

## **Dorsal raphé stimulation modifies striatal-evoked antidromic invasion of nigral dopaminergic neurons in vivo**

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**Summary.** Extracellular single unit recordings were obtained from antidromically identified nigrostriatal dopaminergic neurons in anesthetized rats to determine the effects of dorsal raphé stimulation on the somatodendritic excitability of substantia nigra dopaminergic neurons. Stimulation of the dorsal raphé with a brief train of pulses delivered 7–2 ms prior to the neostriatal-evoked antidromic response significantly reduced the proportion of neostriatal-evoked antidromic responses that consisted of both initial segment and somatodendritic components without significantly altering the neostriatal-evoked post-stimulus inhibitory period. Raphé stimulation alone facilitated post-stimulus neuronal firing in almost half of the cells examined. The raphé-induced decrease in somatodendritic excitability was blocked by the serotonin antagonist, metergoline (0.5–2.0 mg/kg, i.v.), without significantly affecting the rate or pattern of spontaneous activity. The tryptophan hydroxylase inhibitor, parachlorophenylalanine (400 mg/kg, i.p. for three consecutive days), abolished the decrease in somatodendritic excitability following raphé stimulation which could be re-instated by intravenous administration of 5-HTP. The dopamine antagonists haloperidol (25–100 µg/kg, i.v.) and sulpiride (10–30 mg/kg, i.v.) also blocked the effects of dorsal raphé stimulation on somatodendritic invasion. These data suggest that in vivo, serotonin liberated from raphé-nigral terminals facilitates the release of dopamine from nigrostriatal dendrites resulting in a local, autoreceptor-mediated reduction in somatodendritic excitability without affecting the spontaneous firing rate and excitability of the neuron as a whole.

**Key words:** Substantia nigra – Serotonin – Autoreceptor – Dopamine release – Nigrostriatal – Rat

## Introduction

It is well established that dopamine can be released from the dendrites of nigrostriatal dopaminergic neurons

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(Geffen et al. 1976; Paden et al. 1976; Tagerud and Cuello 1979; Glowinski and Cheramy 1981; Cheramy et al. 1981; Nedergaard et al. 1988) and is believed to function, in part, in the local self-regulation of dopaminergic neuronal activity by interacting with somatodendritic autoreceptors (Groves et al. 1975; Bunney and Aghajanian 1978; Bunney 1979). The somatodendritic autoreceptor has been characterized pharmacologically as a dopamine D<sub>2</sub> receptor and acts to inhibit neuronal firing by producing a potassium dependent hyperpolarization (Aghajanian and Bunney 1977; Lacey et al. 1987). Dopaminergic dendrites contain synaptic vesicles that are labeled by the monoamine-selective marker, 5-hydroxydopamine, have been shown to participate as both the pre- and postsynaptic elements of dendro-dendritic synapses in rat substantia nigra, and may represent the morphological substrate for autoreceptor-mediated self-inhibition in dopaminergic neurons (Groves et al. 1975; Wilson et al. 1977; Groves and Linder 1983).

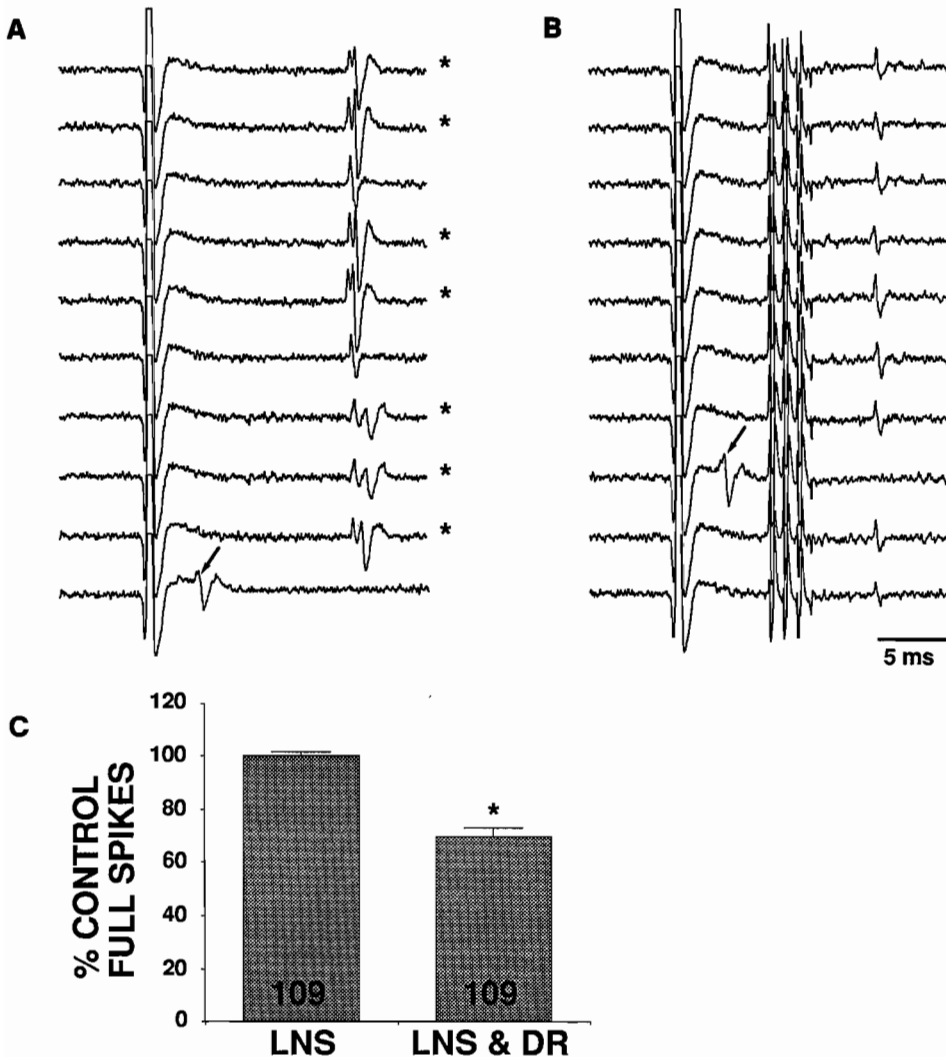
Dopamine released from the dendrites of nigrostriatal dopaminergic neurons which course ventrally into the GABA-rich pars reticulata may also act to influence the activity of non-dopaminergic pars reticulata neurons. In vivo iontophoresis studies suggest that dendritically released dopamine may attenuate the response of reticulata neurons to striatal and other GABAergic afferents, thereby serving a neuromodulatory action within the substantia nigra (Walters et al. 1984; Waszczak and Walters 1983, 1984; Gauchy et al. 1987).

