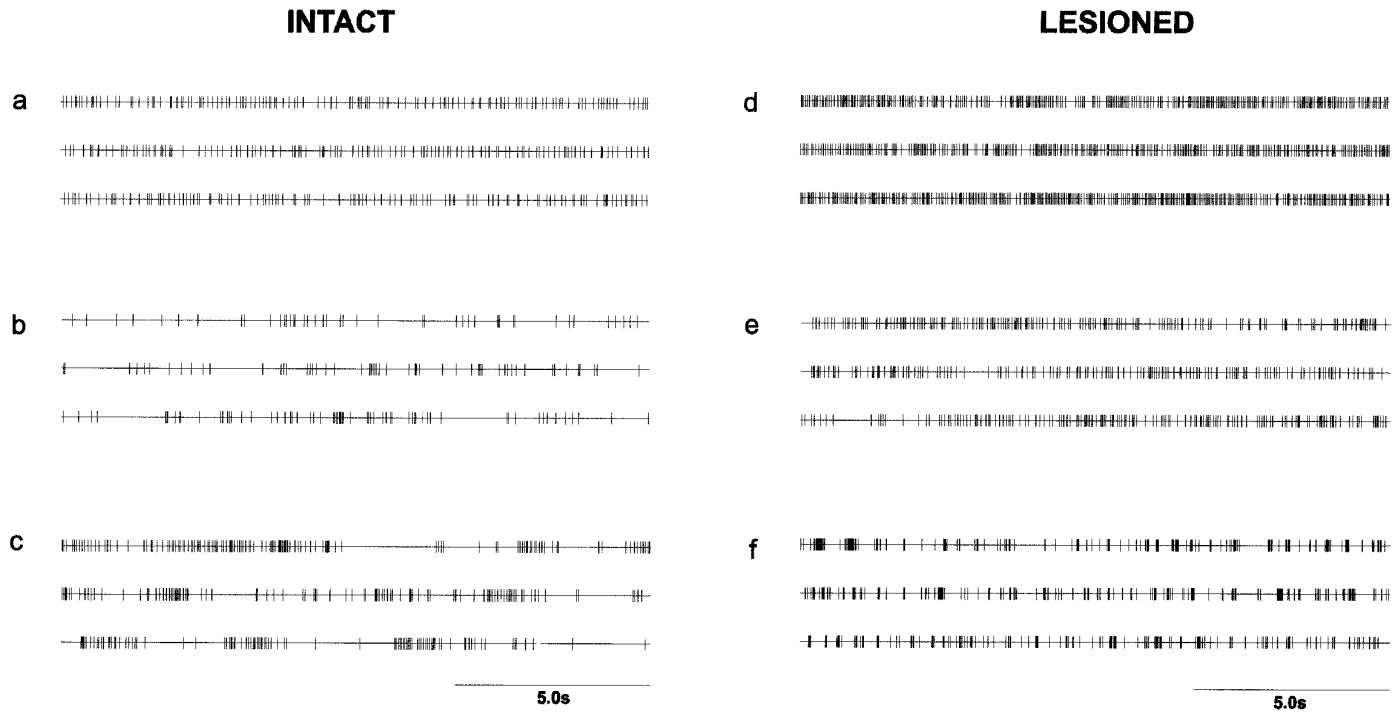

Figure 2. Patterns of firing over a 45 sec period for STN neurons. *Vertical lines* represent spike events. Neurons were selected for presentation to reflect the spectrum of interspike interval coefficient of variation values (a measure of the regularity of spike events) occurring among STN cells from intact (*left*) and lesioned (*right*) rats. The firing patterns of *a* and *d* have low (10th percentile) coefficient of variation values, *b* and *e* have median coefficient of variation values, and *c* and *f* have high (90th percentile) coefficient of variation values. The firing patterns of *b, c,* and *f* are bursting. The firing rates of the presented neurons are as follows: *a*, 8.4 Hz; *b*, 3.8 Hz; *c*, 8.9 Hz; *d*, 21.9 Hz; *e*, 11.7 Hz; and *f*, 14.7 Hz.

0.32 mg/kg. The mean firing rate measured 4–10 min after apomorphine administration (206 ± 24% of basal firing rates; $n = 8$) was significantly different ($p < 0.01$) from the mean rate in intact control rats after administration of saline (101 ± 11% of basal rates; $n = 8$). Only one cell of the eight examined in intact rats after administration of saline showed an alteration (an increase) in firing rate from its basal rate. The dopamine D2 receptor antagonist haloperidol (0.2 mg/kg) reversed the apomorphine-induced increase in firing rate (a change in the apomorphine-induced rate of >30% of basal rate) in six of six cells examined in intact rats (e.g., see Fig. 3, *left top*). Administration of haloperidol (0.2 mg/kg) alone did not alter the mean neuronal firing rate in the STN of intact rats (see Fig. 6, *left*; response was 91 ± 10% of basal rates; $n = 8$), as indicated by ANOVA [$F_{(2,21)} = 1.1$; NS]. On an individual cell basis, haloperidol increased the firing rate of one cell, decreased the firing rate of one cell, and did not alter the firing rates of the other six cells (see Fig. 6, *left*). The excitatory effects of apomorphine on STN neurons in the intact rat and the ability of haloperidol to reverse apomorphine-induced increases in firing rate are consistent with previous observations (Kreiss et al., 1996).

