pair of x-ray–induced DNA damage. The comparatively low concentrations (100 μM) that decrease such repair are comparable to the effective levels of certain potent carbamylating and alkylating agents such as 1,3-bis(2-chloroethyl)-1-nitrosourea (*13*) and 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (*14*). Millimolar concentrations of dimethylsulfate are required to inhibit excision repair of ultraviolet radiation–induced DNA damage (*15*).

The critical factors determining the concentration of HCHO that can be safely handled by the cell remain to be identified. Exogenous HCHO will readily react with respiratory mucus and the exterior surface of the target cells so that only a small fraction of the HCHO reaches the nucleus. Since HCHO is formed endogenously in the cell during demethylation reactions, cells must maintain pathways for its detoxification. Many xenobiotics are also metabolized by demethylation reactions. Formaldehyde is metabolized to formate, but this agent did not cause the formation of DNA single-strand breaks or affect the repair of such breaks induced by x-rays (data not shown).

Since HCHO damages DNA, inhibits DNA repair, and potentiates the cytotoxicity of x-rays in human bronchial epithelial cells, and since the HCHO may act in concert with physical and chemical agents to produce toxic, mutagenic, and carcinogenic effects (*11*), we suggest that the mutagenic and carcinogenic effects of this chemical alone or in combination with other agents should be further investigated.

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Dopamine Modulation of the Effects of γ-Aminobutyric Acid on Substantia Nigra Pars Reticulata Neurons

Abstract. *Studies were conducted to assess whether basal ganglia output neurons originating in the substantia nigra pars reticulata might be affected by dopamine released from dendrites of neighboring substantia nigra pars compacta neurons. Dopamine applied by iontophoresis increased the baseline firing rates of approximately half of the substantia nigra pars reticulata cells tested. The more significant finding, unrelated to the increase in firing, was the ability of dopamine to attenuate the inhibitory responses of these cells to iontophoretically applied γ-aminobutyric acid. These findings suggest a role for dopamine as a neuromodulator and further suggest that it can act at sites beyond the striatum to modify transmission from the basal ganglia to motor nuclei.*

The nigrostriatal dopamine system is involved in several disorders of movement that originate in the basal ganglia—most notably Parkinson's disease. How dopamine influences movement ultimately depends on how it acts within the basal ganglia to modify transmission of information to premotor nuclei outside the basal ganglia. The substantia nigra (SN) pars reticulata, located ventral to the nigral dopamine neurons, functions as one of two basal ganglia output nuclei (*1*), projecting largely to the motor thalamus and superior colliculus (*2*). The SN receives an innervation, in part utilizing γ-aminobutyric acid (GABA), from the striatum and globus pallidus (*3*). Thus, dopamine neurons might affect SN pars reticulata output pathways in two ways: (i) indirectly, by release of dopamine from terminals within the striatum, or (ii) directly, by local release of the transmitter (*4*) from dendrites that extend into the pars reticulata (*5*). Since pars reticulata neurons are strategically involved in the transmission of messages from the basal ganglia to motor effector sites, we conducted studies to determine how these neurons might be affected by dendritically released dopamine. We now report that iontophoretically applied dopamine directly affected the firing rates of approximately half of the pars reticulata neurons. Of potentially greater importance, however, is our observation that dopamine acted as a neuromodulator by markedly and reproducibly diminishing the responses of reticulata cells to the inhibitory transmitter GABA.

Male Sprague-Dawley rats weighing 250 to 300 g were anesthetized with chloral hydrate (400 mg per kilogram of body weight, intraperitoneally) and mounted in a stereotaxic apparatus. Techniques for extracellular, single-unit recording and microiontophoresis were used as described (*6*, *7*). Cells of the SN pars reticulata were identified by criteria described in detail elsewhere (*6*, *8*). These cells could be distinguished from dopamine neurons of the SN pars compacta by their location and by differences in the shape of their action potentials, discharge frequencies, and firing patterns. Efforts were made to antidromically activate the cells used in these studies from the ventromedial nucleus (VM) of the thalamus. Pulses, 200 μsec in duration and 0.2 to 0.5 mA in intensity, were delivered from a bipolar stimulating electrode stereotaxically positioned in the left VM nucleus (ipsilateral to the recording site). Three criteria were used to establish whether cells could be excited antidromically: (i) stable latency of the antidromic response, (ii) ability of cells to follow stimulus frequencies greater than 300 Hz, and (iii) collision of antidromic and spontaneous spikes.

