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Self-Inhibition by Dopaminergic Neurons

An alternative to the "neuronal feedback loop" hypothesis for the mode of action of certain psychotropic drugs.

Philip M. Groves, Charles J. Wilson, Stephen J. Young, George V. Rebec

The mechanisms of action of amphetamine and the antipsychotic drugs have been the subject of intense empirical and theoretical interest for a number of years. Amphetamine appears to act, in large measure, by promoting the release of catecholamines from central and peripheral nerve endings and blocking their reuptake across the presynaptic membrane (*1, 2*). Antipsychotic drugs such as the phenothiazines and butyrophenones are currently believed to act in part by blocking catecholaminergic transmission, especially synaptic transmission in dopaminergic pathways in the central nervous system (*3–5*). These findings support the view that alterations in catecholaminergic transmission in the central nervous system may be significant in drug-induced or idiopathic psychotic disorders (*6, 7*).

One prominent behavioral effect of amphetamine administration in experimental animals is an induction of "stereotyped behaviors" which, in the rat, include compulsive stereotyped biting, licking, gnawing, and sniffing (*8, 9*). When amphetamine is administered for periods of days or weeks, various components of these stereotyped behaviors become progressively more intense (*10, 11*) while a variety of the other effects of amphetamine, such as anorexia and hyperthermia, show evidence of tolerance following long-term administration (*12*). In humans, long-term amphetamine abuse may result in a clinical disorder termed amphetamine psychosis, which is sufficiently similar to paranoid schizophrenia that the former has been regarded as a valuable heuristic model for the latter, and the progressive augmentation of stereotyped behavior that occurs in experimental animals following long-term amphetamine administration has been regarded as a useful experimental model for understanding the mechanisms by which amphetamine psychosis develops (*5, 7, 9, 11, 13*).

It is currently believed, on the basis of several lines of evidence (*14, 15*), that the stereotyped behavior produced by amphetamine is dependent in part on the integrity of catecholaminergic transmission in a pathway arising principally from cell bodies in the pars compacta of the substantia nigra in the brainstem and projecting ipsilaterally to the caudate-putamen, often termed the nigro-neostriatal bundle (*16*). This pathway has also been implicated in the etiology of Parkinson's disease, in which it shows progressive degeneration (*17*). Amphetamine acts on the nigro-neostriatal projection in part by releasing dopamine from the terminals of this pathway and blocking its reuptake across the presynaptic membrane (*2, 18, 19*). Release of dopamine from these terminals by amphetamine is accompanied by a marked inhibition of the activity of many neurons in the caudate-putamen (*20–24*), which is consistent with the role of dopamine as an inhibitory neurotransmitter (*24–26*). Many antipsychotic drugs block dopaminergic transmission in the nigro-neostriatal system and produce increased neuronal firing in elements postsynaptic to dopaminergic nerve terminals (*23*).

The "neuronal feedback loop" hypothesis, proposed initially by Carlsson and Lindqvist (*27*), is one of several theoretical models currently used to account for the regulation of dopaminergic transmission and biosynthesis in the nigro-neostriatal pathway (*28, 29*). Systemic administration of pharmacological agents that facilitate dopaminergic transmission, such as amphetamine, produce a marked inhibition of neuronal activity in the neostriatum (*20–23*) and a similar depression of neuronal firing in dopaminergic neurons in the substantia nigra (*21, 30, 31*). In contrast, systemic treatments with agents that block dopaminergic transmission, such as the antipsychotic drug haloperidol, produce an increase in neuronal firing in the caudate-putamen and in the dopaminergic neurons of the substantia nigra; such agents also block the depression of neuronal firing in both regions produced by prior amphetamine administration (*23, 29, 30, 32*). The effects of amphetamine and the antipsychotic drugs on the activity of dopaminergic neurons of the substantia nigra, located principally within the pars compacta region of this nucleus, have been presumed to occur at least in large measure by means of a neuronal feedback loop from the basal ganglia to the substantia nigra (*4, 21, 29,*

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30, 33). Conceptually, this hypothetical neuronal feedback loop may be viewed as a negative feedback mechanism by which increased release of dopamine from dopaminergic terminals in the neostriatum, and therefore increased stimulation of dopaminergic postsynaptic receptors, leads to decreased impulse flow in dopaminergic neurons. Blockade of dopaminergic transmission in the caudate-putamen, on the other hand, results in decreased stimulation of neostriatal dopamine receptors and increased firing of postsynaptic elements in the caudate-putamen and dopaminergic neurons of the substantia nigra.

Nigro-Striatal Relations and the Self-Inhibition Hypothesis

We have developed a theoretical view of nigro-striato-nigral interconnections and the mechanisms of action of amphetamine and the antipsychotic drugs which is based on a novel integration of information available in the literature and the results of a series of experiments designed to test these theoretical formulations. The anatomical, physiological, and pharmacological evidence currently available in the literature and the results of our experiments suggest that the inhibition of neuronal activity produced in the pars compacta of the substantia nigra by systemic amphetamine may be achieved not by means of a neuronal feedback loop, but rather by the independent effect of amphetamine on a dopaminergic inhibitory mechanism intrinsic to the substantia nigra. Similarly, the antipsychotic dopamine antagonists may produce an increase in neuronal firing rates by the simultaneous but independent blockade of dopaminergic transmission in the neostriatum and pars compacta of the substantia nigra. Although there is a neuronal feedback loop from the basal ganglia to the substantia nigra, the weight of our evidence suggests that with respect to dopaminergic transmission, this is a positive feedback mechanism. According to our hypothesis, this feedback loop consists of cholinergic interneurons innervated by ascending dopaminergic projections; these excitatory interneurons drive descending inhibitory elements that release gamma-aminobutyric acid (GABA) onto dopaminergic neurons in the substantia nigra. The effects of this positive feedback loop would be masked by the simultaneous effects of systemically administered amphetamine and the dopamine antagonists on the independent mechanisms of dopaminergic transmission in the caudate-putamen and pars compacta of the substantia nigra. We propose that this tonic dopaminergic inhibition intrinsic to the substantia nigra may consist of a mode of neurohumoral regulation involving release of dopamine from dendrites of dopaminergic neurons, which acts on postsynaptic receptors to produce inhibition of neuronal firing—that is, self-inhibition. This self-inhibition hypothesis and our hypothesized striato-nigral feedback loop are illustrated schematically in Fig. 1.