In contrast to intact rats, administration of apomorphine in lesioned rats generally decreased STN neuronal firing rates, as shown in Figure 3 (*right*). The firing rates of seven cells examined in the STN of lesioned rats were decreased by the administration of apomorphine at 0.32 mg/kg; the firing rate of one cell was increased and of one cell was not altered, as shown in Figure 3 (*right bottom*). The mean response to apomorphine in the STN of lesioned rats was a decrease to 59 ± 16% of basal firing rates ($n = 9$), which significantly differed ($p < 0.01$) from the response to apomorphine in the STN of intact rats. The mean response to apomorphine in lesioned rats was not statistically different from the mean response to saline in lesioned rats (100 ± 9% of basal rates; $n = 7$). However, whereas eight of nine cells in lesioned rats showed an alteration in firing rate after apomorphine administration, only one of seven cells did so after saline administration (a decrease). Haloperidol (0.2 mg/kg) reversed apomorphine-induced decreases in STN neuronal firing rates in five of six cells examined in lesioned rats (e.g., see Fig. 3, *right top*) but did not reverse the apomorphine-induced increase observed in the one cell. Administration of haloperidol (0.2 mg/kg) alone did not alter either individual cell responses or the mean firing rate of STN neurons in lesioned rats (97 ± 6% of basal rates; $n = 9$), as indicated by ANOVA [$F_{(2,21)} = 0.46$; NS] and shown in Figure 6 (*right*).

Response to administration of L-DOPA

Administration of the dopamine precursor L-DOPA in intact rats pretreated with a decarboxylase inhibitor did not alter the mean neuronal firing rate in the subthalamus, as shown in Figure 4 (*left*). Of the eight cells examined in intact rats, three did not change, three decreased, and two increased their firing rates after administration of L-DOPA at 100 mg/kg (see Fig. 4, *left bottom*). The mean response to L-DOPA (91 ± 14% of basal rates; $n = 8$) in intact rats did not differ from the response in intact, saline-treated control rats. Haloperidol (0.2 mg/kg) reversed decreases in STN neuronal firing rates induced by L-DOPA in two of three cells and reversed increases in two of two cells examined in intact rats.

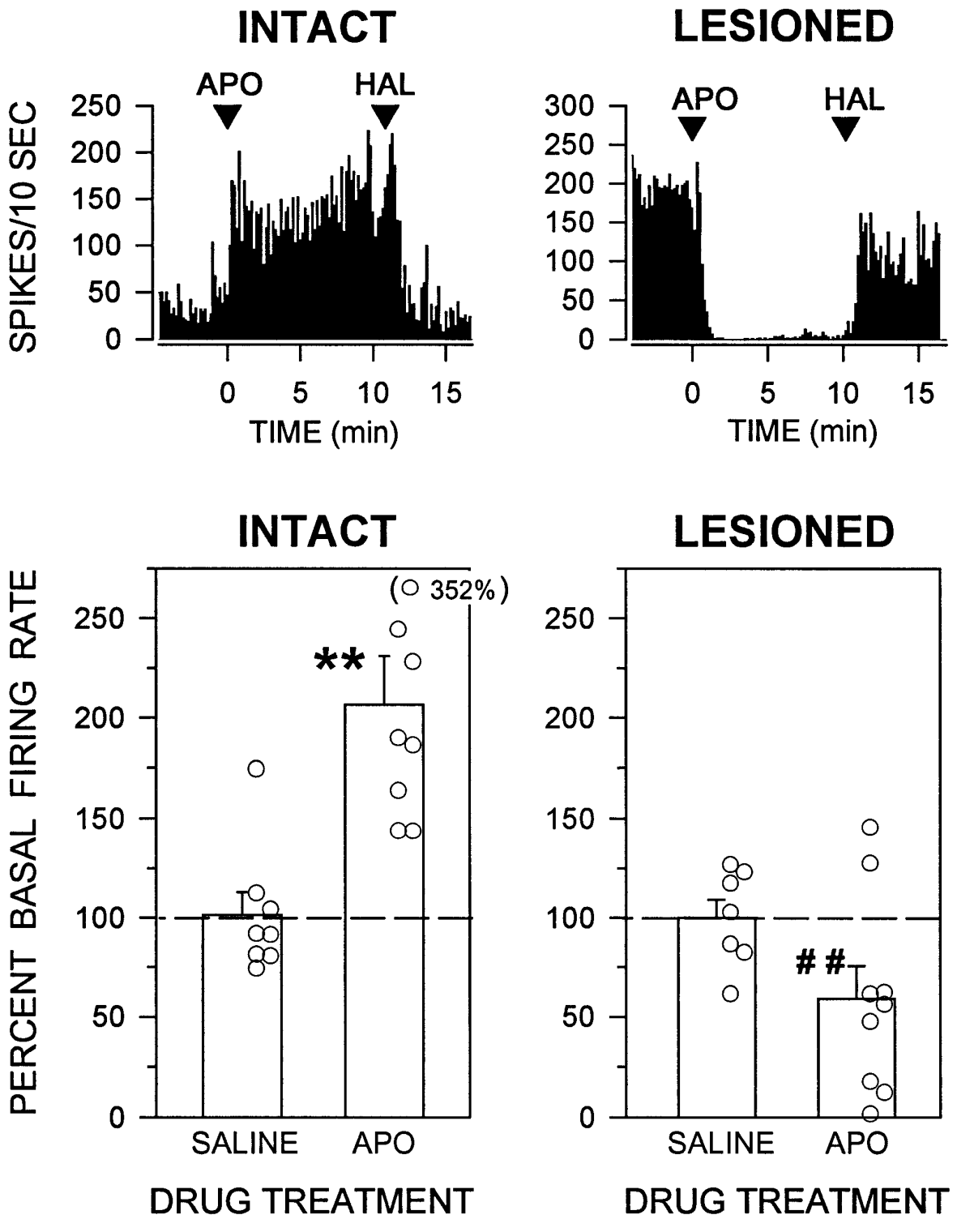

Figure 3. Effects of apomorphine on firing rates of STN neurons in the intact (*left*) and lesioned (*right*) rat. *Top*, Histograms, each illustrating the effects of apomorphine (*APO*; 0.32 mg/kg, i.v.) on a single STN neuron. Haloperidol (*HAL*; 0.2 mg/kg, i.v.) reversed the effects of apomorphine in these two cells. *Arrows* indicate the time at which the drug was administered. *Bottom*, The mean response (bar height), SEM (error bar), and individual responses (*open symbols*), all expressed as a percent of basal values, after administration of saline or apomorphine (0.32 mg/kg, i.v.). The *data point* in *parentheses* indicates a value that exceeds the scale of the *y*-axis. For reference, the *dashed line* indicates 100% of the basal firing rate. The *asterisks* indicate a significant difference from the saline-treated intact group, and the *number signs* indicate a significant difference from the apomorphine-treated intact group ($p < 0.01$).

