

Self-inhibition By Dopaminergic Neurones: Disruption By (+/-)-*alpha*-methyl-*p*-tyrosine Pretreatment Or Anterior Diencephalic Lesions*

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ABSTRACT

(+)-Amphetamine sulphate infused directly into the substantia nigra, pars compacta, at low rates and in low volumes and concentrations, produces a marked and prolonged inhibition of neuronal activity. This effect was significantly reduced or abolished following (+/-)-*alpha*-methyl-*p*-tyrosine pretreatment or following electrolytic lesions in the diencephalon anterior to the substantia nigra. These results confirm previous reports of similar effects of such treatments on the depression of neuronal firing produced by systemically administered amphetamine. Further, our results suggest that (+/-)-*alpha*-methyl-*p*-tyrosine and anterior diencephalic lesions are affecting an indirect dopaminergic inhibitory mechanism intrinsic to the substantia nigra. Such evidence may also be regarded as consistent with the view that amphetamine produces an inhibition of dopaminergic neuronal activity by affecting release of dopamine from dendrites of dopaminergic neurones, i.e. by affecting self-inhibition by dopaminergic neurones.

INTRODUCTION

A variety of theoretical mechanisms has been developed to account for the regulation of catecholaminergic neuronal activity and biosynthesis, one common purpose of which has been to reconcile the degree of catecholamine receptor stimulation with the extent of transmitter biosynthesis and release in catecholaminergic neurones (e.g. Aghajanian and Bunney, 1974; Anden, 1974; Costa and Meek, 1974; Groves and Rebec, 1976). One theoretical hypothesis which has been applied effectively and extensively to the dopaminergic nigro-neostriatal pathway is the postsynaptic neuronal feedback loop hypothesis, proposed by Carlsson and Lindqvist (1963). According to this hypothesis, a postsynaptic negative feedback mechanism exists in the form of a neuronal feedback loop from the basal ganglia to dopaminergic neurones in the substantia nigra. By means of this loop, facilitation of dopaminergic transmission in the neostriatum results in decreased impulse flow in dopaminergic neurones, while blockade of dopaminergic transmission in the caudateputamen produces increased impulse activity in dopaminergic neurones of the substantia nigra.

In support of this concept is evidence showing that agents which result in stimulation of dopaminergic postsynaptic receptors in the neostriatum, such as amphetamine, produce a marked inhibition of neuronal firing in the dopaminergic pars compacta of the substantia nigra,

while agents that block dopaminergic transmission in the neostriatum, such as haloperidol or chlorpromazine, lead to increased firing by dopaminergic neurones of the substantia nigra (e.g. Bunney, Walters, Roth and Aghajanian, 1973; Aghajanian and Bunney, 1973, 1974; Rebec and Groves, 1975). In addition, *alpha*-methyl-*p*-tyrosine pretreatment, which inhibits the synthesis of catecholamines and appears to block amphetamine-induced release of dopamine from dopaminergic terminals in the neostriatum (Spector, Sjoerdsma and Udenfriend, 1965; Besson, Cheramy, Gauchy and Glowinski, 1973; Chieuh and Moore, 1974; Von Voigtlander and Moore, 1973), blocks the depression of dopaminergic neuronal activity to subsequent intravenous amphetamine administration (Bunney et al., 1973). Further, diencephalic transections anterior to the substantia nigra also block the depression of dopaminergic neuronal activity produced by intravenously administered amphetamine (Aghajanian and Bunney, 1973). Such transections are presumed to sever the neuronal feedback pathway from the neostriatum to the substantia nigra, thus cutting the postsynaptic feedback loop by which amphetamine has been theorized to produce its inhibitory effect on dopaminergic neuronal activity.

We have recently developed the view that systemically administered amphetamine produces a depression of neuronal activity in the pars compacta of the substantia nigra not by means of a neuronal feedback loop, but rather by releasing dopamine from the dendrites of dopaminergic neurones to act on dendritic and/or somatic receptors to produce inhibition of neuronal firing, i.e. a novel mode of neurohumoral regulation that we have termed self-inhibition (Groves, Wilson, Young and Rebec, 1975). In these experiments, local infusion of amphetamine into the caudate-putamen failed to produce a depression of neuronal activity in pars compacta of the substantia nigra, as predicted by the neuronal feedback loop hypothesis; and local infusion of haloperidol into the caudate-putamen failed to produce the increase in dopaminergic neuronal activity predicted by the neuronal feedback loop hypothesis. Indeed, such treatments typically resulted in the opposite of these predicted effects. Local infusion of low concentrations of amphetamine into the substantia nigra, on the other hand, produced a marked inhibition of neuronal firing in neurones of pars compacta, while local infusion of low concentrations of haloperidol into this region produced marked increases in dopaminergic neuronal activity. This evidence supports the hypothesis that these drugs have effects on dopaminergic neuronal firing rates by an action intrinsic to the substantia nigra, i.e. by affecting self-inhibition by dopaminergic neurones.