There is a wide variety of evidence suggesting the existence of the elements proposed in the theoretical model illustrated in Fig. 1. Dopaminergic neurons originate in the substantia nigra, pars compacta, and ascend to terminate ipsilaterally within the caudate-putamen in a wide variety of mammals (*16*). Dopaminergic projections to the caudate-putamen appear to end at least in part on cholinergic interneurons that are intrinsic to the basal ganglia (*34*).

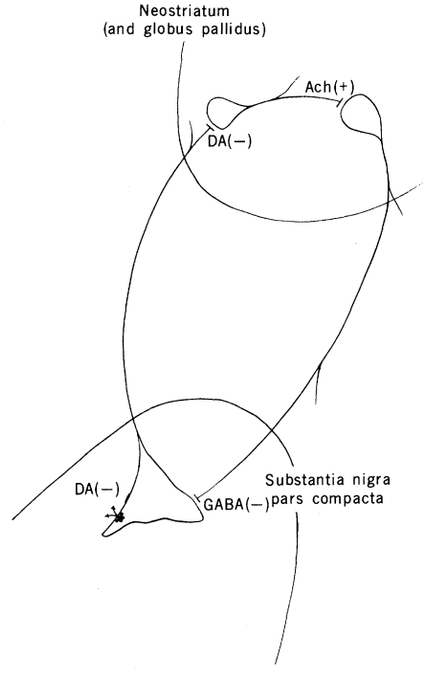

Fig. 1. Schematic illustration of a novel theoretical view of dopaminergic neurons of the substantia nigra and their functional interconnections with neurons of the basal ganglia. Dopaminergic neurons release dopamine (DA) from axon terminals in the caudate-putamen to inhibit (−) the activity of cholinergic interneurons. These interneuronal elements release acetylcholine (Ach), which is excitatory (+), on striatal efferent neurons which, in turn, project to dopaminergic neurons in the substantia nigra. The striatal efferents release gamma-aminobutyric acid (GABA), which is inhibitory (−). In addition, dopaminergic neurons are inhibited by dopamine released from their dendrites in response to amphetamine administration. Thus, systematically administered amphetamine produces an inhibition of neuronal firing in dopaminergic neurons as well as neurons in the caudate-putamen due to the simultaneous release of dopamine in both areas. A variety of other afferent, efferent, and intrinsic excitatory and inhibitory connections of the basal ganglia and substantia nigra are not shown.

Facilitation of dopaminergic transmission produces an increase in the concentration of acetylcholine in the corpus striatum, presumed to be secondary to the inhibition of impulse activity in cholinergic interneurons. Similarly, agents that block dopaminergic transmission result in a decrease in acetylcholine concentration and increased release of acetylcholine from the caudate-putamen, presumed to occur by means of increased firing by cholinergic interneurons released from dopaminergic inhibition (*35, 36*). It has also been reported that, in contrast to the marked inhibitory effects typically seen following iontophoretic application of dopamine to neurons in the caudate-putamen (*24–26*), iontophoretic application of acetylcholine to neurons in the caudate-putamen produces an excitation in many of the responsive neurons (*26*). Although our hypothesized connection between cholinergic striatal interneurons and descending inhibitory striato-nigral efferents has not been demonstrated, a striato-nigral projection has been known for some years (*36, 37*), and direct electrical excitation of neurons in the corpus striatum produces an inhibition of neuronal firing in the substantia nigra which can be blocked by systemically administered picrotoxin, in part a GABA antagonist (*38*). Further, this descending pathway from the corpus striatum to the substantia nigra contains and transports GABA (*36, 39*), and it has been shown that this substance can be released in the substantia nigra of the rat in vitro (*40*). Iontophoretic application of GABA to neurons in the substantia nigra also produces inhibition of neuronal firing which is blocked by picrotoxin (*41, 42*).

The self-inhibitory mechanism illustrated schematically in Fig. 1 is supported by several observations in addition to our experimental results. The pioneering work of Aghajanian and Bunney (*42*) has established that iontophoretic application of dopamine or other directly acting dopamine agonists to dopaminergic neurons in the substantia nigra produces inhibition of neuronal firing which is blocked by systemically administered dopamine antagonists (such as haloperidol). They suggested that dopaminergic neurons possess receptors for dopamine. In addition, the more recent anatomical evidence of Bjorklund and Lindvall (*43*) has revealed that dopaminergic neurons in the substantia nigra possess numerous small swellings or varicosities along their dendrites which contain high amounts of dopamine and, according to these authors (*43*, p. 535), "would be consistent with a hitherto unknown function of DA as a transmitter in a dendro-dendritic synapse." Indeed, Hadju *et al.* (*44*) identified accumulations of "synaptic vesicles"

in some of the dendritic varicosities of neurons in the pars compacta of the substantia nigra (*45*).