Despite the evidence that dopaminergic dendrites can release dopamine, and that dendritically released dopamine plays a role in the regulation of neuronal activity within substantia nigra, the in vivo physiological conditions that evoke the release of dendritic dopamine remain somewhat elusive. It does not appear that dopamine is released simply when the neuron fires, since levels of dopamine release in substantia nigra and neostriatum are often inversely related, with the nigral release often seemingly independent of neuronal firing (Cheramy et al. 1981; Glowinski and Cheramy 1981). In vitro, dopaminergic dendrites have been shown to exhibit at least two different types of voltage-dependent calcium spikes

that have been proposed as possible mechanisms underlying dendritic dopamine release (Llinás et al. 1984; Kita et al. 1986; Nedergaard et al. 1988; Harris et al. 1989), but the endogenous trigger for these calcium spikes remains unclear.

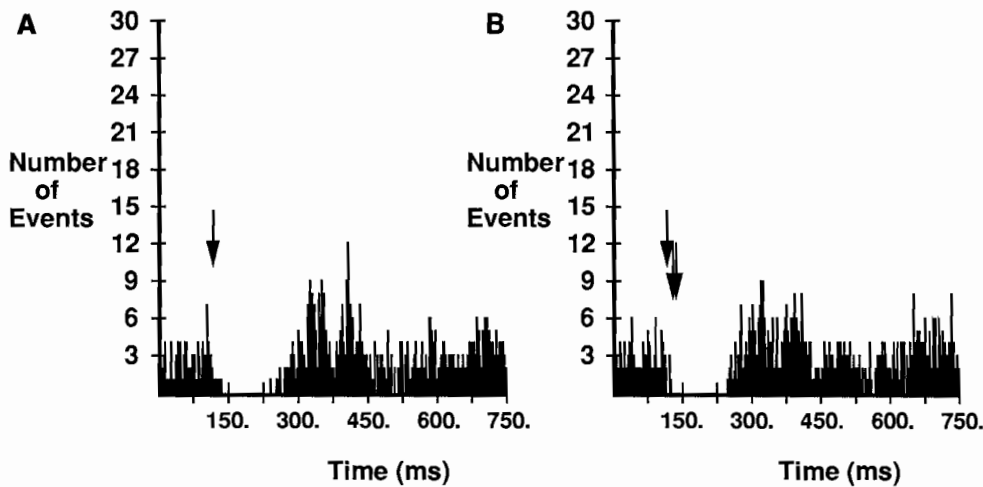
Anatomical studies have identified a serotonergic projection from the dorsal raphe nucleus to substantia nigra in several mammalian species (Dray et al. 1976; Fibiger and Miller 1977; Imai et al. 1986; Mori et al. 1987; Lavoie et al. 1988; Mori and Sano 1988), believed to arise from collaterals of the raphe-striatal projection (Van der Kooy and Hattori 1980; Imai et al. 1986), and immunoelectron microscopic evidence has shown serotonergic boutons synapsing onto dopaminergic den-

drites (Nedergaard 1988). Serotonin has been found to enhance the release of dopamine from the terminals of nigrostriatal neurons in rat striatal slices (Blandina et al. 1988, 1989) and synaptosomes (de Belleruche and Bradford 1980) as well as from substantia nigra in vivo (Glowinski and Cheramy 1981) and the ventral tegmental area in vitro (Beart and McDonald 1982). In vitro intracellular recordings from nigral dopamine neurons have shown that iontophoretic application of serotonin results in an increase in the amplitude of dendritic calcium spikes evoked by direct intracellular depolarization (Nedergaard et al. 1988) suggesting the possibility that serotonin released from serotonergic afferents in vivo may modulate nigral dopamine release through a local



**Fig. 1A-C.** Dorsal raphe stimulation reduces somatodendritic invasion of neostrially-elicited antidromic action potentials in nigral dopaminergic neurons. **A** Neostriatal (NS) stimulation evokes antidromic responses consisting of either a full IS-SD spike or an IS only spike. In this example, 7 of the 9 antidromic responses consist of the IS-SD spike (asterisks). **B** Dorsal raphe (DR) stimulation consisting of a train of 3 pulses (250  $\mu$ A, 250  $\mu$ s) delivered 7-5 ms before the NS-evoked antidromic response dramatically decreased the frequency of IS-SD antidromic responses (0 for 9 in this example). **A, B** Each consist of 10 consecutive stimulus presenta-

tions from one representative nigrostriatal neuron. Positivity is upwards, and collisions with spontaneous spikes are indicated by arrows. **C** Summary histogram of effect of dorsal raphe stimulation on percent full (IS-SD) spikes. DR stimulation caused a significant reduction in the proportion of neostriatal-evoked antidromic responses expressed as percent control (neostriatal stimulation only;  $t=9.6$ ,  $df=108$ ,  $p<0.05$ ). Asterisk indicates a significant difference from control stimulation ( $p<0.05$ ). Numbers within bars represent number of cells tested



**Fig. 2A, B.** Dorsal raphé stimulation does not significantly alter the mean neostriatal-evoked inhibitory period. Peri-stimulus time histograms from a single neuron illustrating similar post-stimulus inhibitory periods during neostriatal stimulation alone (A) and concomitant neostriatal and dorsal raphé stimulation (B). Arrows indicate the time at which the neostriatal (single arrows) and raphé (double arrows) stimuli were delivered. Bin width = 2 ms, 161 trials per condition

action on dopaminergic dendrites. The present study examined the effects of dorsal raphé stimulation on the somatodendritic excitability of antidromically activated nigrostriatal dopamine neurons *in vivo* to determine whether serotonin exerts a modulatory action on dopaminergic neurons similar to that observed *in vitro*. Portions of this work have been reported previously in abstract form (Trent and Tepper 1989).

## Material and methods

### Subjects

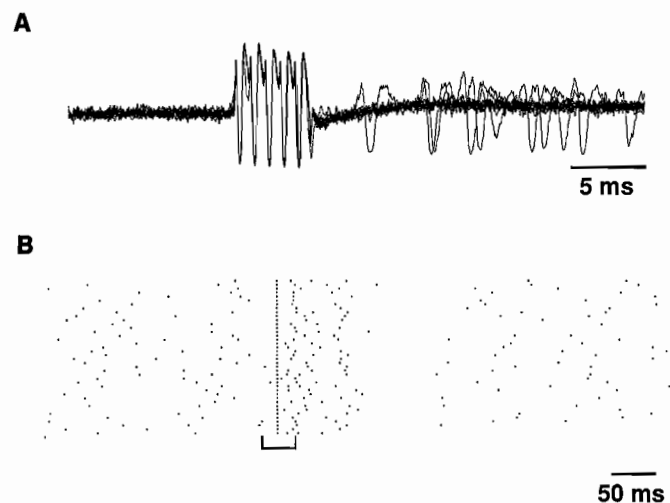
Sixty-six male Sprague-Dawley rats (Charles River or Institute of Animal Behavior, Rutgers) weighing between 200 and 460 g were anesthetized with urethane (1.3 g/kg, *i.p.*). The femoral vein was cannulated, and animals were installed into a stereotaxic frame. Body temperature was maintained at  $37 \pm 1^\circ \text{C}$  and the electrocardiogram was continuously monitored for the duration of the experiment. All wound edges and contact points between the animal and the stereotaxic frame were infiltrated with lidocaine ointment (5%) or solution (2%). All animals and were treated in strict accordance with guidelines set forth in the PHS manual, "Guide for the Use and Care of Laboratory Animals".