The ability of applied dopamine to modify responses of reticulata cells to other transmitters applied iontophoretically was evaluated as follows. Repeated 30-second iontophoretic pulses of GABA, glycine, acetylcholine, or glutamic acid were applied at a fixed current, separated by 30-second periods of baseline activity. After completion of at least three applications of a transmitter, dopamine (10 nA) was simultaneously applied by iontophoresis during three or more additional pulses of the original

transmitter. This system also made it possible to assess whether dopamine, at the ejection current used, altered baseline firing rates (spontaneous firing between 30-second drug pulses).

Comparison of baseline activities showed that iontophoresis of dopamine caused slow increases in firing rates of many, but not all, pars reticulata cells tested. Of 41 neurons studied, 20 were stimulated by 20 percent or more. Five cells were slightly inhibited. The average change in baseline activity for all cells monitored was an increase of 28 ± 5 percent ($P < .001$). In some instances, dopamine-induced excitations persisted for 5 minutes or longer after iontophoresis of dopamine was stopped. Our observation that iontophoretically applied dopamine can stimulate reticulata neurons is consistent with the findings of Ruffieux and Schultz (*9*).

In addition to an ability to excite some cells, dopamine applied by iontophoresis consistently and markedly attenuated inhibitory responses of reticulata neurons to iontophoretic pulses of GABA (Fig. 1, a and c). The attenuations of GABA responses were not dependent on, nor did they simply correspond in magnitude to, dopamine-induced increases in baseline firing. Indeed, we considered dopamine to have attenuated inhibitory responses to GABA only if dopamine caused an increase in firing during GABA pulses that exceeded any dopamine-induced increases in baseline firing between pulses. By this criterion, it was necessary for GABA inhibitions to be diminished by dopamine to a greater degree (in spikes per second) than baseline activity was increased (in spikes per second). In cases when dopamine caused increases in baseline firing, this method of evaluation (comparing absolute changes in numbers of spikes) was a