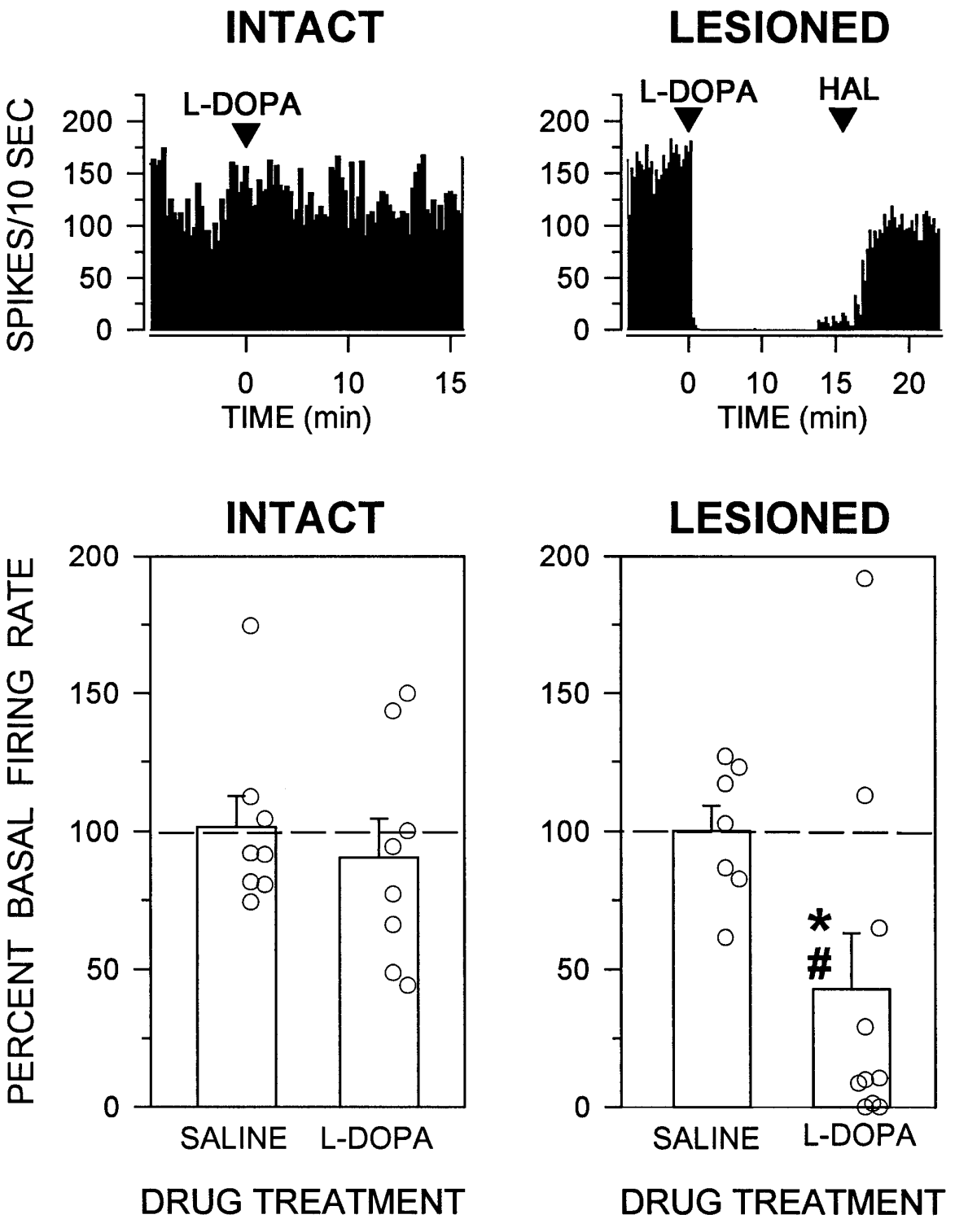

Figure 4. The effects of L-DOPA on firing rates of STN neurons in benserazide-pretreated intact (*left*) and lesioned (*right*) rats. *Top*, Histograms, each illustrating the effects of L-DOPA (100 mg/kg, i.v.) on a single STN neuron. Haloperidol (*HAL*; 0.2 mg/kg, i.v.) reversed the effects of L-DOPA in the lesioned rat. *Arrows* indicate the time at which the drug was administered. *Bottom*, The mean response (bar height), SEM (error bar), and individual responses (*open symbols*), all expressed as a percent of basal values, after administration of saline or L-DOPA (100 mg/kg, i.v.). For reference, the *dashed line* indicates 100% of the basal firing rate. The *asterisk* indicates a significant difference from the saline-treated lesioned group ($p < 0.05$), and the *number sign* indicates a trend toward a significant difference from the L-DOPA-treated intact group ($p = 0.057$).