### Stimulating and recording

After removal of the scalp, small burr holes were drilled overlying the anterior-lateral neostriatum (1.0 mm anterior to bregma, 3.7 mm lateral to the midline) and the dorsal raphé nucleus (0.0 mm posterior to lambda, 0.0 mm lateral to the midline) for the insertion of stimulating electrodes. Bipolar stimulating electrodes having a tip separation of approximately 150  $\mu\text{m}$  and *in vitro* impedances of approximately 10  $\text{K}\Omega$ , were formed from 100  $\mu\text{m}$  diameter enamel-coated stainless steel wires (California Fine Wire). After releasing some cerebrospinal fluid by puncturing the atlanto-occipital membrane, stimulating electrodes were lowered to appropriate depths below the cortical surface for the neostriatum (4.0 mm) and the dorsal raphé nucleus (6.0 mm or 6.2 mm at  $15^\circ$ ) and cemented in place with cyanoacrylate glue and dental cement. A recording hole approximately 3.0 mm in diameter was drilled above the substantia nigra at coordinates 2.0 mm anterior to lambda and 2.0 mm lateral to the midline. Glass micropipettes with tip sizes ranging from 0.5–1.5  $\mu\text{m}$  were pulled on a Narishige vertical pipette puller, filled with 2% Pontamine Sky Blue in 2 M NaCl and possessed *in vitro*

impedances of 4–10  $\text{M}\Omega$ . Constant current electrical stimuli were generated with a Winston A-65 timer/stimulator and SC-100 constant current stimulus isolators. Neostriatal stimuli consisted of monophasic square wave pulses of 150–2000  $\mu\text{A}$  intensity and 100–500  $\mu\text{s}$  duration and were delivered at a rate of 0.67 Hz. Dorsal raphé stimulation consisted of a train of 1–5 monophasic pulses of 250–1000  $\mu\text{A}$  intensity and 100–250  $\mu\text{s}$  duration and were delivered from 7 to 2 ms prior to the neostriatal-evoked antidromic response. Single unit extracellular recordings were amplified with a Neurodata IR183 preamplifier and displayed on a Tektronix 5113A storage oscilloscope. All data were recorded on magnetic tape for off-line analysis.

Dopaminergic neurons were identified by their triphasic extracellular waveforms characterized by a prominent notch in the initial positive phase and having a duration of 2–5 ms, slow spontaneous activity in an irregular firing pattern and long latency antidromic responses evoked from neostriatum (Deniau et al. 1978; Guyenet and Aghajanian 1978; Grace and Bunney 1983a; Tepper et al. 1984, 1987). The antidromic nature of striatal-evoked responses was determined by collision extinction with spontaneously occurring spikes.



**Fig. 3A, B.** Effect of dorsal raphé stimulation alone on antidromically identified nigrostriatal unit activity. A 10 consecutive superimposed sweeps corresponding to trials 5–14 in B illustrating facilitation following a train of 5 stimuli (250  $\mu\text{A}$ , 250  $\mu\text{s}$ ) to dorsal raphé. B Raster display of same neuron constructed from 40 consecutive trials illustrating raphé facilitatory effect at a longer time scale. Bracket indicates time domain of digitized traces in A

## Drugs

All drugs were dissolved in 0.9% saline and were administered intravenously through a femoral catheter. The drugs used in this study and their concentrations were as follows: metergoline, 0.1–2.0 mg/kg (Farmitalia); haloperidol lactate, 25–100  $\mu$ g/kg (McNeil Pharmaceuticals); 5-hydroxy-L-tryptophan, 20 mg/kg (Sigma) and L(-)-sulpiride, 10–30 mg/kg (Research Biochemicals Inc.). For some experiments, DL-parachlorophenylalanine methyl ester HCl (Sigma) was dissolved in 0.9% saline and rats were administered 400 mg/kg, i.p. daily for three days prior to recording.

## Data analysis

Data were analyzed off-line with a Macintosh II computer equipped with a National Instruments NB-MIO16L multifunction board. Spike trains of spontaneous activity were analyzed for firing rate, burst occurrence and composition (Grace and Bunney 1983a; Tepper et al. 1990) and first order interval histograms and autocorrelations were constructed. Orthodromic responses evoked from neostriatal and/or dorsal raphe stimulation were characterized by measuring the magnitude and duration of inhibitory and excitatory responses from peri-stimulus time histograms generated from spike trains. Bin widths of 2 ms were employed for all analyses. Baseline firing (i.e., the number of spikes per bin) was determined from the pre-stimulus time period. Measurements were taken from user-specified time frames following the stimulus until firing returned to within ten percent of baseline activity in order to calculate the magnitude and duration of evoked responses. Data were analyzed

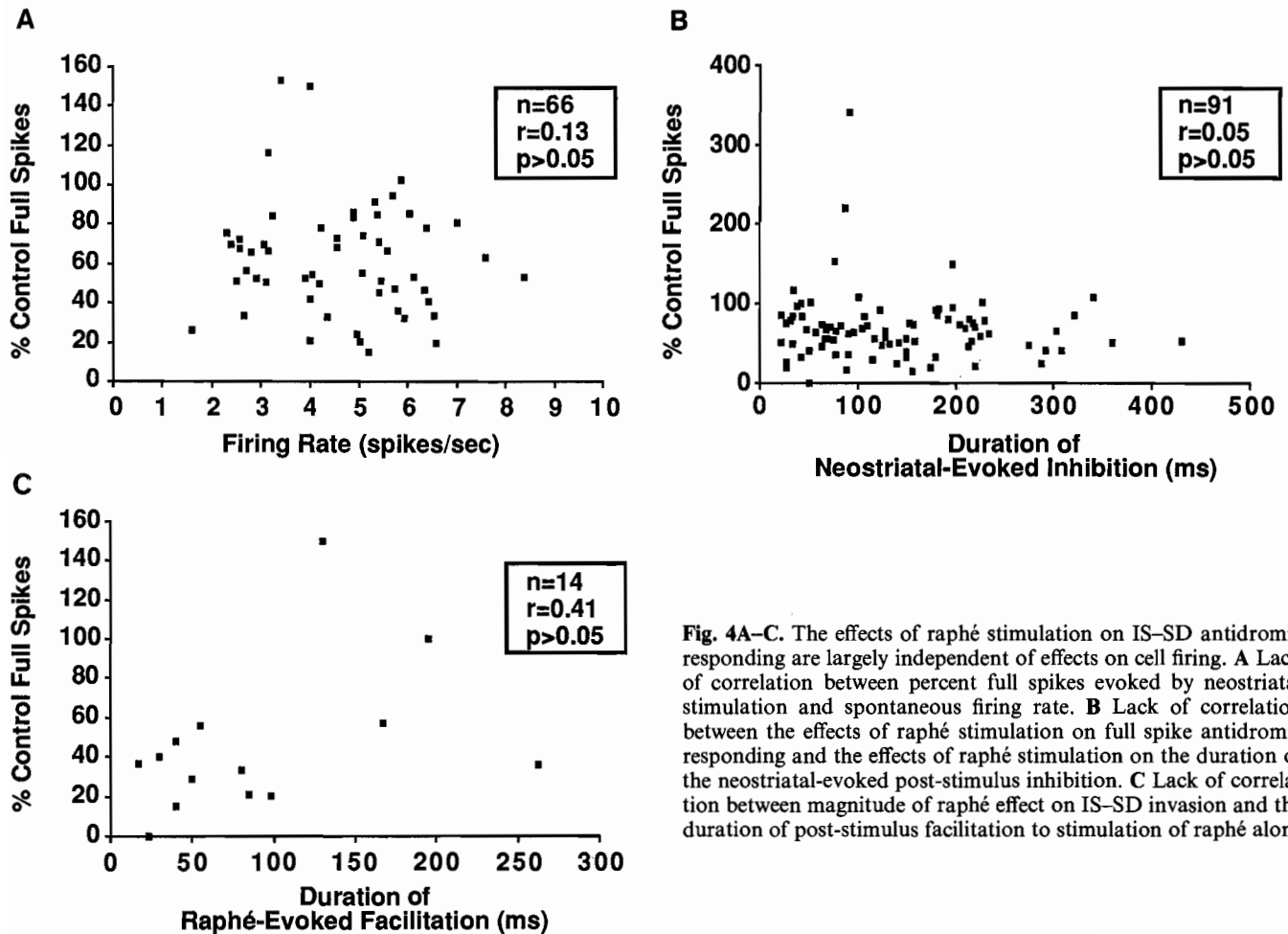
statistically with an analysis of variance and differences between individual groups were tested with Scheffé's F test at the  $p < 0.1$  level of significance.