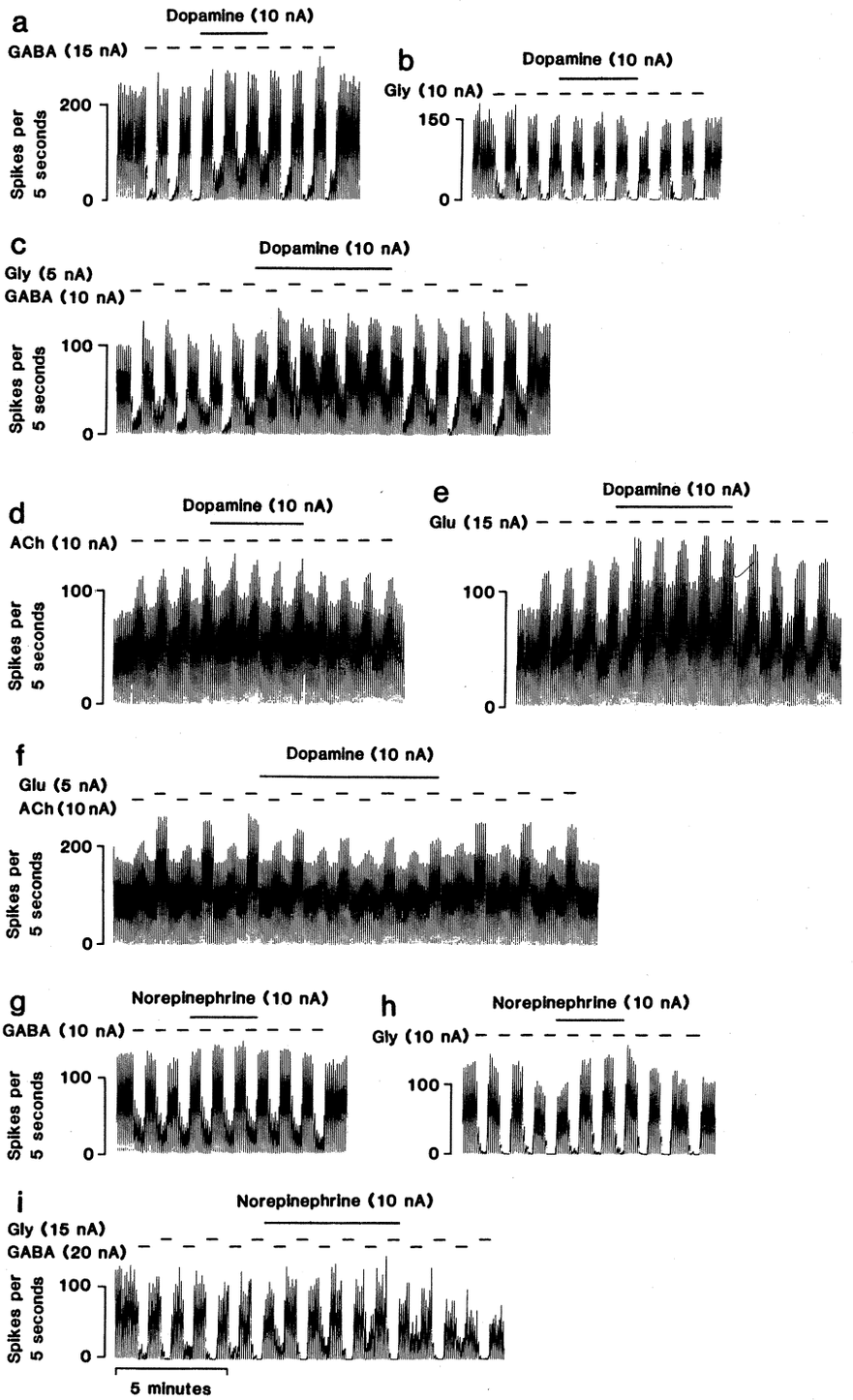

Fig. 1. Rate meter recordings of interactions between iontophoretically applied transmitters on activity of substantia nigra pars reticulata neurons. (a and c) Dopamine sometimes caused increases in baseline firing, but consistently attenuated inhibitory responses of cells to GABA. (b and c) Dopamine both potentiated (b) and attenuated (c) responses to glycine (*Gly*). Similarly, excitatory responses to acetylcholine (*ACh*) and glutamic acid (*Glu*) were not consistently affected by applied dopamine. (d and f) Acetylcholine-elicited excitations were generally unchanged during dopamine application. (e) Dopamine caused similar increases in baseline firing and firing during glutamic acid iontophoresis while (f) responses to glutamic acid were attenuated by dopamine. (g and i) GABA-induced inhibitions were either unaffected (g) or attenuated (i) by iontophoretically applied norepinephrine. (h and i) Glycine responses, in the cases shown, were unaffected by norepinephrine. Glass micropipettes with four side chambers and a central recording barrel were used for iontophoresis. Drug solutions were as follows: GABA (0.001*M* in 0.2*M* NaCl, *p*H 4), glycine (0.1*M*, *p*H 4), acetylcholine (0.2*M*, *p*H 5), glutamic acid (0.02*M*, *p*H 8.6), dopamine hydrochloride (0.2*M*, *p*H 4), and norepinephrine bitartrate (0.2*M*, *p*H 4). The impedance of the recording barrel, filled with 2*M* NaCl containing 2 percent Pontamine sky blue, ranged from 2 to 7 megohms. One side barrel (impedance, 15 to 50 megohms) was routinely filled with 4*M* NaCl and served as a balance channel. The three remaining drug-filled barrels typically had impedances of 65 to 100 megohms. Drugs were ejected with positive currents, except for glutamic acid, which required a negative current. The ejection currents selected for GABA and glycine could inhibit firing by at least 50 percent, but not totally. Iontophoretic currents chosen for acetylcholine and glutamic acid increased firing by at least 15 percent. GABA was usually paired with glycine and acetylcholine was usually paired with glutamic acid in these trials. Between periods of iontophoresis, a negative retaining current of 10 nA was passed through all drug barrels, except for that of glutamic acid, which received a positive retaining current of 10 nA. Bars above records indicate the duration of the designated ejection current for each drug.

more stringent determinant of modulatory interactions than a comparison of percentage changes in firing would be. No underlying mechanism of interaction between the two test transmitters was implied by our use of the more stringent criterion.