In contrast to intact rats, administration of L-DOPA in lesioned rats pretreated with the decarboxylase inhibitor benserazide produced dramatic decreases in STN neuronal firing rates, as shown in Figure 4 (*right*). L-DOPA (100 mg/kg) fully inhibited the firing of six cells (rate decreases of >90% of basal rates), decreased firing by a more moderate degree in two cells, did not change the firing in one cell, and increased the firing in one cell. The mean firing rate of STN neurons in lesioned rats after L-DOPA administration was 43 ± 20% of basal firing rates ($n = 10$). This decrease in rate was significantly different ($p < 0.05$) from the mean response in lesioned control rats after administration of saline. In addition, the mean response to L-DOPA in the lesioned rat showed a trend (Student's t test, $p = 0.057$) toward being significantly different from the mean response to L-DOPA in intact rats. Haloperidol (0.2 mg/kg) reversed the L-DOPA-induced decrease in STN neuronal firing rates in six of eight cells examined in lesioned rats (e.g., see Fig. 4, *right top*).

Response to administration of SKF 38393

Administration of the dopamine D1 receptor agonist SKF 38393 increased STN neuronal firing rates in the intact rat, as shown in Figure 5 (*left*). SKF 38393 at 20 mg/kg increased the firing rates of eight cells and did not alter the firing rate of one cell. A lower dose of SKF 38393 (10 mg/kg) increased the firing rates of four cells and did not alter the firing rates of four cells. The mean firing rate of STN neurons in intact rats after the higher dose (20 mg/kg) was 239 ± 37% of basal rates ($n = 9$), whereas the mean rate after the lower dose of SKF 38393 (10 mg/kg) was 165 ± 23% of basal firing rates ($n = 8$). Only the higher dose of SKF 38393 produced a significant increase in the mean firing rate of STN neurons in the intact rat compared with saline-treated controls, as determined by ANOVA [$F_{(2,24)} = 6.5$; $p < 0.01$] followed by Dunnett's *post hoc* test ($p < 0.01$). The dopamine D1 receptor antagonist SCH 23390 (0.5 mg/kg) reversed SKF 38393-induced increases in firing rate in five of seven cells examined in intact rats (e.g., see Fig. 5, *left top*). The excitatory effects of SKF 38393 on STN neurons in the intact rat and the ability of SCH 23390 to reverse SKF 38393-induced increases in firing rate are consistent with observations from a previous study (Kreiss et al., 1996). In addition, previous observations (Kreiss et al., 1996) have demonstrated that administration of SCH 23390 (0.5 mg/kg, i.v.) alone did not alter the mean neuronal firing rate in the STN of intact rats.

Administration of SKF 38393 also increased STN neuronal firing rates in the lesioned rat, as shown in Figure 5 (*right*). Whereas SKF 38393 at 10 mg/kg did not significantly alter the mean firing rate of STN neurons in the intact rat, this dose did significantly increase ($p < 0.01$) the firing rates of STN neurons in the lesioned rat (compared with saline-treated control lesioned rats) to a mean of 220 ± 35% of basal rates ($n = 16$). Of the 16 cells examined in the lesioned rat, 10 cells increased, 1 cell decreased, and 5 cells did not alter their firing rates after administration of SKF 38393 at 10 mg/kg. In these lesioned rats, the dopamine D1 receptor antagonist SCH 23390 (0.5 mg/kg) reversed SKF 38393-induced increases in firing rate in five of the five cells examined (e.g., see Fig. 5, *right top*) and reversed a SKF 38393-induced decrease in one cell. Administration of SCH 23390 (0.5 mg/kg, i.v.) alone did not alter the mean neuronal firing rate in the STN of lesioned rats (response was 89 ± 3% of basal rates; $n = 5$; data not shown).

Response to administration of SKF 38393 after pretreatment with haloperidol

The possible role of endogenous D2 receptor tone in the expression of D1 receptor-mediated effects was investigated by administering the dopamine D2 receptor antagonist haloperidol 5 min before administration of SKF 38393 (data not shown). Pretreatment with haloperidol (0.2 mg/kg) significantly attenuated the ability of the dopamine D1 receptor agonist (20 mg/kg) to increase the mean firing rate in intact rats. After haloperidol pretreatment, SKF 38393 increased the firing rates of only three of nine STN cells examined in the intact rat, decreased the firing rate of one cell, and did not alter the firing rates of five cells. The mean response in the STN of intact rats to SKF 38393 (20 mg/kg) after pretreatment with haloperidol was 117 ± 15% of pre-SKF 38393 rates ($n = 9$), which differed significantly ($p < 0.01$) from the response after SKF 38393 (20 mg/kg) administration without haloperidol pretreatment.

Pretreatment with haloperidol in the lesioned rat did not significantly block the ability of SKF 38393 to alter STN neuronal firing rates (data not shown). After pretreatment with haloperidol (0.2 mg/kg), SKF 38393 (10 mg/kg) altered the firing rates of six of nine cells examined in lesioned rats: three cells increased and three cells decreased their firing. The mean neuronal firing rate after SKF 38393 administration with haloperidol pretreatment in lesioned rats was 131 ± 41% of pre-SKF 38393 rates ($n = 9$), which was not significantly different from the response after SKF 38393 administration without haloperidol pretreatment. In these experiments involving haloperidol pretreatment, SCH 23390 (0.5 mg/kg) reversed SKF 38393-induced firing rate increases in two of three cells and reversed SKF 38393-induced firing rate decreases in one of three cells examined.

Response to administration of quinpirole

Administration of the dopamine D2 receptor agonist quinpirole alone did not alter neuronal firing rates, on average, in the STN of either intact or lesioned rats, as shown in Figure 6. The mean neuronal firing rate after administration of quinpirole alone in intact rats (116 ± 14% of basal rates; $n = 6$) was not different from the mean of saline-treated controls, as indicated by ANOVA [$F_{(3,27)} = 1.27$; NS]. Of the six cells examined in the STN of intact rats, only one cell had a firing rate that was altered (increased) by administration of quinpirole at 0.26 mg/kg. In lesioned rats, the mean STN neuronal response to administration of quinpirole at 0.26 mg/kg (87 ± 14% of basal rates; $n = 6$) did not differ from the response in the corresponding saline-treated control group (see Fig. 6, *right*). Of the six cells that were examined in the lesioned rat, only two had firing rates that were altered (decreased) by quinpirole.