Tests of Hypothesis

To test the theoretical formulations outlined in Fig. 1, we infused amphetamine or haloperidol (dissolved in sterile physiological saline at *p*H 6.8 to 7.1) directly into the caudate-putamen or substantia nigra at low rates, and in low volumes and concentrations, while recording changes in spontaneous neuronal firing rates in these two structures simultaneously (*46*). Data reported here were derived from a series of experiments carried out on 83 male Sprague-Dawley albino rats weighing 250 to 450 grams at the time of experimentation. Surgery was performed while animals were anesthetized with ether, all points of surgical and stereotaxic contact were infiltrated thoroughly with procaine hydrochloride and lidocaine anesthetic ointment, supplemented at intervals of 1 to 2 hours. The preparation was then immobilized with *d*-tubocurarine and artificially respired, as described in detail elsewhere (*22, 47*). Heart rate (typically 340 to 380 beats per minute), body temperature (typically between 36.5° and 37.5°C), and breath-by-breath expired carbon dioxide (typically 4 ± 0.5 percent) were monitored continuously. A 32-gauge stainless steel infusion cannula connected to a Hamilton microsyringe by means of Teflon tubing was lowered stereotaxically into the caudate-putamen or substantia nigra, along with an extracellular glass-coated tungsten microelectrode having a tip diameter of from less than 1 to 2 micrometers and impedance of 0.5 to 3.0 megohms. A similar microelectrode was then lowered into the structure not being infused, so that changes in neuronal activity produced by local infusion into one of these areas could be monitored in both structures simultaneously. We usually allowed 60 minutes or more to elapse after electrode placement to isolate neuronal activity with the microelectrodes and to allow firing to stabilize. Effective concentrations and volumes of agents infused into the neostriatum or substantia nigra were determined in preliminary experiments by comparison of the effects of intraperitoneally administered haloperidol (1 to 2 mg/kg) or amphetamine (0.5 to 2 mg/kg) on neuronal activity in the caudate-putamen and substantia nigra with different concentrations and volumes of these agents infused directly into the neostriatum or substantia nigra. At the end of each experiment the animal was killed by pentobarbital injection and small d-c lesions marked electrode tip placements. The subject was then perfused with normal saline followed by formalin, and the brain was removed, frozen, sectioned, and stained with cresyl violet for localization of electrode tip placements and the tip of the infusion cannula.

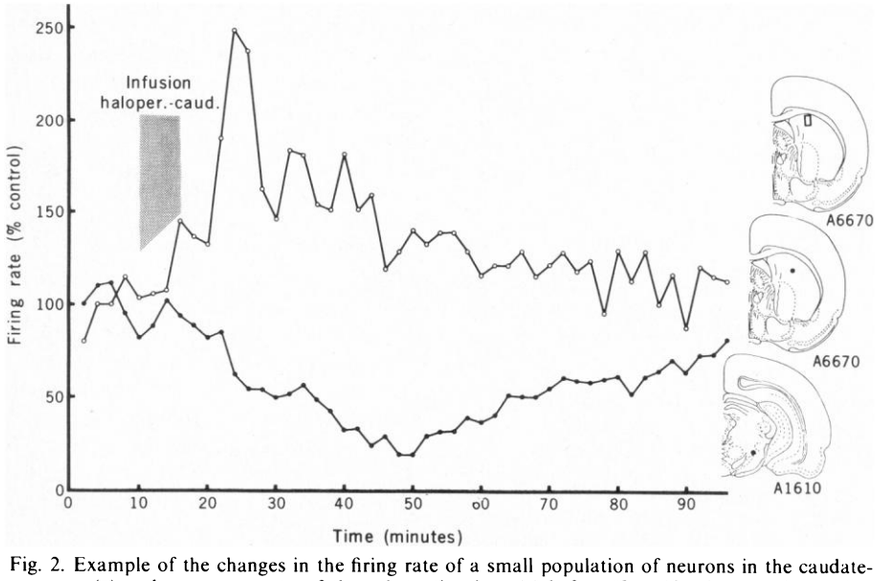

Fig. 2. Example of the changes in the firing rate of a small population of neurons in the caudate-putamen (○) and pars compacta of the substantia nigra (●) before (first 10 minutes), during (indicated by the shaded bar), and after local infusion of $5 \times 10^{-5}M$ haloperidol directly into the caudate-putamen. The spontaneous rate at the recording electrode in the caudate-putamen was 106 spikes per minute, while in the substantia nigra the mean firing rate was 331 spikes per minute, based on the 10-minute sample of neuronal firing shown above before drug infusion. Here and in Figs. 3 to 6 these mean predrug firing rates are represented as 100 percent and all changes in activity are plotted as percentages of control firing rate for each structure. The position of the infusion cannula and its approximate angle of entry are illustrated in the histological drawing at the upper right. The middle drawing illustrates the approximate position of the microelectrode tip in the caudate-putamen (●), and the lower drawing illustrates the approximate position of the tip of the recording microelectrode in the substantia nigra. The histological sections and their stereotaxic positions in Figs. 2 to 6 are after Koenig and Klippel (*61*).

In our initial series of experiments, haloperidol, an antipsychotic drug that blocks dopaminergic transmission, was infused directly into the caudate-putamen while the spontaneous firing rates of single or small populations of neurons (two to eight, approximately) were recorded simultaneously from the caudate-putamen and substantia nigra ($N = 11$; N means the number of animals in the experiments). Figure 2 illustrates, for one animal, the results of infusion of $5 \times 10^{-5}M$ haloperidol for a period of 6 minutes into the caudate-putamen at a rate of 1.4 μl/hour; the total infusion volume was 0.14 μl. Whereas the neuronal feedback loop hypothesis predicts that such local blockade of dopaminergic transmission in the caudate-putamen would result in an increase in neuronal activity in both the caudate-putamen and substantia nigra, our theoretical formulation predicts an increase in neuronal firing rates in the caudate-putamen, but a simultaneous inhibition of neuronal activity in the substantia nigra due to activation of the descending inhibitory projection from the basal ganglia to the substantia nigra. As shown in Fig. 2, when haloperidol infused directly into the caudate-putamen produced a marked increase in neuronal activity in the neostriatum, similar to its effect following intraperitoneal or intravenous administration, a simultaneous depression of neuronal activity typically occurred in the pars compacta of the substantia nigra, consistent with the theoretical view outlined in Fig. 1. Similar effects were obtained during simultaneous recording from nondopaminergic regions of the substantia nigra (*48*).

In a second series of experiments, we infused amphetamine directly into the caudate-putamen while recording changes in neuronal activity simultaneously from a site near the infusion cannula and from the substantia nigra ($N = 15$). Whereas the neuronal feedback loop hypothesis predicts a depression of neuronal activity in the caudate-putamen and substantia nigra from this procedure, our model predicts a depression of firing rate in the caudate-putamen, but a release from descending GABA-mediated inhibition projected from the striatum to substantia nigra resulting in increased impulse activity in neurons of the substantia nigra.