In the experiments described here, we report that

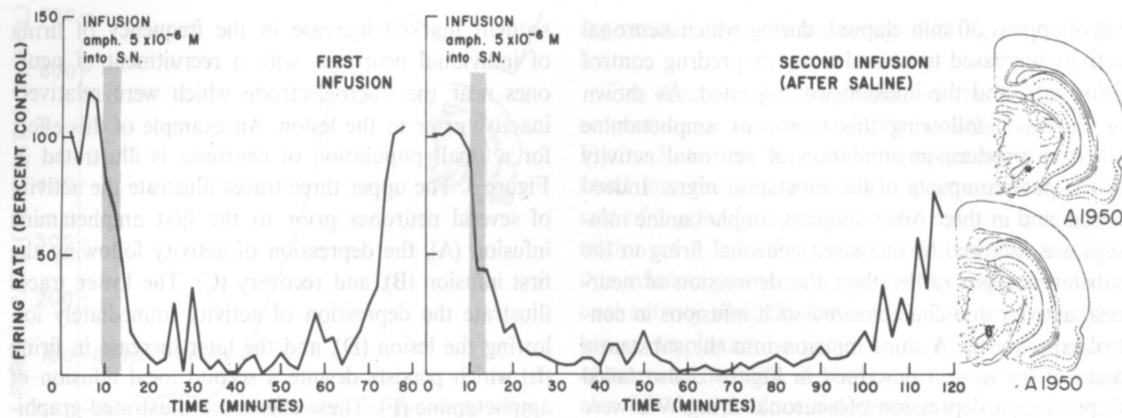


Fig. 1. The depression of neuronal firing rate in the substantia nigra, pars compacta (S.N.) produced by local infusion of (+)-amphetamine. Following recovery of neuronal activity from the effects of the first infusion, a second identical infusion was given. The microelectrode tip placement (black dot) and the position of the tip of the infusion cannula (black rectangle) are illustrated to the right of the graphs. Histological sections and identifying numbers are after KONIG and KLIPPEL (1963).

the effects of low concentrations of locally administered amphetamine on self-inhibition by dopaminergic neurones can be disrupted by alpha-methyl-p-tyrosine pretreatment or anterior diencephalic lesions, thus paralleling the results of such treatments on dopaminergic neuronal activity in response to systemically administered amphetamine.

METHODS

Experiments were carried out on 19 male, Sprague-Dawley albino rats weighing between 250 and 400 g at the time of experimentation. Animals were housed individually on a 12 hr light-dark cycle with food and water available ad lib.

Subjects were anaesthetized with ether, and placed in a stereotaxic instrument having blunt, atraumatic ear bars (Kopf Instruments). A midsagittal incision exposed the calvarium, and two small holes were drilled overlying the substantia nigra and anterior diencephalon of the left side of the brain. A 32-gauge infusion cannula connected by means of teflon tubing to a Hamilton microsyringe in a Harvard Instruments infusion pump was lowered into the region of the substantia nigra, along with a glass-coated tungsten microelectrode having a tip diameter of approx. 1 μ m and impedance of from 0.5 to 3.0 M ohm. The infusion cannula and microelectrode were lowered simultaneously utilizing a double-electrode carrier assembly and the distance between cannula tip and microelectrode tip