## Histology

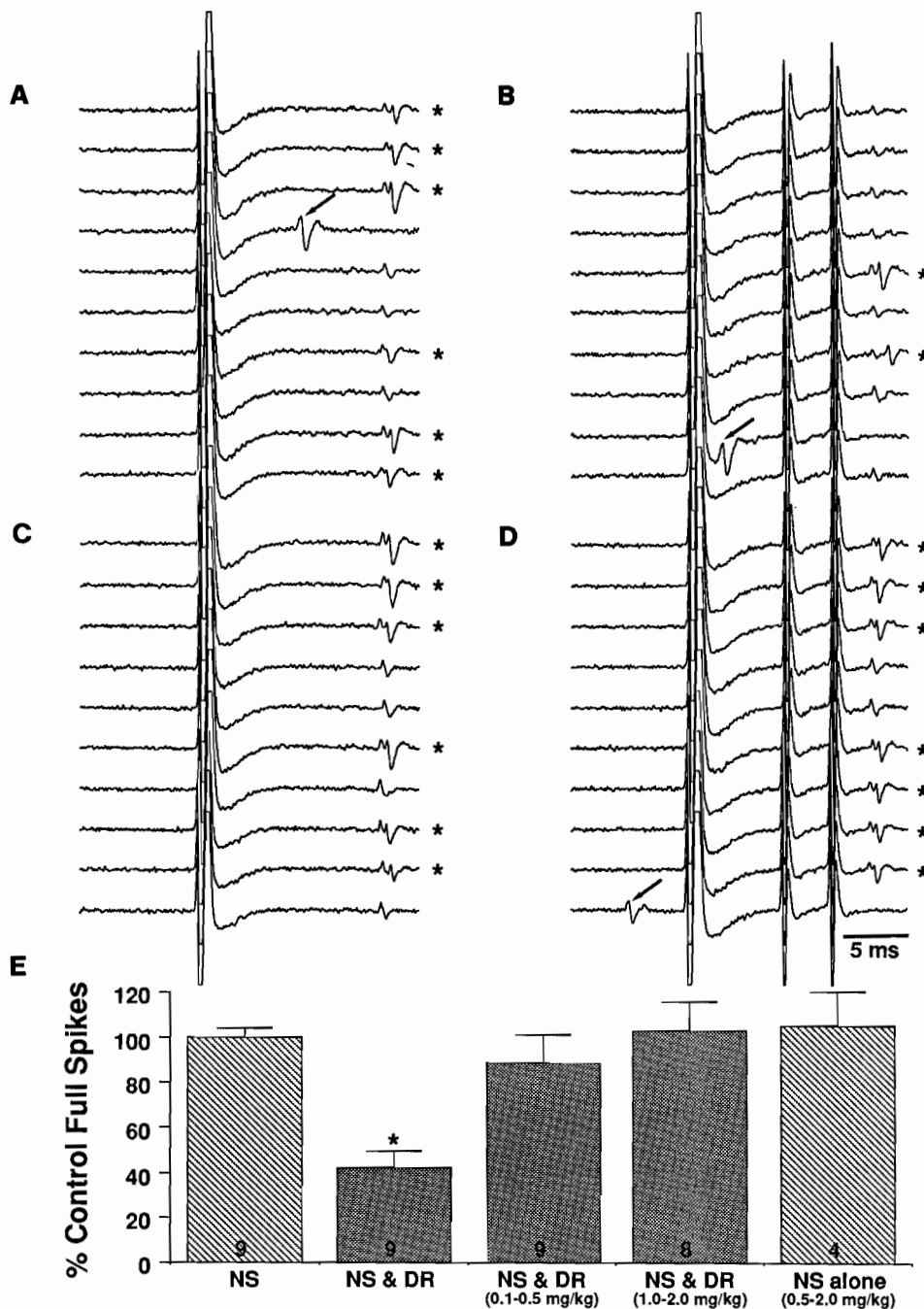
At the end of each experiment, small lesions were created at the stimulating sites by passing 500  $\mu$ A direct current through each stimulating electrode for 1–2 s and the recording site was marked with Pontamine Sky Blue by passing  $-20 \mu$ A for 20–30 min through the recording electrode. Animals were administered a lethal dose of urethane and perfused transcardially with a saline rinse followed by 10% buffered formalin or 4% paraformaldehyde and 0.2% glutaraldehyde in 0.15 M phosphate buffer. Brains were post-fixed, sectioned and stained with neutral red for histological verification of both stimulating and recording sites according to the atlas of Paxinos and Watson (Paxinos and Watson 1986).