Response to administration of quinpirole after pretreatment with SKF 38393

Because concurrent stimulation of dopamine D1 and D2 receptors by apomorphine had a significant effect on STN neuronal firing in both intact and lesioned rats, the effects of stimulating dopamine D2 receptors after previous stimulation of dopamine D1 receptors were examined. When administered 10 min after SKF 38393 in intact animals, quinpirole did not significantly alter the mean firing rate of STN cells (see Fig. 6, *left*). In a subset of the previously described group of intact animals treated with SKF 38393 (20 mg/kg, i.v.), a dose of quinpirole (0.16 mg/kg, i.v.) was administered. In this subset of animals ($n = 6$), the mean firing

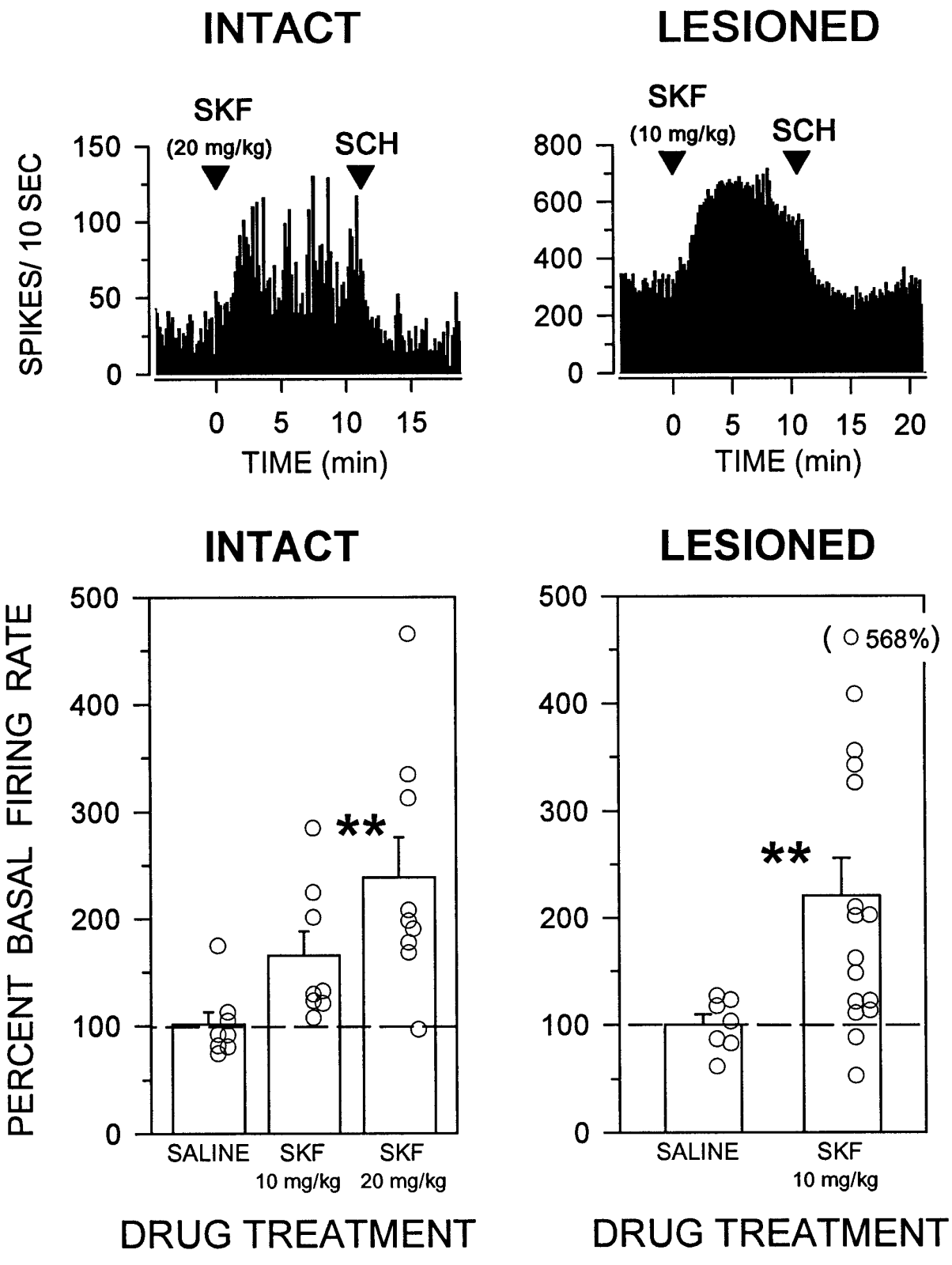

Figure 5. The effects of SKF 38393 on firing rates of STN neurons in the intact (*left*) and lesioned (*right*) rat. *Top*, An approximate twofold increase in firing rate produced by SKF 38393 (*SKF*; intact, 20 mg/kg, i.v.; lesioned, 10 mg/kg, i.v.), which were reversed by SCH 23390 (*SCH*; 0.5 mg/kg, i.v.). *Arrows* indicate the time at which the drug was administered. *Bottom*, The mean response (bar height), SEM (error bar), and individual responses (*open symbols*), all expressed as a percent of basal values, after administration of saline (*SAL*) or SKF 38393 (SKF; 10 or 20 mg/kg, i.v.). The *data point* in *parentheses* indicates a value that exceeds the scale of the *y*-axis. For reference, the *dashed line* indicates 100% of the basal firing rate. The *asterisks* indicate a significant difference from the corresponding saline-treated group ($p < 0.01$).