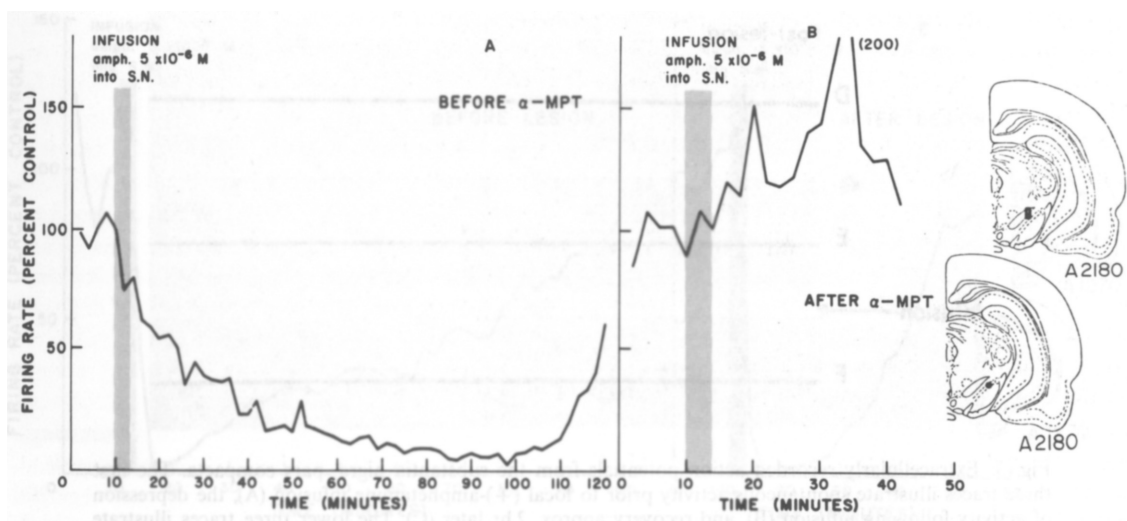


Fig. 2. The effects of local infusion of (+)-amphetamine into the substantia nigra, pars compacta (S.N.), on neuronal activity prior to (A) and following (B) alpha-MPT (200 mg/kg i.p.). The approximate positions of the tips of the microelectrode and infusion cannula are illustrated to the right as in Figure 1.

was set to between approx. 100 and 500 μm . In addition, a stainless steel, 0.4 mm o.d. lesion electrode, coated with enamel to within 0.25 mm of its tip, was lowered into the anterior diencephalic region in some animals. The scalp and points of surgical contact were then infiltrated thoroughly with procaine hydrochloride (Novocain) and local anaesthetic ointment (Xylocaine) supplemented at intervals of from 1 to 2 hr. The subject was immobilized with (+)-tubocurarine and artificially respired by means of a rubber cone fitted snugly over the snout. Eye-drops (Visine) were applied periodically to prevent corneal drying. Heart rate (typically 340-380 beats/min), body temperature (typically 36.5-37.5°C), and breath-by-breath expired carbon dioxide (typically 4 +/- 0.5%) were monitored continuously, the latter by means of a Beckman LB-2 gas analyzer.

In all of the experiments reported here, amphetamine was infused into the substantia nigra at rates of from 1 to 2 $\mu\text{l/hr}$, for periods ranging from 2 to 6 min, and in concentrations of $5 \times 10^{-5} \text{ M}$ or $5 \times 10^{-6} \text{ M}$ (concentration

of free base) in sterile physiological saline at neutral pH, while single or multiple neurone activity was recorded simultaneously. Neuronal firing rates were counted online by means of a Schmidt-trigger circuit and high-speed printer-counter on a minute-by-minute basis throughout the experiment. Action potentials were amplified by means of Tektronix 122 pre-amplifiers and 3A9 differential amplifiers in a Tektronix 565 oscilloscope. Data were displayed at high sweep speed on the face of the oscilloscope, and at low sweep speed on a Federal 18" screen monitor scope, and could be heard through an audio monitor. Results of experiments in which well isolated action potentials were recorded from single neurones and those in which action potentials from small populations of neurones (ranging typically from 3-5 neurones as determined by visual observation of extracellular spike amplitudes and waveforms) were identical. In some animals ($n = 7$), (+/-)-alpha-methyl-p-tyrosine methyl ester (alpha-MPT, Sigma) was administered at a dose of 200 mg/kg intraperitoneally while in others ($n = 9$), electrolytic lesions