The results of one such experiment are illustrated in Fig. 3. In this experiment, *d*-

amphetamine sulfate was infused directly into the caudate-putamen in a concentration of $5 \times 10^{-5}M$ (concentration of free base) at a rate of 1 μl/hour for a period of 4 minutes, resulting in a total infusion volume of approximately 0.07 μl. As shown in Fig. 3, this resulted in a transient potentiation followed by a marked and long-lasting inhibition of neuronal firing in the caudate-putamen in this animal, and this was accompanied by a transient depression and subsequent dramatic increase in neuronal activity in the substantia nigra (*49*). The increase in firing rate, which we believe is due to the release of these neurons from descending GABA-mediated inhibition, was often transient (10 to 30 minutes) and would yield to a period of relative silence in neurons of pars compacta; the firing rate usually returned subsequently to about the level observed before the drug was administered, as illustrated in the example in Fig. 3. In several animals ($N = 3$) we were able to reverse the period of inhibition of neuronal firing with systemically administered haloperidol (1 to 2 mg/kg, intraperitoneally).

This apparently intrinsic effect did not appear to characterize neurons in nondopaminergic regions of the substantia nigra where descending striato-nigral projections are also known to terminate (*36, 37*). We recorded the effects of amphetamine infused directly into the caudate-putamen on changes in neuronal activity recorded simultaneously in the caudate-putamen and in the nondopaminergic pars reticulata or pars lateralis of the substantia nigra ($N = 4$), and the results of one of these experiments are illustrated in Fig. 4. Amphetamine, infused in a concentration of $5 \times 10^{-5}M$ at a rate of 1 μl/hour for a period of 4 minutes, produced a marked and long-lasting inhibition of neuronal activity at the recording site in the caudate-putamen, and this was accompanied by a mirror-image increase in neuronal firing rate in pars reticulata of the substantia nigra, with no evidence of the subsequent inhibitory process that characterized the majority of neurons in the dopaminergic pars compacta of the substantia nigra (*49*). While it is tempting to speculate that this inhibitory process is related to the dendritic release of dopamine from active dopaminergic neurons, a variety of other intrinsic regulatory properties of these neurons remains to be explored (*50*).

In order to test the hypothesis that systemically administered amphetamine and haloperidol achieve their effects on dopaminergic neuronal firing rates by an action on dopaminergic transmission intrinsic to the substantia nigra, we completed several other series of experiments in which haloperidol ($N = 8$) or amphetamine ($N = 14$) was infused directly into the substantia nigra, while the effects of this infusion were recorded in both the substantia nigra and the caudate-putamen. Our model predicts that haloperidol infused directly into the substantia nigra should produce an increase in neuronal firing rates of dopaminergic neurons due to blockade of the self-inhibitory mechanism. We also expected that increased impulse activity in dopaminergic neurons would be reflected in the caudate-putamen by a simultaneous depression of neuronal firing due to the release of dopamine from nigro-neostriatal axonal endings. The results of one of these experiments are illustrated in Fig. 5. In this experiment, haloperidol was

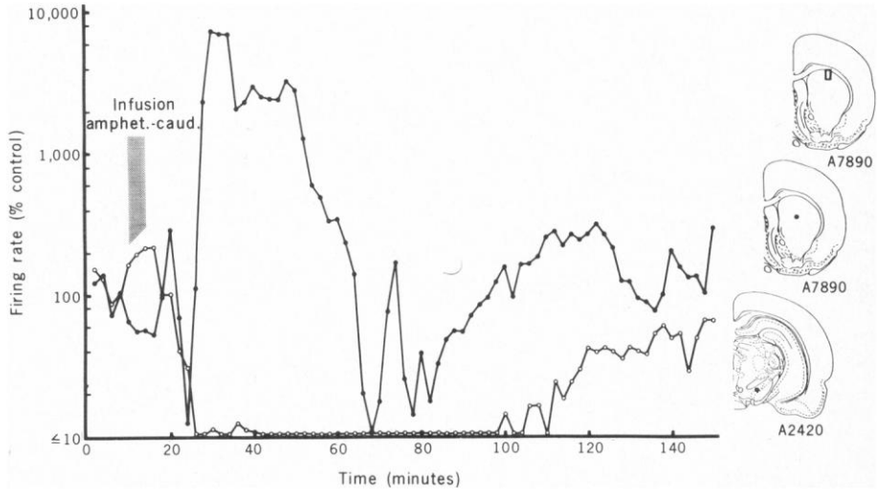

Fig. 3. An example of the changes in the firing rate of a small population of neurons in the caudate-putamen (○) and pars compacta of the substantia nigra (●) for 10 minutes before, during (indicated by the shaded bar), and after local infusion of $5 \times 10^{-5}M$ *d*-amphetamine directly into the caudate-putamen. Before infusion the mean rate of neuronal firing in the caudate-putamen was 98 spikes per minute, while in the substantia nigra the mean population firing rate was 32 spikes per minute, each of which is represented by 100 percent. The position of the infusion cannula and its approximate angle of entry are illustrated in the histological drawing at the upper right, while the positions of the recording microelectrode tips in the caudate-putamen and substantia nigra are illustrated in the middle and lower drawings, respectively. Percentage alterations in firing rate were sufficiently large that a semilogarithmic scale was necessary.