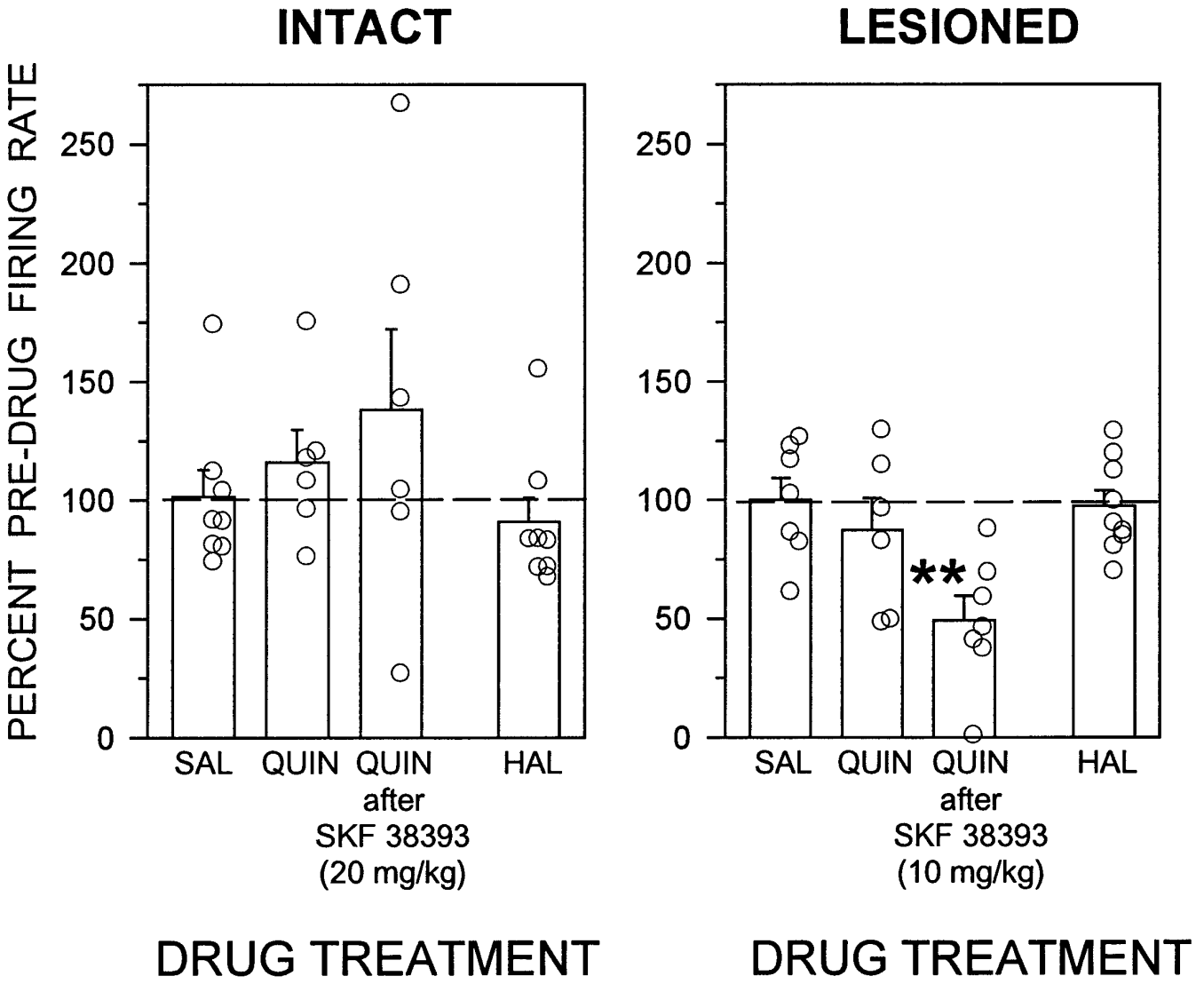

Figure 6. The effects of quinpirole and haloperidol on firing rates of STN neurons in the intact (*left*) and lesioned (*right*) rat. The graphs illustrate the mean response (bar height), SEM (error bar), and range of individual responses (*open symbols*). Data are expressed as a percent of basal values after administration of saline, quinpirole (*QUIN*; 0.26 mg/kg, i.v.), and haloperidol (HAL; 0.2 mg/kg, i.v.). However, data for rats administered quinpirole 10 min after SKF 38393 administration (QUIN after SKF 38393; quinpirole, 0.16 mg/kg, i.v.; SKF 38393 doses as indicated) are expressed as a percent of SKF 38393-induced firing (i.e., the 4 min immediately before administration of quinpirole). For reference, the *dashed line* indicates 100% of predrug and saline firing rate. The *asterisks* indicate a significant difference from the corresponding saline-treated group ($p < 0.01$).

rate after SKF 38393 administration was 216 ± 52% of basal rates, and the mean firing rate after the subsequent injection of quinpirole was 287 ± 118% of basal rates. Of the five cells in this subset that had significantly elevated firing rates after the injection of SKF 38393, one cell decreased, two cells increased, and two cells did not alter their firing rates after quinpirole administration. Quinpirole increased the firing rate of the one cell of this subset that did not show an altered firing rate after the administration of SKF 38393.

When administered 10 min after SKF 38393 in lesioned animals, quinpirole significantly decreased the mean firing rate of STN cells. In a subset of the previously described group of lesioned animals treated with SKF 38393 (10 mg/kg, i.v.), a dose of quinpirole (0.16 mg/kg, i.v.) was administered (see Fig. 6, *right*). In this subset of animals ($n = 7$), the mean firing rate after SKF 38393 administration was 178 ± 34% of basal rates, and the mean firing rate after the subsequent injection of quinpirole was 95 ± 28% of basal rates. Overall, quinpirole reduced the mean firing rate of STN neurons in lesioned rats pretreated with SKF 38393 by 51 ± 10% of the pre-quinpirole firing rate, which is significantly different from the effects both of saline-treated controls ($p < 0.01$) and of quinpirole-only-treated lesioned rats ($p < 0.05$), as determined by ANOVA [$F_{(3,28)} = 5.98$; $p < 0.01$] followed by Newman–Keuls *post hoc* test. Of the four cells in this subset that had significantly elevated firing rates after the injection of SKF 38393, three cells decreased their firing rates and one cell did not alter firing after quinpirole administration. Quinpirole also significantly decreased the firing rate of the three cells of this subset that did not show an altered firing rate after the administration of SKF 38393.