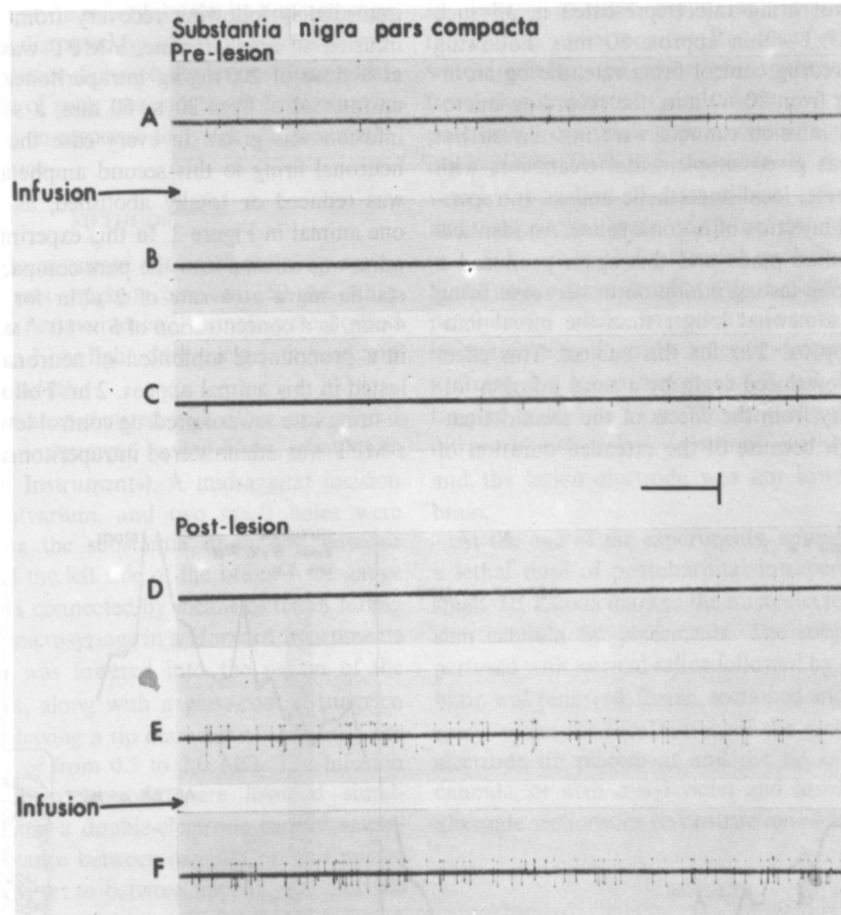


Fig. 3. Extracellularly recorded action potentials from the substantia nigra, pars compacta. The first three traces illustrate spontaneous activity prior to local (+)-amphetamine infusion (A), the depression of activity following infusion (B), and recovery approx. 2 hr later (C). The lower three traces illustrate the same activity within 4 min after a lesion was made in the anterior diencephalon (D), the subsequent recruitment and increase in firing (E), and the lack of effect of local infusion of $5 \times 10^{-6} \text{ M}$ (+)-amphetamine into the substantia nigra following such lesions (F). The calibration marks represent 1 sec (horizontal) and 200 μV (vertical).

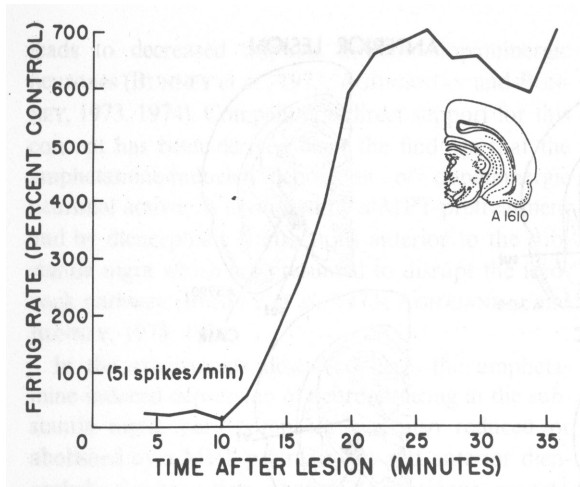


Fig. 4. Changes in spontaneous neuronal firing rates in the substantia nigra pars compacta following an ipsilateral anterior diencephalic lesion at time zero on the graph. The microelectrode tip placement is illustrated to the right with a small black dot.

were made in the anterior diencephalon using a Grass d.c. lesion maker at 1.0 mA for durations ranging from 5 to 15 sec. In control animals (n = 3), intraperitoneal saline injections were administered and the lesion electrode was not lowered into the brain.

At the end of the experiments, animals were given a lethal dose of pentobarbital intraperitoneally and small d.c. lesions marked the microelectrode and infusion cannula tip placements. The subject was then perfused with normal saline followed by formalin. The brain was removed, frozen, sectioned and stained with cresyl violet for localization of the recording microelectrode tip placement and the tip of the infusion cannula, or with cresyl

violet and luxol fast blue on alternate sections for reconstruction of lesion damage.