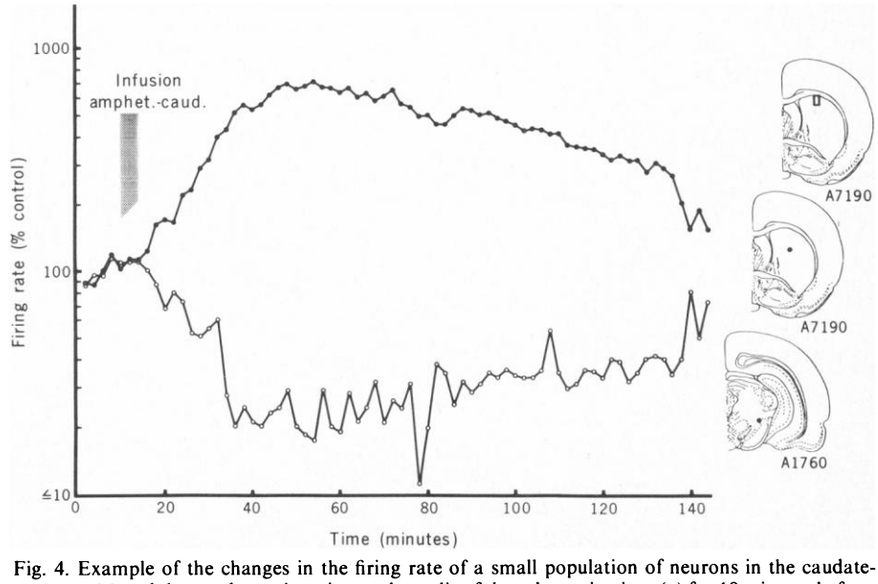

Fig. 4. Example of the changes in the firing rate of a small population of neurons in the caudate-putamen (○) and the nondopaminergic pars lateralis of the substantia nigra (●) for 10 minutes before, during (indicated by the shaded bar), and after local infusion of $5 \times 10^{-5}M$ *d*-amphetamine directly into the caudate-putamen. The mean firing rate before drug infusion was 180 spikes per minute at the recording site in the caudate-putamen, while in the substantia nigra, pars lateralis, the predrug firing rate was 207 spikes per minute. The position of the infusion cannula and its approximate angle of entry are illustrated in the histological drawing at the upper right. The middle and lower drawings illustrate the approximate positions of the tips of the recording electrodes in the caudate-putamen and substantia nigra, respectively.

infused directly into the substantia nigra, pars compacta, in a concentration of $1 \times 10^{-9}M$ at a rate of 1 μl/hour for a duration of 4 minutes, resulting in a total infusion volume of 0.07 μl. This produced a marked increase in neuronal firing in the substantia nigra, pars compacta, and a simultaneous inhibition of neuronal activity usually occurred in the caudate-putamen (*51*). In a final series of experiments, amphetamine was infused directly into the pars compacta of the substantia nigra while neuronal firing rates there and in the caudate-putamen were simultaneously recorded. An example of the results of this infusion is illustrated in Fig. 6. Amphetamine was infused directly into the pars compacta of the substantia nigra in a concentration of $1 \times 10^{-5}M$ at a rate of 1 μl/hour for a duration of 6 minutes, resulting in a total infusion volume of 0.1 μl. As shown in Fig. 6, this procedure resulted in a dramatic and long-lasting inhibition of neuronal activity in the pars compacta of the substantia nigra, and a simultaneous increase in neuronal firing rates typically occurred at the remote recording site in the caudate-putamen (*52*).

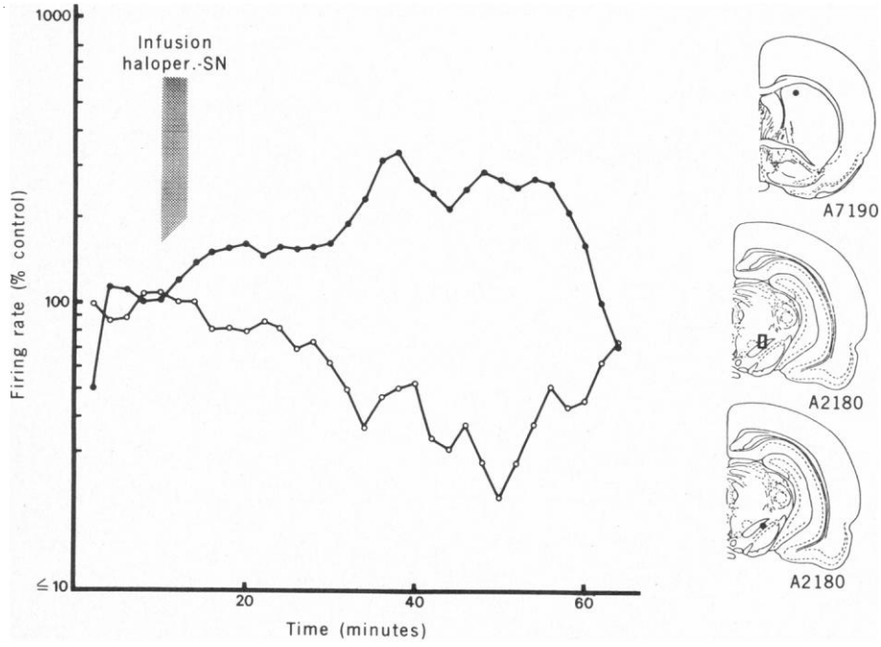

Fig. 5. Example of the changes in the firing rate of a small population of neurons in the substantia nigra (●) and caudate-putamen (○) for 10 minutes before, during (indicated by the shaded bar), and after local infusion of $1 \times 10^{-9}M$ haloperidol directly into the substantia nigra. Before drug infusion the mean firing rate at the recording electrode in the caudate-putamen was 410 spikes per minute, while in the substantia nigra it was 140 spikes per minute. The approximate position of the tip of the microelectrode in the caudate-putamen is illustrated in the histological drawing to the upper right, while the approximate position of the tip of the infusion cannula and the recording electrode in the substantia nigra are shown in the middle and lower drawings, respectively.