## Results

Dopaminergic neurons were identified by previously established criteria (Deniau et al. 1978; Guyenet and Aghajanian 1978; Grace and Bunney 1983a; Tepper et al. 1984, 1987) and possessed slow firing rates averaging  $4.5 \pm 0.2$  spikes/s (mean  $\pm$  SEM;  $n=66$ ), and exhibited long latency ( $15.2 \pm 0.4$  ms;  $n=112$ ) antidromic activa-



**Fig. 4A–C.** The effects of raphe stimulation on IS–SD antidromic responding are largely independent of effects on cell firing. **A** Lack of correlation between percent full spikes evoked by neostriatal stimulation and spontaneous firing rate. **B** Lack of correlation between the effects of raphe stimulation on full spike antidromic responding and the effects of raphe stimulation on the duration of the neostriatal-evoked post-stimulus inhibition. **C** Lack of correlation between magnitude of raphe effect on IS–SD invasion and the duration of post-stimulus facilitation to stimulation of raphe alone



**Fig. 5A–E.** Intravenous administration of metergoline prevents the raphe-induced decrease in percent full antidromic spikes. **A** Stimulation of neostriatum alone elicits 6 full spike responses out of 9. **B** Concurrent stimulation of neostriatum and dorsal raphe with a train of two pulses (250  $\mu$ A, 250  $\mu$ sec) reduces full spike responding to 2 out of 10. Note the increased IS–SD delay in the 7th trace. **C, D** Following 0.5 mg/kg metergoline i.v., there is no change in the proportion of full spikes elicited by neostriatum alone (**C**); however, the effects of concurrent raphe stimulation (**D**) are completely blocked. **A–D** Consist of 10 consecutive stimulus presentations from one representative nigrostriatal neuron. Positivity is upwards. Full spike responses are indicated by asterisks and collisions with spontaneous spikes are indicated by arrows. **E** Dose-dependent nature of the blockade of the raphe-induced decrease in percent full antidromic responses by intravenously administered metergoline. Numbers within the bars represent the number of cells tested at each cumulative dose, both before and after metergoline administration. Doses are listed within parentheses. Note that metergoline does not affect IS–SD invasion to neostriatal stimulation alone. Asterisk indicates significant difference from control (NS) stimulation (Scheffé,  $p < 0.1$ )

tion from the neostriatum. As reported previously, antidromic responses of nigral dopamine neurons to stimulation of neostriatum consisted of either an initial segment (IS) spike only, or a full IS-somatodendritic (IS–SD) spike with the IS-only response predominating (Guyenet and Aghajanian 1978; Grace and Bunney 1983a, b; Tepper et al. 1984) as shown in Fig. 1A. The neostriatal stimulating current was set to the minimum value that elicited antidromic responses on 100% of non-collision trials, and 50–100 stimuli delivered. Antidromic responses were classified as IS or IS–SD, and the proportion of full IS–SD antidromic responses divided by total antidromic responses was used as an index of somatodendritic excitability. Only neurons exhibiting at least

15% IS–SD spikes evoked from neostriatal stimulation were studied further.

The mean neostriatal threshold for evoking 100% antidromic responses was  $1.6 \pm 0.1$  mA. Stimulation of neostriatum alone (control condition) yielded  $36.4 \pm 1.8\%$  IS–SD responses ( $n = 109$  cells), i.e., almost 64% of all neostriatal-evoked antidromic responses consisted of the IS spike only. Stimulation of dorsal raphe was delivered 7 to 2 ms before the striatal-evoked antidromic response. The timing of this stimulus was determined from the distance between the dorsal raphe and the substantia nigra (approximately 3 mm) and from the estimated conduction velocity of dorsal raphe fibers reported in other studies ( $\sim 0.6$  m/s; Dray et al. 1976;

Fibiger and Miller 1977; Park et al. 1982; Sawyer et al. 1985). Other delays (i.e., 12 to 5, 10 to 4, 10 to 5, 5.5 to 2.5, 7.5 to 4.5 ms before the antidromic response) were tested, but the delay of 7 to 2 ms yielded the most consistent and dramatic effect on the striatal-evoked antidromic response. Dorsal raphé stimulation significantly reduced the proportion of IS-SD responses to  $24.5 \pm 1.6\%$  ( $69.4 \pm 3.9\%$  of the control proportion of full spikes;  $t=9.6$ ,  $df=108$ ,  $p<0.05$ ) as shown for one representative neuron in Fig. 1B, suggesting that the stimulus produced a decrease in the somatodendritic excitability of nigrostriatal neurons. The raphé induced reduction in IS-SD spikes was seen in 87 (79.8%) of the 109 cells studied while an increase was observed in 5 (4.6%) cells and no effect ( $100 \pm 10\%$  control) was seen in 17 cells (15.6%). This effect was reversible in all cases upon cessation of the raphé stimulation. The mean change in IS-SD antidromic responding produced by raphé stimulation for all cells is plotted in Fig. 1C.

Peri-stimulus time histograms constructed from spike trains during stimulation of neostriatum alone and concurrent stimulation of neostriatum and dorsal raphé revealed that the dorsal raphé-induced decrease in the proportion of IS-SD antidromic responses occurred without a significant alteration in the mean neostriatal-evoked post-stimulus inhibitory period ( $142.1 \pm 10$  ms vs.  $142.7 \pm 12.9$  ms) as illustrated by the histograms in Fig. 2. Although dorsal raphé stimulation did not exert a significant effect on the neostriatal-evoked post-stimulus inhibitory period, an initial excitatory response to stimulation of the dorsal raphé alone was elicited in fourteen of twenty-nine neurons (48.3%) in which this parameter was examined. This raphé-evoked facilitation showed a mean onset latency of  $11.7 \pm 1.2$  ms, persisted for  $90.9 \pm 19.6$  ms, during which time the mean number of spikes per bin was increased to  $321.7 \pm 41.3\%$  of pre-stimulus firing levels, as illustrated for one neuron in Fig. 3. There were no significant correlations between the percent of full IS-SD antidromic spikes to striatal stimulation and the spontaneous firing rate of the neuron (measured in the absence of any stimulation), between the percent change in the proportion of IS-SD antidromic spikes and the duration of the post-stimulus inhibition following concurrent neostriatal and raphé stimulation, or between the effects of the raphé stimulus alone on post stimulus inhibition or excitation and the change in IS-SD antidromic responding, as illustrated in Fig. 4.

Attempts were made to reverse the effects of raphé stimulation by systemic administration of the serotonergic antagonist, metergoline, at incremental doses from 0.1 to 2.0 mg/kg, i.v. in 9 animals. Metergoline produced a dose-dependent blockade of the raphé-induced reduction in IS-SD spikes in 9 out of 9 cells, as illustrated for one representative neuron in Fig. 5. Metergoline given at 0.1–0.5 mg/kg, i.v. effected a partial blockade of the decrease in IS-SD antidromic responding, and completely abolished the raphé effect on somatodendritic antidromic invasion at a dose of 1.0 mg/kg or higher. Metergoline had no effect on the proportion of IS-SD antidromic responses elicited by neostriatal stimulation alone. Even at the highest cumulative dose (2.0 mg/kg),

metergoline did not significantly affect the rate or pattern of spontaneous activity of nigrostriatal neurons ( $3.0 \pm 1$  spikes/s vs.  $3.5 \pm 0.8$  spikes/s;  $n=3$ ). In some cases the neuron being examined was lost during metergoline administration therefore precluding the measurement of post-drug responses from that cell. In order to determine the effects of the drug in these situations, recording sessions continued until another antidromic neuron was found. An additional 9 neurons recorded within thirty minutes after metergoline administration also failed to display the raphé effect in response to maximal raphé stimulation which elicited  $105 \pm 35.8\%$  control full spikes.