RESULTS

Amphetamine, when administered by means of local infusion into the substantia nigra, pars compacta, produced a marked inhibition of neuronal firing. This effect was achieved at low concentrations and low volumes as illustrated in Figure 1. In this typical control experiment, amphetamine was infused into the substantia nigra, pars compacta, in a concentration of 5×10^{-6} M, at a rate of 2 μ l/hr for a period of 4 min, which is illustrated in Figure 1 by the shaded bar, and this procedure resulted in a marked depression of neuronal activity which recovered to near predrug control firing rate (represented in all such figures as 100%) within approx. 80 min. Following recovery to predrug control firing rate, during an interval ranging from 20-60 min, the recording microelectrode and infusion cannula were not moved but the animal was given supplemental treatments with (+)-tubocurarine, local anaesthetic and an intraperitoneal control injection of isotonic saline. An identical infusion was then made and this again produced a marked and long-lasting inhibition of neuronal firing which lasted somewhat longer than the initial infusion, being approx. 2 hr for this subject. This effect could also be produced again by a third infusion following recovery from the effects of the second treatment, although because of the extended duration of this effect, the time-course for recovery to predrug control firing rate was not determined.

In one group of experimental animals, amphetamine was infused directly into the substantia nigra as in the control experiments described above, but immediately following recovery from the first local infusion of amphet-

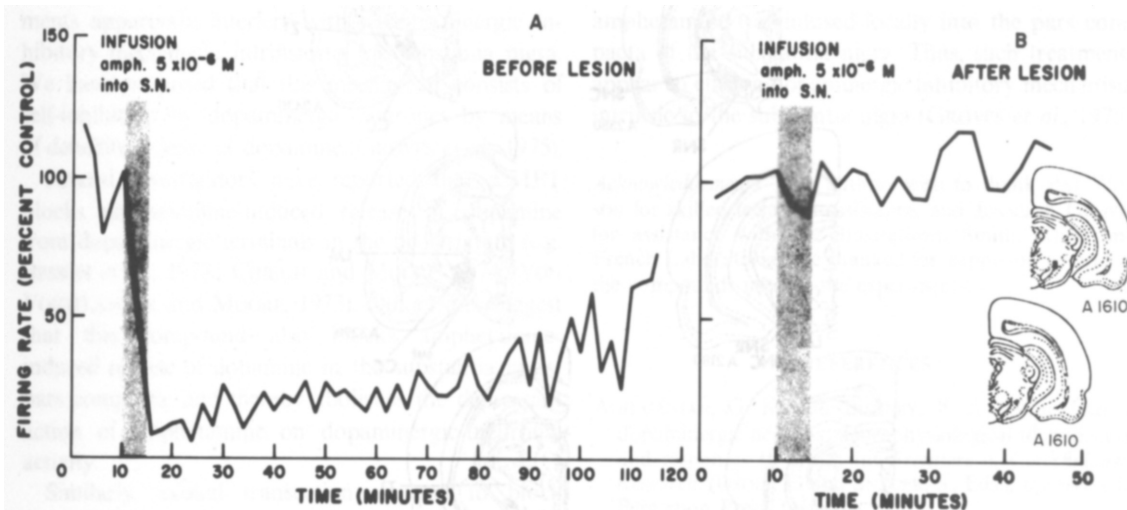


Fig. 5. The effects of local infusion of 5×10^{-6} M (+)-amphetamine into the substantia nigra, pars compacta on neuronal activity prior to (A) and following (B) an ipsilateral, diencephalic lesion anterior to the substantia nigra. The approximate positions of the tips of the infusion cannula and microelectrode are illustrated to the right of the graph as in Figure 1.