DISCUSSION

Results of the present study support predictions based on basal ganglia organization (Albin et al., 1989; DeLong, 1990) concern-

ing the effects of dopamine cell loss and dopamine agonist administration on neuronal activity of the STN in the parkinsonian state; however, the results challenge some of the premises on which these predictions were made. In accordance with predictions, neuronal activity in the subthalamus of rats with lesions of the nigrostriatal pathway was found to be significantly elevated; the mean firing rate of STN neurons in lesioned rats was 62% faster than that in intact rats. These observations are consistent with previous studies conducted in dopamine-depleted rodents (Robledo and Féger, 1991; Hassani et al., 1996) or primates (Miller and DeLong, 1987; Bergman et al., 1994; Wichmann et al., 1995; Vila et al., 1996). Examination of firing pattern using the discharge density analysis of Kaneoke and Vitek (1996) revealed that compared with STN cells of intact rats: (1) STN cells of lesioned rats had a more regular firing pattern; and (2) a smaller percent of the cells were bursting. A reduction of burstiness among STN cells of dopamine-depleted rats contrasts with previous reports (Hollerman and Grace, 1992; Hassani et al., 1996). One explanation is a difference in burst analysis methods. Another explanation is that the previous studies examined STN neuronal firing in the presence of general anesthetic agents. Earlier work from this laboratory has demonstrated that anesthetics significantly alter the regulation of STN neuronal activity (Kreiss et al., 1996), and more specifically, preliminary studies revealed that none of the STN cells in intact chloral hydrate-treated rats ($n = 8$) were bursting when the spike trains were analyzed using the discharge density analysis described above.

The prediction that neurons of the STN would become more active in the dopamine-depleted state is based on the hypothesis that loss of dopamine in the striatum would lead to a reduction in the activity of the inhibitory GABAergic pallidosubthalamic pathway (Miller and Delong, 1987; Albin et al., 1989; Robledo and Féger, 1991). In accordance with this hypothesis, the activity of pallidal neurons was found to be decreased in the lesioned animal (Miller and Delong, 1987; Pan and Walters, 1988; Filion and Tremblay, 1991). Moreover, the activity of STN neurons was observed to be enhanced after lesion of the external globus pallidus (Ryan and Clark, 1992; Ryan et al., 1992; but see Hassani et al., 1996). On the other hand, some aspects of pallidal neuronal activity seem to be augmented under conditions of chronic dopamine depletion; neuronal bursting activity is increased (Pan and Walters, 1988), and levels of mRNA for the GABAergic metabolic enzyme GAD67 are elevated (Kincaid et al., 1992; Soghomonian and Chesselet, 1992; Delfs et al., 1995).

Mechanisms other than decreased pallidal input may also contribute to the hyperactivity of STN neurons in rats with dopamine cell lesions. One contributing factor could be the loss of dopaminergic tone at receptors of nigral projections to the subthalamus (Delfs et al., 1995). However, this hypothesis implies that dopamine receptors in the STN exert an inhibitory effect on STN neuronal activity, which is at odds with results from this laboratory that demonstrate that locally infused dopaminergic agonists excite STN neurons in the intact rat (Kreiss et al., 1996). Another factor that could contribute to the increased activity of STN neurons in the lesioned rat is an enhancement of excitatory input (Overton and Greenfield, 1995; Hassani et al., 1996), putatively from the cortex that is a major source of glutamatergic afferents (Afsharpour, 1985; Rouzaire-Dubois and Scarnati, 1987; Canteras et al., 1990). Observations that stimulation of cortical neurons increased bursting activity (Kitai and Deniau, 1981; Fujimoto and Kita, 1993) and c-*fos* expression in the STN (Wan et al., 1992) are supportive of this hypothesis. However, preliminary studies from this laboratory found that the glutamatergic antagonists (5*R*,10*S*)-(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]-cyclohepten-5,10-imine and 1,2,3,4-tetrahydro-6-nitro-2 ,3-dioxo-benzo[*f*]quinoxaline-7-sulfonamide, did not alter average neuronal firing rates in the subthalamus of either the intact or lesioned rat (Thompson and Walters, 1993; Allers et al., 1996).

In addition to supporting the prediction that STN neurons in the dopamine-depleted animal are hyperactive, results of the present study also support the prediction that dopaminergic agents can reduce STN neuronal activity in these animals. Neuronal firing rates in the subthalamus of the 6-hydroxydopamine-lesioned rat were significantly reduced by systemic administration of the dopamine precursor L-DOPA and the nonselective dopamine receptor agonist apomorphine. What was surprising about these observations was that the effects of the agonists in the intact rat were quite different. The mean firing rate of STN neurons in the intact rat was not altered by L-DOPA and was significantly increased by apomorphine (present study and Kreiss et al., 1996). The increased effectiveness of L-DOPA in the parkinsonian state could be caused by receptor supersensitization, a phenomenon believed to underlie the alterations of motor output characteristic of the dopamine-depleted animal (Ungerstedt, 1971; Schoenfeld and Uretsky, 1973; Miller and Beninger, 1991). Another reason L-DOPA could be more efficacious in the lesioned state is because there are fewer dopaminergic uptake sites to compensate for increased levels of extracellular dopamine (Abercrombie et al., 1990; Orosz and Bennett, 1992). However, neither a supersensitization of receptors nor an increase in drug efficacy can explain the dramatic conversion of the excitatory effect of apomorphine in the subthalamus of the intact rat to its inhibitory effect in the subthalamus of the lesioned rat.