The raphé-induced decrease in somatodendritic invasion of neostriatal-evoked antidromic responses was also blocked by the dopamine antagonists, haloperidol (25–100  $\mu\text{g}/\text{kg}$ , i.v.;  $n=7$ ) and sulpiride (10–30 mg/kg, i.v.;  $n=5$ ). Haloperidol returned the somatodendritic excitability to  $114.3 \pm 10.2\%$  of control and sulpiride similarly reversed the raphé-induced decrease in percent control full spikes to  $109.3 \pm 12.4\%$  as shown in Fig. 6. Like metergoline, the spontaneous activity of nigrostriatal dopaminergic neurons was not altered by either haloperidol ( $3.1 \pm 0.4$  spikes/s vs.  $2.7 \pm 0.1$  spikes/s,  $n=3$ ) or sulpiride ( $4.0 \pm 0.5$  spikes/s vs.  $3.9 \pm 0.2$  spikes/s,  $n=2$ ).

In two animals, endogenous serotonin was depleted by three daily injections of the tryptophan hydroxylase inhibitor para-chlorophenylalanine (pCPA; 400 mg/kg, i.p.; Fibiger and Miller 1977; Wang and Aghajanian 1977; Sloviter et al. 1978). After a number of stable recordings were obtained from dopaminergic neurons in the pCPA-treated animals and the effects of raphé stimulation examined, serotonin was replenished by systemic administration of its immediate precursor 5-hydroxytryptophan (5-HTP; 20 mg/kg, i.v.; Wang and Aghajanian 1977; Sloviter et al. 1978). Dorsal raphé stimulation had no effect on the percent full antidromic responses recorded from eight dopaminergic neurons in pCPA-treated animals ( $29.3 \pm 4.5\%$  vs.  $28.5 \pm 7.6\%$ ). In these animals, the basal activity (e.g., firing rate and pattern) and antidromic response properties of the dopaminergic

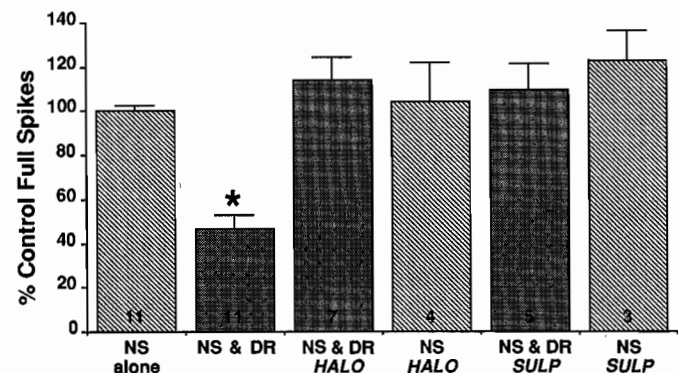
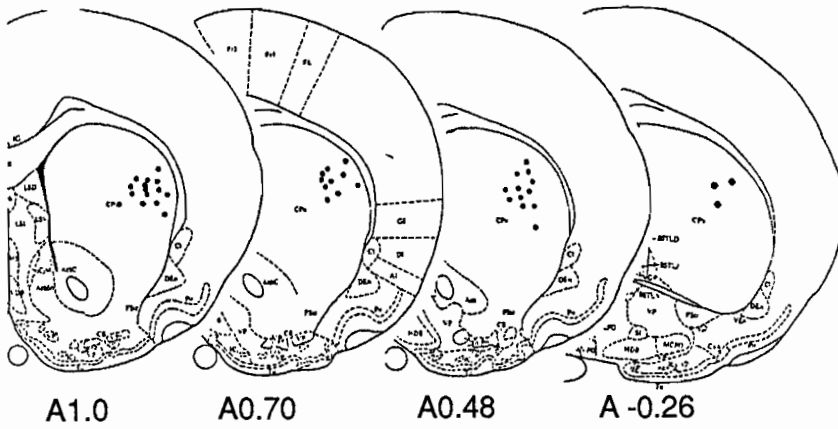
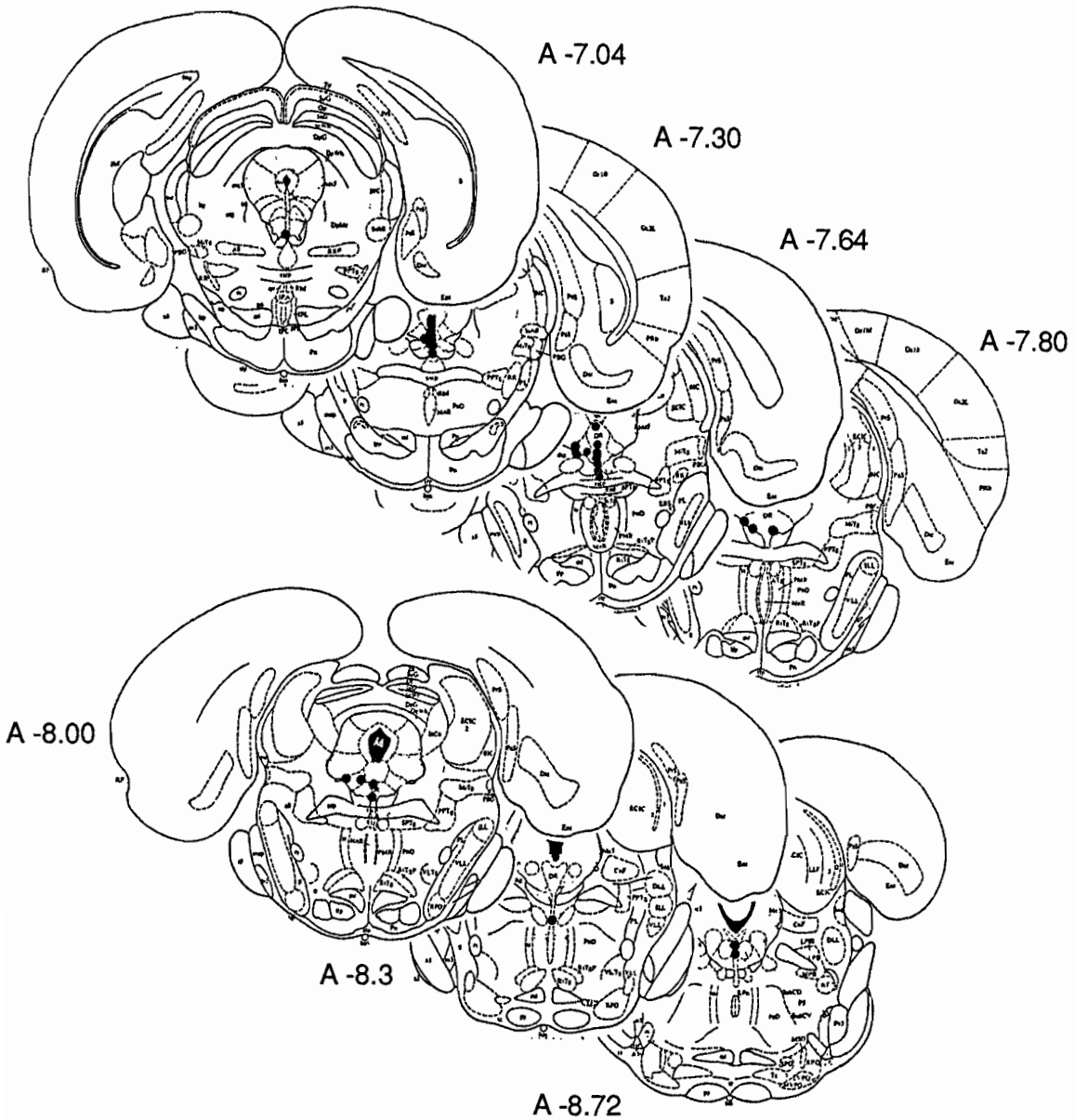