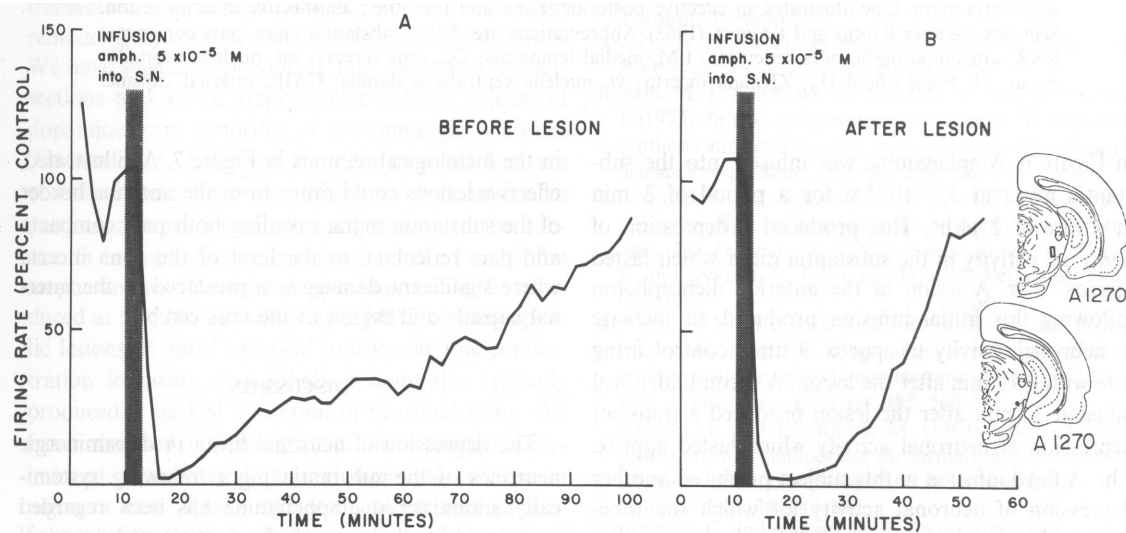


Fig. 6. The effects of local infusion of 5×10^{-5} M (+)-amphetamine into the substantia nigra, pars compacta, on neuronal activity prior to (A) and following (B) an ipsilateral, diencephalic lesion anterior to the substantia nigra. The approximate positions of the tips of the infusion cannula and microelectrode are illustrated to the right of the graph as in Figure 1.

amine, alpha-MPT was administered at a dose of 200 mg/kg intraperitoneally. Following an interval of from 20 to 60 min, a second identical infusion was given. In every case the depression of neuronal firing to this second amphetamine infusion was reduced or totally abolished, as illustrated for one animal in Figure 2. In this experiment, amphetamine was infused into the pars compacta of the substantia nigra at a rate of 2 μ l/hr for a duration of 4 min, in a concentration of 5×10^{-6} M. This resulted in a pronounced inhibition of neuronal firing which lasted in this animal approx. 2 hr. Following a return of firing rate toward predrug control levels, 200 mg/kg alpha-MPT was administered intraperitoneally. An interval of approx 30 min elapsed, during which neuronal activity increased to several times its predrug control firing rate, and the infusion was repeated. As shown in Figure 2, following this treatment amphetamine failed to produce an inhibition of neuronal activity in the pars compacta of the substantia nigra. Indeed in this and in three other subjects, amphetamine infusion was followed by increased neuronal firing in the substantia nigra rather than the depression of neuronal activity that characterized such infusions in control experiments. A third infusion into the substantia nigra of the subject described in Figure 2 also failed to produce a depression of neuronal firing. We were able in several animals to produce a transient depression of neuronal firing by local amphetamine infusion following alpha-MPT pretreatment, although this effect lasted only for periods of from 5 to 25 min, and was achieved following infusion of a higher concentration of amphetamine, 5×10^{-5} M.

Electrolytic lesions anterior to the substantia nigra in the diencephalon typically had dramatic effects on neuronal activity in the pars compacta of the substantia nigra, consisting of an initial depression and subsequent marked

increase in the frequency of firing of individual neurones, with a recruitment of neurones near the microelectrode which were relatively inactive prior to the lesion. An example of this effect for a small population of neurones is illustrated in Figure 3. The upper three traces illustrate the activity of several neurones prior to the first amphetamine infusion (A), the depression of activity following the first infusion (B), and recovery (C). The lower traces illustrate the depression of activity immediately following the lesion (D), and the later increase in firing (E) which persists despite a second local infusion of amphetamine (F). These effects are illustrated graphically in Figure 4. Firing rate of a small population of neurones in the substantia nigra, pars compacta, is plotted as a function of time following diencephalic damage. As shown, after the lesion was made there was a period of quiescence near the microelectrode, although Figure 4 illustrates the longest such depression that was observed. More commonly, this effect lasted several minutes or less. Soon after this immediate depression, however, neuronal activity began to show a marked increase in frequency typically reaching several times the frequency of firing prior to the lesion.

The effect of this treatment on the response to amphetamine is illustrated for another subject in Figure 5. In this experiment, amphetamine was infused into the substantia nigra, pars compacta, at a rate of 2 μ l/hr for a duration of 5 min, in a concentration of 5×10^{-6} M. Before diencephalic damage, this infusion produced a marked depression of neuronal activity which lasted approx. 2 hr. Following the lesion, amphetamine failed to produce a depression of neuronal activity. A third infusion in this animal also failed to produce a depression of neuronal activity in pars compacta.