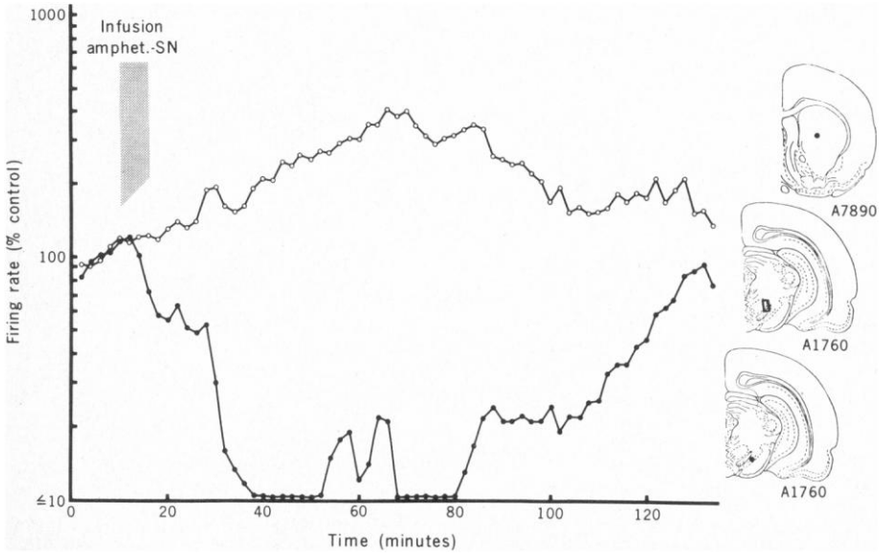

Fig. 6. Example of changes in the firing rate of a small population of neurons in the substantia nigra, pars compacta (●), and caudate-putamen (○) for 10 minutes before, during (indicated by shaded bar), and after local infusion of $1 \times 10^{-5}M$ *d*-amphetamine directly into the substantia nigra, pars compacta. Before drug infusion the mean rate of activity at the recording electrode in the caudate-putamen was 108 spikes per minute, while in the substantia nigra it was 529 spikes per minute. Approximate positions of the microelectrode recording tips and the tip of the infusion cannula are illustrated in the histological drawings to the right, as in Fig. 5.

Discussion

We believe that the results of these experiments, coupled with the wide variety of evidence cited earlier, are inconsistent with the neuronal feedback loop hypothesis but are consistent with the theoretical formulation illustrated in Fig. 1. Thus, the depression of neuronal activity produced in the substantia nigra, pars compacta, by systemically administered amphetamine may be achieved not by means of a neuronal feedback loop, but by means of the independent effect of amphetamine on dopaminergic transmission in this area. Similarly, the increase in neuronal firing rates in the neostriatum and pars compacta of the substantia nigra produced by systemic administration of many antipsychotic drugs may be achieved by blockade of the two independent mechanisms of dopaminergic transmission in these areas. The effects of the positive feedback loop arising from within the basal ganglia and projecting to the substantia nigra would be masked by the independent but simultaneous effects of these agents on dopaminergic transmission in the neostriatum and substantia nigra.

Aghajanian and Bunney (*42*) have made several observations which appear to be inconsistent with our hypothesis. They found that administration of amphetamine microiontophoretically to individual dopaminergic neurons in the substantia nigra produced only minimal depressions of neuronal firing at low ejection currents, when compared to the marked inhibition of neuronal firing produced by similarly iontophoretically applied dopamine or other direct-acting dopamine agonists to these neurons, or to the marked inhibition produced in the caudate-putamen by similar iontophoretic applications of amphetamine. These results suggested that amphetamine did not act directly to inhibit the firing of dopaminergic neurons and that it did not act indirectly within the substantia nigra, for example, by releasing dopamine from recurrent axon collaterals of dopaminergic neurons. They also observed that transections of the pathways

connecting the basal ganglia and substantia nigra abolished the depressant action of systemically administered amphetamine on dopaminergic neuronal activity. On the basis of these findings, they were led to conclude that the depression of neuronal firing in dopaminergic neurons following systemic amphetamine administration was not due to an action of amphetamine within the substantia nigra. Rather, the depression of firing of dopaminergic neurons was attributed to a neuronal feedback loop from the basal ganglia to the substantia nigra, a view later reiterated by Rebec and Groves (*21*).

While we agree with the conclusion of Bunney and Aghajanian (*53*) that "it would appear from the relative lack of response of DA cells to microiontophoretically injected d-AMP that recurrent collaterals are not involved in the marked slowing of these cells by low doses of systemically administered d-AMP," we believe that their evidence is not inconsistent with the view that amphetamine acts by releasing dopamine from dendrites to inhibit firing of dopaminergic neurons. The lack of effect of intravenously administered amphetamine on neuronal activity in pars compacta of the substantia nigra following diencephalic transections could have been secondary to the trauma of surgical disruption of nigro-neostriatal axons (*19, 54*). Further, while there is little convincing anatomical evidence for the existence of extensive recurrent dopaminergic axon collaterals in the substantia nigra, considering both light and electron microscopic evidence there appears to be a growing body of evidence suggesting that dopamine may be released from dendrites of dopaminergic neurons (*43–45*), and such a tonic dopaminergic inhibitory mechanism is consistent with the low firing rates characteristic of these cells as well as our observations on the effects of local infusion of amphetamine or haloperidol into the substantia nigra. Also significant are the observations of Parizek *et al.* (*55*) who, utilizing autoradiographic techniques, have shown that radioactively labeled catecholamines are taken up almost exclusively by somata and dendrites of neurons in pars compacta of the substantia nigra. Electron microscopic observations revealed that axon terminals were only rarely labeled.

Our theoretical hypothesis of nigrostriato-nigral functional interconnections is consistent with behavioral, biochemical, and pharmacological evidence suggesting the existence of an antagonistic dopamine-acetylcholine interaction in the basal ganglia. For example, the marked stereotyped behavior in rats and other species produced by amphetamine administration, and which is dependent in part on transmission in the nigro-neostriatal dopaminergic projection as well as the efferent pathways from the basal ganglia (*14, 15, 56*), is potentiated by anticholinergic drugs and weakly antagonized by agents that facilitate cholinergic transmission (*57*). It has been known for some years that scopolamine, an anticholinergic drug, is useful to some extent in treating Parkinson's disease, at least in cases that are not advanced, while facilitation of cholinergic transmission with physostigmine, for example, exacerbates the condition of such patients (*58*), and this disease is associated in part with an apparent reduction in the efficacy of dopaminergic transmission in the nigro-neostriatal system (*17*). Our view is also consistent with the recent demonstration by Kim and Hassler (*59*) that intraperitoneal administration of haloperidol to rats produces apparent increased release of GABA in the substantia nigra.