Fig. 6. Intravenous administration of haloperidol (25–100  $\mu\text{g}/\text{kg}$ ) or 1-sulpiride (15–30 mg/kg) reverses the decrease in somatodendritic excitability induced by dorsal raphé stimulation. Numbers within the bars represent number of cells tested, both before and after haloperidol administration. Asterisk indicates a significant difference from control (NS) stimulation (Scheffé,  $p<0.1$ )

**A** Neostriatal Stimulating Sites



**B** Dorsal Raphé Stimulating Sites



**Fig. 7A-B.** Reconstruction of (A) neostriatal and (B) dorsal raphé stimulating sites verified histologically from 80  $\mu$ m frozen sections stained with neutral red according to Paxinos and Watson (1986)



neurons encountered did not significantly differ from that of controls. In the two pCPA-treated animals, 5-HTP was administered while recording from an antidromic cell to restore endogenous serotonin levels. 5-HTP reinstated the raphé effect as indicated by a decrease in percent full spikes evoked by raphé stimulation ( $57.4 \pm 5.9\%$  control full spikes;  $n=2$ ). In these animals, an additional nine cells were recorded after 5-HTP administration. All nine cells also displayed the raphé-induced decrease in percent full antidromic responses. Pooling all cases together, 5-HTP caused a 29.4% decrease in percent control full antidromic responses to concurrent neostriatal and raphé stimulation ( $n=11$ ) compared to those evoked before the serotonin precursor was administered. 5-HTP did not alter dopaminergic basal activity as indexed by mean spontaneous firing rate, examination of autocorrelograms of spontaneous spike trains, or statistical analyses of burst firing.

## Discussion

In nigrostriatal dopaminergic neurons the failure of the antidromic spike to invade the somatodendritic region of the neuron is quite common, even at modest rates of stimulation (Guyenet and Aghajanian, 1978; Deniau et al. 1978; Grace and Bunney 1983a, b; Tepper et al., 1984; Grace 1987; Tepper et al. 1987). Several reasons have been proposed for this failure in dopaminergic neurons including the coactivation of an IPSP by the antidromic stimulus, the absence of a slow depolarization of the somadendrite that occurs during orthodromic impulse conduction and the fact that the threshold for action potential initiation is likely lower at the IS (spike-generating) region than at the somadendrite (Grace and Bunney 1983a, b; Llinás et al. 1984). Due to the morphological organization of dopamine neurons in which the fine, unmyelinated axon arises from a major dendritic branch at distances ranging from 30–100  $\mu\text{m}$  from the soma (Grace and Bunney 1983b; Tepper et al. 1987; Grace and Onn, 1989), the IS region may not be isopotential with the somadendrite and the current source provided by the IS action potential may not be sufficient to reliably depolarize the considerably larger somadendrite to spike threshold.

Intermittent invasion of the somadendrite by antidromic spikes has been exploited previously by Matsuda and Jinnai to analyze afferent synaptic interactions in neostriatal neurons (Matsuda and Jinnai 1980). These authors demonstrated that antidromic responses of neostriatal neurons evoked from substantia nigra or entopeduncular nucleus that consisted of IS-only spikes could be transformed into full-spike responses by appropriately timed concurrent stimulation of excitatory afferents from cerebral cortex or thalamus. They concluded that extracellular recordings of intermittent invasion of the somadendrite by antidromic potentials can accurately reflect changes in membrane potential due to synaptic activation, with depolarization resulting from excitatory synaptic activity favoring the expression of full antidromic spikes (Matsuda and Jinnai 1980).

Using the proportion of antidromic responses that consist of both IS and SD components as a measure of somatodendritic excitability, our data indicate that stimulation of the dorsal raphé produces a decrease in the somatodendritic excitability of nigrostriatal neurons. That this effect is due, at least in part, to serotonin release in substantia nigra is indicated by the reversal of the effect by the serotonin antagonist, metergoline, and by the abolition of the effect in rats depleted of serotonin by pCPA treatment and subsequent reinstatement of the effect by 5-HTP administration.

The simplest explanation for these findings is that stimulation of the serotonergic input acts to hyperpolarize the somatodendritic region of the dopaminergic neuron through a metergoline sensitive serotonin receptor, and that this hyperpolarization (or perhaps the underlying conductance increase) decreases the excitability of the neuron as a whole and thereby depresses IS–SD invasion. This explanation is, however, inconsistent with several of our findings. First, although raphé stimulation decreased the frequency of full antidromic spikes, this effect occurred in the absence of any change in the probability of neuronal firing as measured by the duration and magnitude of the inhibitory period following neostriatal stimulation in peri-stimulus time histograms. When the post-stimulus inhibitory periods following neostriatal stimulation alone were compared to those following concurrent stimulation of neostriatum and dorsal raphé, there were no differences. Consistent with this finding, administration of metergoline did not affect spontaneous activity, despite blocking the reduction in full spike antidromic responding, nor did depletion of endogenous serotonin or subsequent repletion with 5-HTP affect firing rate. In addition, in approximately 50% of the neurons so tested, the initial response to presentation of the raphé stimulus alone was an increase in firing, not a decrease. Finally, the raphé effect was also abolished by haloperidol over a dose range at which this drug is a relatively selective dopamine receptor antagonist, as well as by the  $D_2$ -selective antagonist, sulpiride. Thus, it is unlikely that these findings simply reflect an overall neuronal hyperpolarization induced by serotonergic afferents. Instead, we propose the following explanation.

Stimulation of the dorsal raphé activates a serotonergic raphé-nigral pathway synapsing on dopaminergic neurons. Synaptically released serotonin activates a serotonin receptor on the nigral somadendrite which triggers a *local* dendritic release of dopamine, either by dendritic depolarization or by a direct action on a somatodendritic calcium conductance. The dendritically released dopamine in turn activates somatodendritic  $D_2$  dopamine autoreceptors which hyperpolarize the somatodendritic membrane locally and results in conduction failure of the SD component of the antidromic response due to the dendritic hyperpolarization, underlying increase in potassium conductance or both.