The results of all tests for interactions between dopamine and GABA were evaluated from a 45° equivalence plot of the data (Fig. 2a) (*10*). Points (closed circles) above the 45° line represent cells that fulfilled our criterion for dopamine-mediated attenuation of inhibitory responses to GABA. Of the 19 cells tested, only two (points touching the 45° line) exhibited essentially equal increases in baseline firing and firing during GABA applications. The other 17 points (above the 45° line) displayed attenuations of GABA responses that were greater than changes in baseline activity. Dopamine also reduced inhibitory responses to GABA in cases when dopamine had little or no significant effect on baseline firing rates (points nearest to the vertical axis). For many reticulata cells, the attenuation continued for several minutes after iontophoresis of dopamine was stopped; other neurons recovered their responses to GABA more quickly (Fig. 1, a and c).

Both the stimulation of baseline firing and the attenuation of GABA-mediated responses by dopamine were less pronounced at an ejection current of 5 nA than at 10 nA ($N = 5$); at 2 nA there were no effects, or responses were further diminished ($N = 6$).

The consistent modulatory interaction of dopamine and GABA appeared to be specific for these two transmitters. Iontophoretically applied dopamine had variable effects on responses of cells to glycine, in some instances attenuating and in others potentiating glycine-evoked inhibitions (Fig. 1, b and c). The variability of the interaction between dopamine and glycine was apparent from the similar distribution of points (open circles) above and below the 45° equivalence line (Fig. 2a). Similarly, no consistent modulatory interactions were detected between dopamine and either of two transmitters known to stimulate reticulata cell firing, acetylcholine and glutamic acid (Fig. 1, d to f). The inconsistent nature of the interactions between acetylcholine or glutamic acid and dopamine was reflected by nearly equal numbers of points on the two sides of a 45° equivalence line (Fig. 2b).

The specificity of dopamine for stimulating reticulata cell firing and attenuating inhibitory responses to GABA was evaluated by substituting norepinephrine, a structurally related catecholamine neurotransmitter, for dopamine in the test procedure. Norepinephrine and dopamine applied iontophoretically at equimolar concentrations and ejected at the same current (10 nA) caused similar average increases in baseline activity (28 ± 9 percent, $N = 18$, $P < .01$). Norepinephrine did not, however, consistently modify responses to either GABA or glycine (Fig. 1, g to i), as evidenced by points on either side of the equivalence line for each test pair (Fig. 2c). When 0.2*M* NaCl was substituted for dopamine and ejected at 10 nA, average baseline firing rates were not significantly changed ($N = 12$), and responses to GABA were variably but usually only slightly affected (attenuated by more than 10 percent for two cells, potentiated by 10 percent or more for three cells, and generally unaffected for seven cells). These findings suggest that the rate-increasing effect produced by dopamine may be produced by other catecholamine transmitters, but the consistent modulation of reticulata cell responses to GABA appears to be specific for dopamine and cannot be attributed simply to chloride effects due to prolonged application of the balance current during iontophoresis of dopamine.