The existence of "presynaptic" anatomical specializations in the dendrites of neurons in many areas of the mammalian nervous system has been established for more than a decade, including neurons at all levels of the neuraxis. While little is known concerning the functional role of such presynaptic dendritic specializations, the possibility of release of neurotransmitters or other substances from regions of the nerve cell other than presynaptic axonal endings has been suggested by a number of workers on the basis of results obtained with anatomical as well as neurophysiological and biochemical techniques (*4, 60*). If the mechanism of dendritic release of dopamine in response to amphetamine is correct, as suggested by our experimental results and the other evidence cited above, it may open a new avenue of inquiry relevant to the mechanisms by which amphetamine as well as other agents achieve their pharmacological effects on neuronal activity and behavior. Such a mechanism may also bear on the means by which such dramatic behavioral effects occur in response to chronic amphetamine intoxication, including the augmentation of stereotyped behavior that develops following long-term amphetamine administration and perhaps the amphetamine psychosis that occurs following chronic amphetamine abuse in humans.

References and Notes

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45. J. Juraska, C. J. Wilson, and P. M. Groves (in preparation) have confirmed the existence of varicosities in the dendrites of neurons in pars compacta of the substantia nigra by using the rapid Golgi method. C. J. Wilson, P. M. Groves, and E. Fifkova (in preparation) have verified the existence of accumulations of synaptic vesicles in apparent dendritic profiles of neurons in pars compacta of the substantia nigra. Although the presence of vesicles in the dendrites of these neurons is consistent with the possibility of dendro-dendritic synapses between dopaminergic neurons in the substantia nigra, we believe that the evidence currently available is equally consistent with the view that dopamine is released directly into the extracellular space to inhibit neuronal activity.
46. We have performed autocorrelation and cross-correlation analyses on simultaneously recorded spike trains from single neurons in the caudate-putamen and substantia nigra (C. J. Wilson, S. J. Young, P. M. Groves, in preparation). The results of such analyses can reveal a variety of possible afferent and efferent as well as intrinsic interconnections [G. P. Moore, J. P. Segundo, D. H. Perkel, H. Levitan, *Biophys. J.* **10**, 876 (1970); R. J. MacGregor, S. W. Miller, P. M. Groves, *Exp. Neurol.* **47**, 581 (1975)].
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48. Increased neuronal activity in the caudate-putamen following local infusion of haloperidol was achieved with concentrations of 1×10^{-5} to $5 \times 10^{-5}M$ and injection volumes ranging from 0.40 to 0.05 μl. Under these conditions, effects in the caudate-putamen similar to those following administration of haloperidol systemically were achieved in 13 out of 18 experiments carried out in 11 experimental animals. In cases where more than one experiment was performed on the same preparation, these were separated by two or more hours. Effects inconsistent with those produced by systemic haloperidol consisted of no effect of the infusion in four experiments and a depression of neuronal firing in the caudate-putamen in one case. Of the 13 experiments in which increased neuronal activity occurred in the caudate-putamen to local infusion of haloperidol, simultaneous depressions of neuronal firing similar to that illustrated in Fig. 2 occurred in ten cases (four placements later localized to pars compacta and six to pars reticulata), while in three others firing rates in the substantia nigra increased. In two of these latter cases there was no evidence of recovery of neuronal firing at the time recovery occurred in the caudate-putamen, and attempts to replicate these exceptions proved unsuccessful. The lack of recovery suggests that such changes were unrelated to the infusion, although all three instances could have been produced by a local anesthetic effect very near the tip of the infusion cannula, as noticed subsequently during infusions directly into the substantia nigra (*51*). Criteria developed to distinguish drug-induced changes from spontaneous variations in firing at the infusion site required an alteration in firing rate within 15 minutes and a return to within 60 percent of predrug firing rate within 180 minutes following infusion.
49. The depression of neuronal firing following local infusion of amphetamine into the caudate-putamen occurred consistently with concentrations ranging from 5×10^{-5} to $5 \times 10^{-4}M$ and volumes ranging from approximately 0.12 to 0.05 μl. The depression was preceded in 6 of 15 subjects by a brief initial potentiation of neuronal firing similar to the effects of moderate doses of amphetamine (such as 1 to 2 mg/kg) given intraperitoneally, and this was often accompanied by an initial transient mirror-image depression of neuronal firing as well as the potentiation and inhibition phenomena in the substantia nigra, pars compacta. Both of these effects are illustrated in the example shown in Fig. 3. Simultaneous recordings in the substantia nigra were successfully obtained from 11 subjects and revealed either an approximate mirror image of the response in the caudate-putamen or an initial potentiation followed by inhibition and recovery. Substantia nigra units responding in the former manner were found at recording placements in both pars reticulata and pars lateralis ($N = 4$), and along the ventral border of pars compacta ($N = 2$), while units showing the inhibitory phenomenon were found only in and along the border of pars compacta ($N = 5$).
50. Accommodative or adaptive properties as well as other intrinsic mechanisms capable of regulating repetitive firing [D. Bradley and G. Somjen, *J. Physiol. (Lond.)* **156**, 75 (1961); R. Granit, D. Kernell, G. K. Shortress, *ibid.* **168**, 911 (1963); K. Sasaki and T. Otani, *Jpn. J. Physiol.* **11**, 443 (1961); T. Bullock, in *Structure and Function in the Nervous Systems of Invertebrates*, T. Bullock and O. Horridge, Eds. (Freeman, San Francisco, 1965), vol. 1, pp. 125–180; D. Kernell, *Acta Physiol. Scand.* **65**, 65 (1965); *ibid.*, p. 87; N. P. Rang and J. M. Ritchie, *J. Physiol. (Lond.)* **196**, 183 (1968); D. J. Mishelevich, *Exp. Neurol.* **25**, 401 (1969); M. Kuno, J. T. Miyahara, J. N. Weekley, *J. Physiol. (Lond.)* **210**, 839 (1970); W. Spencer and R. April, *Short-Term Changes in Neural Activity and Behavior*, G. Horn and R. Hinde, Eds. (Cambridge Univ. Press, New York, 1970), p. 433] remain to be explored.
51. For infusions directly into the substantia nigra the recording electrode and infusion cannula were often placed in very close proximity (for example, 50 to 100 μm apart), as opposed to our caudate-putamen infusions, in which the recording electrode was commonly 500 to 1000 μm from the infusion site. At very close separations in several animals we observed local anesthetic effects of haloperidol at concentrations of $1 \times 10^{-7}M$ and higher, consisting of a transient decline in the amplitude of extracellularly recorded action potentials. Similar local anesthetic effects have been noted for haloperidol applied to peripheral nerves [P. Seeman, A. Staiman, M. Chau-Wong, *J. Pharmacol. Exp. Ther.* **190**, 123 (1974)]. Increased neuronal activity without evidence of local anesthetic effects at the recording electrode near the infusion cannula was obtained from eight subjects with concentrations of haloperidol ranging from 1×10^{-8} to $5 \times 10^{-10}M$ and infusion volumes of approximately 0.04 to 0.14 μl. Of these, depressions of neuronal firing were revealed at the recording site in the caudate-putamen in five subjects, while in the other three, no significant changes occurred in the caudate-putamen. In the latter cases, the recording electrode in the caudate-putamen may have been recording activity from neurons not innervated by dopaminergic projections, or the infusion may not have invaded the substantia nigra, pars compacta, in sufficient concentrations to produce an acceleration of activity in all dopaminergic neurons projecting to the caudate-putamen. An influence of haloperidol on nondopaminergic projections to the caudate-putamen directly (for example, from pars reticulata), or by way of several indirect routes (for example, reticular formation through cerebral cortex or thalamic nuclei), may also contribute to such effects (*20*, *36*, *37*).
52. Infusions of amphetamine into the substantia nigra consistently produced inhibition of neuronal firing