This interpretation is consistent with the available data. A serotonergic raphé-nigral projection has been identified at both the light (Dray et al. 1976; Fibiger and Miller 1977; Van der Kooy and Hattori 1980; Imai et al. 1986; Mori et al. 1987; Mori and Sano 1988; Nedergaard

et al. 1988) and electron microscopic levels (Mori et al. 1987; Nedergaard et al. 1988), and high levels of both serotonin (Palkovits et al. 1974) and tryptophan hydroxylase (Brownstein et al. 1975) in substantia nigra suggest a significant serotonergic input. Local application of serotonin or serotonergic agonists to substantia nigra increases nigral or ventral tegmental area dopamine release both in vivo (Glowinski and Cheramy 1981) and in vitro (Beart and McDonald 1982). Release of dopamine in neostriatum is also increased by serotonin agonists (de Belleruche and Bradford 1980; Blandina et al. 1988, 1989), presumably by a direct action on dopaminergic nerve terminals, and intracellular recordings from neostriatal neurons reveal a serotonergic EPSP arising from raphé stimulation (Park et al. 1982).

Previous reports of the effects of serotonergic agonists on nigral dopaminergic neuronal activity have not revealed robust effects, and even potent serotonin agonists are largely without effect on spontaneous activity except at high doses (e.g. Bunney 1979). In a recent report, systemic administration of 5HT<sub>1A</sub> receptor agonists was found to increase the firing of slow (i.e., < 4 spikes/s) but not fast firing nigrostriatal dopaminergic neurons while 5HT<sub>1B</sub> receptor agonists exerted weak inhibitory effects on neuronal firing (Kelland et al. 1990). Stimulation of the dorsal raphé at low to moderate frequencies for 2 min continuously was found to inhibit the subsequent spontaneous activity of slowly firing neurons. These effects were described by the authors as "subtle at best". In the present study, no correlation was observed between the effects of raphé stimulation on the reduction in IS-SD antidromic responding and spontaneous firing rate, consistent with a dissociation between the effects of raphé input on somatodendritic excitability and neuronal firing.

In another study stimulation of dorsal raphé produced a marked inhibition of nigral dopaminergic neurons that was eliminated by pCPA-pretreatment, but pCPA was without effect on the rate or pattern of spontaneous activity (Fibiger and Miller 1977). Although the lack of changes in the rate or pattern of spontaneous activity following metergoline or pCPA treatment that we observed is consistent with these previous reports (Fibiger and Miller 1977; Kelland et al. 1990), in our experiments, stimulation of the raphé alone resulted in an initial excitatory response in 48.3% of the cases. The reason(s) for this discrepancy are not immediately obvious. It is possible that slight differences in the positions of the raphé stimulating electrodes, or in the stimulus parameters used could have biased the character of the observed responses by a differential stimulation of non-serotonergic inhibitory raphé efferents (Park et al. 1982; Sawyer et al. 1985). It is also conceivable that differences in stimulus strength could lead to a condition where the evoked release of dopamine acting through somatodendritic autoreceptors could mask the initial excitation responsible for its release.

Our explanation of the effects of raphé stimulation on dopamine neuron physiology in vivo is consistent with the findings by Greenfield and associates who report that iontophoretic application of serotonin facilitates a vol-

tage dependent calcium conductance in dopaminergic dendrites in vitro that may be essential for dendritic release of dopamine (Nedergaard et al. 1988). Dendritic dopamine release can be induced by many factors including depolarization, and is both calcium-dependent and tetrodotoxin-resistant (Cheramy et al. 1981; Glowinski et al. 1981). The tetrodotoxin resistance suggests that dendritic release is not dependent on fast sodium spikes which are abolished by tetrodotoxin (Llinás et al. 1984; Kita et al. 1986; Nedergaard et al. 1988), consistent with the dissociation between somatodendritic excitability and neuronal firing in the present report.

Somatodendritic autoreceptors can function as a tonic dopaminergic inhibitory mechanism by suppressing neuronal firing at the level of the soma (Groves et al. 1975; Aghajanian and Bunney 1977; Bunney and Aghajanian 1978; Bunney 1979; Lacey et al. 1987) as demonstrated in vivo and in vitro by the systemic or local administration of dopamine agonists (Groves et al. 1975; Bunney and Aghajanian 1978; Bunney 1979; Lacey et al. 1987), and are often assumed to act physiologically to modulate or "self-inhibit" dopaminergic neuron activity for a short time following an action potential through dendritically released dopamine (Groves et al. 1975). The physiological role played by autoreceptor-mediated self-inhibition is somewhat puzzling, however, since recent in vitro recording experiments indicate that dopaminergic neurons, like other central monoamine neurons, exhibit a calcium-activated potassium conductance that appears to be responsible for the bulk of the post-firing inhibition seen in these neurons (Grace and Bunney 1983a; Kita et al. 1986). Based on our present data, one may speculate that under physiological conditions, perhaps somatodendritic autoreceptors have a function other than to produce a type of neuron-wide post-firing inhibition. When dopamine somatodendritic autoreceptors are stimulated by systemic, iontophoretic or bath application of agonists, many or most of the autoreceptors on the cell being recorded from may be stimulated. However, when dopamine release is elicited by serotonergic afferent input, the action of serotonin and the secondary release of dopamine may be local events, restricted to those portions of the dendritic arborization that are near to the serotonergic synapses. A consequence of this may be that only a relatively small number of somatodendritic autoreceptors are activated and although this number is sufficient to inhibit the propagation of the antidromic spike into that region of the somadendrite (as well as to affect local signal processing), it remains insufficient to affect overall neuronal firing activity.

It has been demonstrated by in vitro intracellular recordings that in the substantia nigra dendritic calcium spikes may occur both in the presence and absence of somatic sodium spikes (Llinás et al. 1984). This would further suggest independent regulation of dendritic spiking and somatic firing as indicated by the poor correlation between changes in dendritic dopamine release which may have resulted from activation of a voltage-dependent dendritic calcium conductance with changes in somatic firing frequency. Furthermore, Greenfield and co-workers report that sectioning of the distal dendrites

of nigral dopamine neurons in pars reticulata results in a loss of the low threshold calcium spike (Harris et al. 1989), suggesting that the low threshold calcium spike is localized to the distal regions of the dendrites. The effects that we observe on IS–SD antidromic invasion may be an *in vivo* correlate of the serotonergic effects on the low threshold calcium spike *in vitro* (Nedergaard et al. 1988) and provide supporting evidence for an independent modulation of dendritic and somatic electroresponsiveness *in vivo* as indicated by the lack of correlation between firing rate and the raphé-induced decrease in full IS–SD antidromic responses and the inability of both raphé stimulation and serotonin antagonists to significantly alter spontaneous activity. These data confirm and extend previous reports on the interaction of dopaminergic and serotonergic systems in the substantia nigra (Nedergaard et al. 1988) and suggest that, *in vivo*, the serotonergic input from the dorsal raphé nucleus may act in a phasic rather than tonic manner to regulate the presynaptic (dopamine-releasing) functions of nigral dopaminergic dendrites locally, without affecting neuronal excitability as a whole.

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