In three animals, in which the concentration of

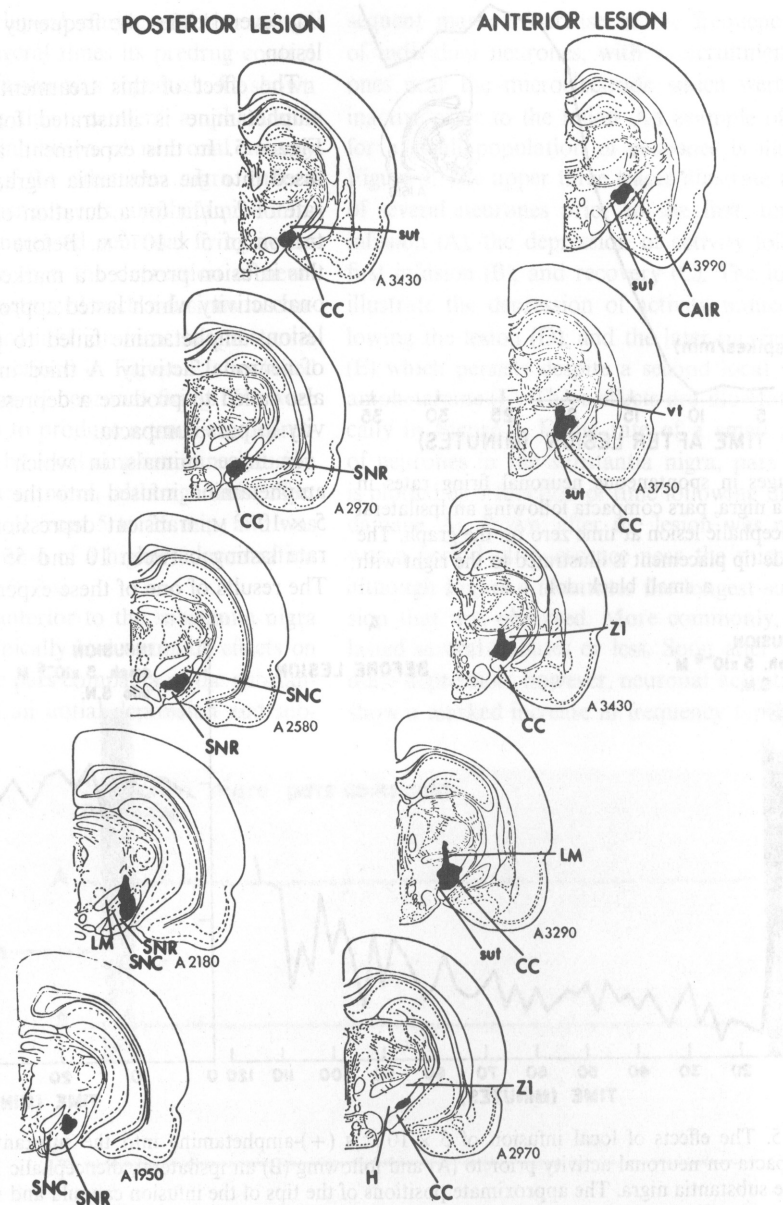


Fig. 7. Reconstructions of two representative electrolytic lesions in the diencephalon, anterior to the substantia nigra. One illustrates an effective posterior lesion and the other, an effective anterior lesion. Sections are after Konig and Klippel (1963). Abbreviations are: SNC, substantia nigra, pars compacta; SNR, substantia nigra, pars reticulata; LM, medial lemniscus; CC, crus cerebri; sut, nucleus subthalamicus; H, Forel's field H2; ZI zona incerta; vt, nucleus ventralis thalamus; CAIR, internal capsule.

amphetamine infused into the substantia nigra was 5×10^{-5} M, transient depressions of neuronal firing rate lasting between 10 and 55 min were produced. The results of one of these experiments are illustrated in Figure 6. Amphetamine was infused into the substantia nigra at 5×10^{-5} M for a period of 3 min at a rate of 2 μ l/hr. This produced a depression of neuronal activity in the substantia nigra which lasted approx. 2 hr. A lesion in the anterior diencephalon following this initial infusion produced an increase in neuronal activity to approx. 9 times control firing rate within 15 min after the lesion. A second, identical infusion 30 min after the lesion produced a transient

depression of neuronal activity which lasted approx. 1 hr. A third infusion in this subject produced another depression of neuronal activity for which the timecourse of recovery was not determined.