The mechanism underlying the observed interaction of GABA and dopa-

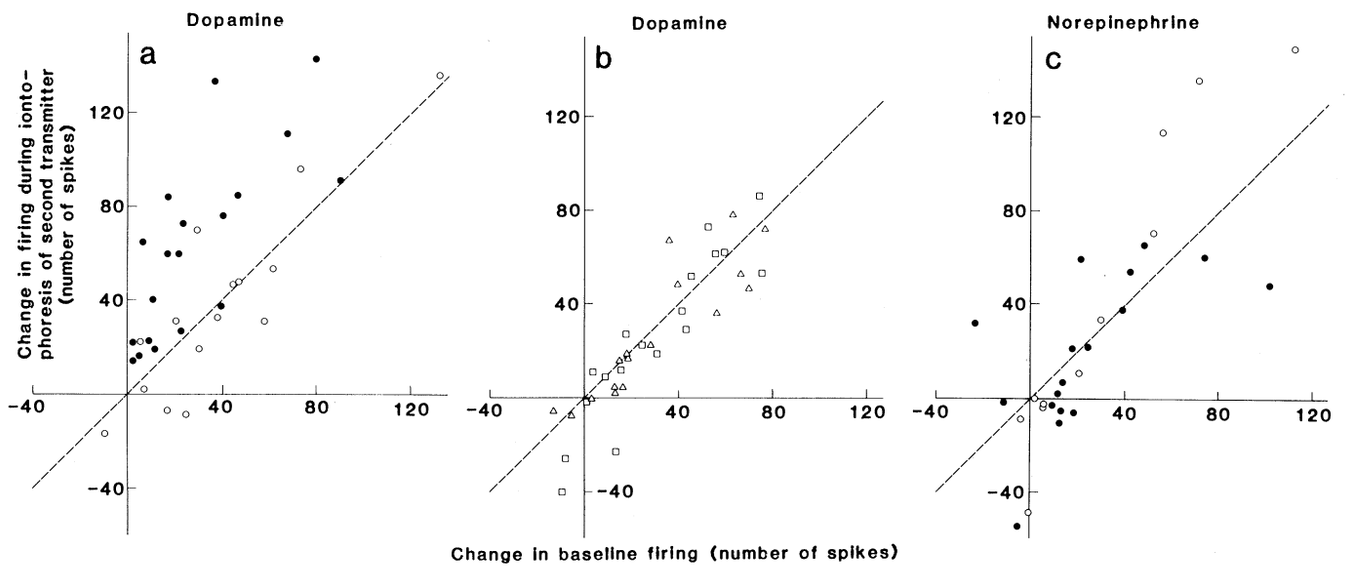

Fig. 2. Equivalence plots of dopamine- and norepinephrine-induced changes in baseline firing rate versus changes in firing during iontophoresis of other transmitters. (a) Interactions of dopamine with GABA (●) and glycine (○). Responses that were attenuated by dopamine are above the 45° line, whereas responses that were potentiated by dopamine are below the line. Dopamine consistently attenuated responses of cells to GABA (see text), but no consistent interaction emerged between dopamine and glycine. Glycine inhibitions were attenuated for four cells, unchanged for five cells, and augmented for six cells. (b) Interactions of dopamine with acetylcholine (△) and glutamic acid (□). Excitatory responses that were attenuated by dopamine are below the equivalence line and potentiated responses are above the line. No consistent interactions were observed for either test pair. Acetylcholine-induced excitations were attenuated for six cells, unchanged for seven cells, and potentiated for four cells. Similarly, glutamic acid–evoked responses were attenuated for six cells, unaffected for seven cells, and augmented for five cells. (c) Interactions of norepinephrine with GABA (●) and glycine (○). As in (a), attenuated responses to GABA or glycine are above the line, and potentiated responses are below the line. Norepinephrine did not consistently modify inhibitions elicited by either GABA or glycine. GABA responses were attenuated for five cells, unchanged for three cells, and potentiated for nine cells. Glycine inhibitions were attenuated for five cells, unchanged for two cells, and potentiated for five cells. Responses to test transmitters were categorized as either attenuated or potentiated by dopamine or norepinephrine if the absolute value of the difference (in number of spikes) between the x and y values for a point exceeded 5 percent of the original baseline firing rate for that cell. Differences of less than 5 percent were interpreted as showing no effect on responses to the test transmitter.

mine and the dopamine-induced increases in firing are not yet understood. However, it seems unlikely that the attenuation of GABA responses by dopamine involves a presynaptic action, such as an ability of dopamine to block release of GABA from striatonigral terminals impinging on reticulata neurons. Such an action would be expected to cause fixed increases in firing over the entire period of dopamine application, rather than the attenuations we observed during GABA pulses. A postsynaptic mechanism, such as a dopamine-mediated change in GABA receptor binding kinetics or in ion currents elicited by the interaction of the two transmitters at the membrane level, may provide a more likely explanation for the apparent modulation. The dopamine-induced increases in baseline firing, however, may reflect a direct excitatory action, a presynaptic action such as that described above, or a dopamine-mediated modulation of the effects of locally released GABA.