in experiments on 14 subjects in concentrations ranging from 5×10^{-6} to $2 \times 10^{-4}M$ and volumes from 0.13 to 0.04 μl, without evidence of local anesthetic effects. In 9 of 14 animals, neuronal firing in the caudate-putamen increased following infusion of amphetamine into the substantia nigra, while in four animals it was unaffected and in one animal activity declined in the caudate-putamen. Infusion of amphetamine into the reticular formation above the substantia nigra, or in pars reticulata of the substantia nigra below pars compacta, usually resulted in increased neuronal activity at the recording electrode in these structures, although the reverse was also seen, and increased neuronal activity typically accompanied these changes at the recording site in the caudate-putamen. It is unclear whether in such instances increased neuronal firing in the caudate-putamen is due to release from dopaminergic inhibition, or increased activity in nondopaminergic elements of the substantia nigra or reticular formation, both of which have access to the caudate-putamen (*51*). Bunney *et al.* (*31*) have reported that the inhibition of dopaminergic neuronal firing produced by intravenously administered amphetamine can be blocked by treatment 15 to 30 minutes prior to amphetamine administration with DL-α-methyl-*p*-tyrosine, a drug that inhibits synthesis of catecholamines [S. Spector, A. Sjoerdsma, S. Udenfriend, *J. Pharmacol. Exp. Ther.* **147**, 86 (1965); M. J. Besson, A. Cheramy, J. Glowinski, *ibid.* **177**, 196 (1971); Weissman *et al.* (*15*)]. We have also been able to reduce or abolish the depression of dopaminergic neuronal firing produced by local infusion of amphetamine with similar pretreatments ($N = 4$). In addition, this compound typically leads to marked increases in spontaneous neuronal activity in both pars compacta of the substantia nigra and the caudate-putamen.

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Reticulocyte Transfer RNA and Hemoglobin Synthesis

Transfer RNA availability may regulate hemoglobin synthesis in developing red blood cells.

David W. E. Smith

In most primates and in some other mammals, such as rabbits, the mature red blood cell of the healthy adult contains approximately 300×10^6 hemoglobin molecules (*1*). In other mammals, which have smaller erythrocytes, and even in lower vertebrates, which generally have larger erythrocytes, the hemoglobin concentration within the cells is about the same as in higher mammals. The amount of hemoglobin is constant from cell to cell, which suggests a program of controlled macromolecular synthesis and degradation during red cell differentiation.

Red cell development takes place in the bone marrow and begins with rapidly dividing precursors that contain no hemoglobin and that synthesize many proteins for mitosis and vegetative life. Differentiation requires several days, during which there are several cell divisions that maintain a population of precursors and produce cells that begin to synthesize hemoglobin. Later in development, when the cells are called polychromatophilic erythroblasts and contain some hemoglobin, DNA and RNA synthesis and cell division cease, and hemoglobin synthesis becomes more rapid. From this stage onward globin synthesis is dependent on preexisting messenger RNA (mRNA), transfer RNA (tRNA), ribosomes, and supernatant factors (*2*).

The nucleus is lost in mammalian erythrocyte differentiation, and the penultimate stage is an anucleate cell called a reticulocyte that still synthesizes protein, over 90 percent of which is hemoglobin (*3*). Although reticulocytes originate in the bone marrow, normally about 1 to 2 percent of circulating red cells are reticulocytes that represent the youngest red cell additions to the peripheral blood. In rabbits under stress, such as bleeding or treatment with phenylhydrazine, a chemical which accelerates red cell destruction, reticulocytes may account for 80 to 90 percent of circulating red cells. These reticulocytes are larger and more active in hemoglobin synthesis than normally circulating reticulocytes and are sometimes called "stress reticulocytes" (*4*). Circulating reticulocytes become mature erythrocytes after 2 to 3 days during which polysomes and ribosomes disappear (*5, 6*) and hemoglobin synthesis ceases. While the reticulocyte is a cell in transition, with its contents and its activity in hemoglobin synthesis changing with age, a certain uniformity of the cells can be achieved by a commonly used schedule of phenylhydrazine injections (*7*), and it is possible to obtain reproducible results in studies on hemoglobin synthesis with cells from different rabbits. The stress reticulocyte preparation is the system in which such important discoveries as the direction of translation (*8*), the mechanism of initiation of protein synthesis in eukaryotes (*9*), and the factors and events involved in peptide chain elongation (*10*) have been made. In this article the experiments discussed have been carried out with this system, except where otherwise indicated.

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