Two representative lesions, showing both posterior and anterior damage to the diencephalon in this group of brain-damaged subjects, are reconstructed in the histological sections in Figure 7. As illustrated, effective lesions could range from the anterior border of the substantia nigra, invading both pars compacta and pars reticulata, to the level of the zona incerta where significant damage was produced to the internal capsule and

DISCUSSION

The depression of neuronal firing in dopaminergic neurones of the substantia nigra following systemically administered amphetamine has been regarded as support for the concept of a postsynaptic neuronal feedback loop from the basal ganglia to the substantia nigra by which amphetamine-induced release of dopamine from dopaminergic terminals in the neostriatum leads to decreased impulse flow in dopaminergic neurones (Bunney et al., 1973; Aghajanian and Bunney, 1973, 1974). Compelling indirect support for this concept has been derived from the findings that the amphetamine-induced depression of dopaminergic neuronal activity is abolished by alpha-MPT pretreatment and by diencephalic transections anterior to the substantia nigra which are presumed to disrupt the feedback pathway (Bunney et al., 1973; Aghajanian and Bunney, 1973, 1974).

In the experiments described here, the amphetamine-induced depression of neuronal firing in the substantia nigra, pars compacta was also reduced or abolished by alpha-MPT pretreatment and anterior diencephalic lesions, thus confirming previous reports. However, since amphetamine was infused directly into the substantia nigra, pars compacta, these blocking effects cannot be due to interference with dopaminergic transmission in the neostriatum. Such treatments apparently interfere with a dopaminergic inhibitory mechanism intrinsic to the substantia nigra. We have proposed that this mechanism consists of self-inhibition by dopaminergic neurones by means of dendritic release of dopamine (Groves et al., 1975).

Several investigators have reported that alpha-MPT blocks amphetamine-induced release of dopamine from dopaminergic terminals in the neostriatum (e.g. Besson et al., 1973; Chieuh and Moore, 1974; Von Voigtlander and Moore, 1973). Our results suggest that this compound also blocks amphetamine-induced release of dopamine in the substantia nigra, pars compacta, and thereby abolishes the depressant action of amphetamine on dopaminergic neuronal activity.

Similarly, axonal transection appears to block amphetamine-induced release of dopamine from dopaminergic terminals in the neostriatum (Besson et al., 1973; Von Voigtlander and Moore, 1973), and Ungerstedt (1974) has suggested that axonal transection produces a similar blocking effect on remaining, intact catecholaminergic axon collaterals. We have suggested previously that diencephalic transections may block amphetamine-induced release of dopamine from dendrites of dopaminergic neurones (Groves et al., 1975) and the data reported here are consistent with this view. While we were unable to produce depressions of neuronal activity in the substantia nigra at a concentration of 5×10^{-6} M amphetamine, transient depressions could be produced at 5×10^{-5} M following alpha-MPT or diencephalic lesions. A third identical infusion at this concentration following diencephalic

lesions also typically produced a marked inhibition of neuronal firing. We have observed concentration-related, amphetamine-induced release of dopamine from the substantia nigra, in vitro (Paden, Wilson and Groves, unpublished observations). Release of dopamine from chopped nigral tissue was obtained with concentrations of amphetamine of 10^{-5} M and higher, but not lower than this value. Thus, axonal transection which must occur when nigral tissue is removed and studied in vitro, does not block release of dopamine at concentrations of amphetamine above 10^{-5} M. However, release of dopamine in vitro does not occur below this value and this is consistent with the evidence reported here that the depression of neuronal activity produced by infusion of 5×10^{-6} M amphetamine is blocked by diencephalic lesions, while that produced by 5×10^{-5} M is only partially blocked.

The blocking effect of diencephalic lesions has been attributed to interruption of striato-nigral efferent fibres presumed to participate in a neuronal feedback loop from the basal ganglia to the substantia nigra (e.g. Bunney and Aghajanian, 1973). Such selective damage to efferent fibres is unlikely in our experimental paradigm. However, what is clear from our experiments is that alpha-MPT and our anterior diencephalic lesions do not remove an influence of amphetamine on neostriatal dopaminergic transmission since amphetamine was infused locally into the pars compacta of the substantia nigra. Thus, such treatments appear to effect a dopaminergic inhibitory mechanism intrinsic to the substantia nigra (Groves et al., 1975).

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