Further investigations will be required to ascertain the precise physiological relevance of the dopamine-mediated modulation. Endogenous release of dopamine by amphetamine also appears to diminish reticulata cell responses to iontophoretically applied GABA (*11*). It will be of interest to determine whether inhibition of reticulata neurons evoked by striatal stimulation, and presumably mediated by GABA, can be similarly attenuated by applied dopamine.

The monoamine neurotransmitters norepinephrine and serotonin have been assigned roles as neuromodulators (*12*), and we have now presented evidence for a modulatory function of dopamine. We have observed similar dopamine-induced attenuations of neuronal responses to GABA in the globus pallidus (*13*), the second output nucleus of the basal ganglia which has been reported to receive a sparse but widespread dopamine innervation from the SN (*14*). Demonstration of the interaction of GABA and dopamine in these two nuclei raises the possibility that dopamine may have a modulatory function in other areas of the central nervous system.

The nigrostriatal dopamine system has traditionally been viewed as influencing movement primarily by release of dopamine at postsynaptic sites within the striatum. Our results suggest that the net effect of dopamine on transmission of motor commands may reflect the combined actions of dopamine within the striatum and the SN. The ability of dopamine to act directly on basal ganglia output neurons to lessen their responses to GABA represents a means by which nigral dopamine neurons could influence transmission of movement-related messages without directly involving the striatum. Specifically, pars reticulata neurons constitute the link in the striatonigrothalamic pathway, both receiving striatal GABA inputs (*15*) and projecting heavily to the motor thalamus (*2*). Many cells included in these studies (55 percent of the cells exhibiting the modulatory interaction) could be antidromically activated from the VM thalamus. In addition, pars reticulata neurons give rise to major projections to other movement-related areas, including the superior colliculus and reticular formation (*2*). These results demonstrate, therefore, that dopamine, released from dendrites within the pars reticulata, could serve an important local function downstream from the striatum in adjusting or fine-tuning the relay of striatal commands to premotor nuclei outside the basal ganglia.

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Diffusion Barrier in the Small Intestine

Smithson *et al.* (*1*) propose evidence for a diffusion barrier in the intestinal glycocalyx capable of impeding the transfer of low molecular weight nutrient precursors (disaccharides and short peptides) toward brush border enzyme sites. They are led to their conclusion in part by calculations of the thickness of "an unstirred layer." They consider the values obtained as "unphysiological" in that nearly half the intestinal volume would represent a zone of "intestinal fluid stasis." The authors propose an additional "important diffusion barrier" aside from aqueous diffusion zones, which presumably would justify shorter diffusion distances.

The authors' rejection of the unstirred layer thickness as unphysiological is based on an erroneous perception of what the thickness means and how it arises hydrodynamically. Analysis shows that for given values of the intestinal dimensions, stream flow rate, and substrate diffusivity, there exist a continuum of "unstirred layers" (more precisely, the diffusion boundary layer) commencing with nearly zero thickness at the stream entrance and growing as a function of the intestinal axis coordinate on passing to the distal end. Strictly speaking, the boundary layer reflects the ever decreasing radial concentration gradient of substrate along the axis coordinate. There is no macroscopic zone of fluid statis; fluid is convected in the axial direction including that containing the radial concentration gradients. For the laminar flow conditions in the experiments and in the case of complete diffusion control, the boundary layer approximation (*2, 3*) gives for the diffusion boundary layer:

$$\delta = 1.4693\left(\frac{DRz}{v_m}\right)^{1/3}$$

where D is the substrate diffusion coefficient, R is the radius (assuming a minimum cylinder), z is the axis coordinate, and v_m is the maximum stream velocity