The excitatory effect of apomorphine in the STN of the intact rat challenges the premises that underlie the prediction that dopaminergic agents would inhibit neuronal activity in the STN. Anatomical considerations have led to the idea that dopamine receptor agonists would alter STN neuronal activity indirectly, predominantly via effects on the striatopallidal pathway. A number of studies have provided evidence that the major dopamine receptor subtype expressed by the striatopallidal neurons is the D2 subtype (Gerfen et al., 1990; Levey et al., 1993; Le Moine and Bloch, 1995; but see Surmeier et al., 1993, 1996). It has been hypothesized that the direct dopamine D1/D2 receptor agonist apomorphine and the indirect agonist L-DOPA would reduce activity in the STN by stimulating striatopallidal D2 receptors, thus reducing GABAergic input to the globus pallidus and thereby enabling pallidosubthalamic neurons to become disinhibited and thus to increase inhibitory input to the subthalamus.

Results from the current study challenge this proposed scenario, as does a comparison of data from previous studies of the globus pallidus and STN (Carlson et al., 1990; Kreiss et al., 1996). In the current study, administration of the dopamine D2 receptor agonist quinpirole alone had no effect on the mean firing rate of neurons in the STN of either 6-hydroxydopamine-lesioned or intact rats. Thus, selective stimulation of dopamine D2 receptors reproduced neither the inhibitory effects of L-DOPA and apomorphine in the lesioned rat nor the excitatory effects of apomorphine in the intact rat. Quinpirole did, however, markedly reduce firing rates of STN neurons in the lesioned rat when administered after a D1 receptor agonist. This finding suggests that activation of the D1 receptor is essential for the inhibitory effects of dopamine

agonists in the lesioned rat. Activation of the D1 receptor is also essential for the effects of apomorphine on STN neuronal activity in the intact rat; the excitatory effects of apomorphine were completely blocked by pretreatment with a D1 receptor antagonist (D. S. Kreiss and J. R. Walters, unpublished observations).

The role of the D1 receptor in the dopaminergic regulation of the subthalamus is complex, as illustrated by the observation that the qualitative shift in the effects of apomorphine from the intact to the lesioned rat was not accompanied by a corresponding alteration in the effects of the selective D1 receptor agonist SKF 38393. Administration of SKF 38393 alone increased STN neuronal firing rates in both 6-hydroxydopamine-lesioned rats and intact rats (Kreiss et al., 1996; present study). Moreover, the excitatory effects of the D1 receptor agonist seemed to be enhanced in the lesioned rat because a dose of SKF 38393 that did not significantly alter the firing of STN neurons in the intact rat significantly increased the firing in the lesioned rat. Augmentation of responses to D1 receptor agonists in the STN of the lesioned rat have been reported previously in studies measuring biochemical indexes of neuronal activation such as c-*fos* expression (Ruskin and Marshall, 1995) and glucose metabolism (Trugman and Wooten, 1987). One aspect of the D1 receptor-mediated effect in the STN that was altered by lesion was the role of the dopamine D2 receptor. In the intact rat, endogenous dopamine D2 receptor tone was necessary for the expression of D1 receptor-mediated effects because blockade of D2 receptors by pretreatment with the D2 receptor antagonist haloperidol prevented SKF 38393-induced increases of STN neuronal firing rates. The necessity of D2 receptor tone for the expression of D1 receptor-mediated effects was abolished in the lesioned state because SKF 38393 increased neuronal firing rates in dopamine-depleted rats.

Results from the present study suggest that regulatory mechanisms affecting the activity of STN neurons in the lesioned state differ from mechanisms most commonly described for dopamine-mediated phenomena involving basal ganglia function (for review, see Waddington and Daly, 1993; White and Hu, 1993; Marshall et al., 1997). In many reports, an interdependence between D1 and D2 receptor-mediated processes has been shown to underlie dopaminergic effects in the intact rodent, whereas an independence of D1 and D2 receptor-mediated effects seems to underlie dopaminergic phenomena in the lesioned rodent. However, a shift from interdependence to independence of D1 and D2 receptor-mediated effects cannot explain the conversion of the excitatory effects of apomorphine in STN of the intact rat to the inhibitory effects of apomorphine in the STN of the lesioned rat. With respect to the subthalamus, removal of dopaminergic input to the basal ganglia seems to alter the net effect of the interaction between the receptor subtypes. One explanation for the altered nature of the relationship between D1 and D2 receptor-mediated processes may be a change in the relative influence of the various afferents that regulate neuronal activity in the STN. Perhaps the inhibitory effect of apomorphine involves an increase in the relative weight of inhibitory input from the external globus pallidus, enabling it to overcome the dopamine D1 receptor-mediated excitatory input (putatively from the cortex). A second possibility is that dopamine D1 and D2 receptor interactions are altered within the STN itself, because both receptor subtypes have been shown to be present within the nucleus (Martes et al., 1985; Bouthenet et al., 1987; Dawson et al., 1988; Fremeau et al., 1991; Kreiss et al., 1996).

In conclusion, the present study provides support for the idea that hyperactivity of STN neurons contributes to the pathophysiology of Parkinson's disease. Neurons of the subthalamus in the lesioned rat were found to have elevated basal firing rates. Moreover, the most common pharmacological approach to reversing parkinsonian symptomology, administration of nonselective dopamine receptor agonists such as L-DOPA, markedly decreased the firing of the hyperactive STN neurons. Results demonstrate that both dopamine D1 and D2 receptors play a critical role in the regulation of STN neuronal activity, thus challenging the hypothesis that effects of dopaminergic agonists on the STN can be explained predominantly by actions at striatal dopamine D2 receptors. Present findings suggest that an integration of D1 and D2 receptor-mediated processes underlies the dopaminergic regulation of STN neuronal activity, both in the intact and dopamine-depleted states.

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