REGULATION OF NIGRO-STRIATAL DOPAMINERGIC NEURONAL ACTIVITY

By

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ABSTRACT

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The purpose of these studies was to determine the location of the dopamine receptors responsible for regulation of the activity of nigro-striatal dopamine-containing neurons. Biochemical estimates of neuronal activity were measured following systemic or intracranial administration of dopamine agonists and antagonists.

Systemic administration of dopamine antagonists (haloperidol, thioridazine, clozapine, sulpiride) increased, and agonists (apomorphine, piribedil) decreased striatal concentrations of dihydroxyphenylacetic acid (DOPAC), the major acid metabolite of dopamine. A similar but less pronounced response to these drugs was observed in substantia nigra.

Since nigral DOPAC concentrations paralleled those in striatum following systemic administration of dopamine agonists and antagonists, dopamine appears to be released in the substantia nigra. Likewise, intranigrally-administered baclofen, a drug which inhibits activity in nigro-striatal neurons, attenuated the increases in nigral and striatal DOPAC concentrations induced by systemically administered haloperidol. On the other hand, dopamine dynamics at dopaminergic cell bodies in substantia nigra and at terminals in the striatum appear to be

different, since intranigrally administered baclofen increased striatal, but not nigral dopamine concentrations.

Stimulation of nigral dopamine receptors does not appear to be a mechanism for control of dopaminergic neuronal activity since intranigral administration of dopamine agonists and antagonists did not produce changes in biochemical indices of neuronal activity similar to those observed after systemic administration of these drugs.

A striato-nigral feedback loop does not appear to control nigro-striatal activity in response to drugs. Neuronal perikarya in the striatum were destroyed with intrastriatal injections of kainic acid. Striatal choline acetyltransferase (ChAT) activity was used as an index of feedback loop destruction. Following such injections, systemic administration of dopamine agonists decreased striatal DOPAC concentrations, while dopamine antagonists increased DOPAC relative to control animals and relative to the contralateral striatum. When the α-methyltyrosine (αMT)-induced decline of dopamine was used as an index of nigro-striatal nerve activity, haloperidol increased neuronal activity on the kainic acid-treated and control sides of the brain.

No increases in nigro-striatal neuronal activity were observed in kainic acid-treated animals when the αMT-induced decline of dopamine, or the accumulation of striatal DOPA were used as indices of neuronal activity. Nigral DOPAC concentrations were also unaffected by kainic acid treatment. These data suggest that kainic acid increases striatal DOPAC concentrations by a mechanism not related to increased neuronal activity. Histological examination of the striata of kainic acid-treated rats revealed evidence of damage to fibers as well as cell bodies.

To eliminate such nonselective actions of kainic acid, striato-nigral neurons were destroyed with knife cuts. Data was collected only from those animals in which approximately 60 percent of the feedback loop, but none of the nigro-striatal fibers were destroyed. Nigral glutamic acid decarboxylase (GAD) activity was used as an index of feedback loop destruction, while the integrity of nigro-striatal neurons was judged by striatal dopamine concentrations. Such knife cuts did not increase striatal DOPAC concentrations. When animals with unilateral knife cuts were given haloperidol systemically, DOPAC concentrations were increased in both knife cut and intact striata, and the increases on the knife cut-treated side were significantly greater than those on the contralateral side. These results suggest that postsynaptic mechanisms, such as a feedback loop, are not essential for regulation of nigro-striatal neuronal activity in response to dopamine antagonists, and imply that dopamine antagonists have different pre- and postsynaptic effects.

To determine if control of nigro-striatal activity is mediated by striatal mechanisms, intrastriatal injections of dopamine antagonists were made. These injections produced small but significant increases in striatal, but not nigral DOPAC concentrations. These increases persisted in animals pretreated with knife cuts of the striato-nigral fibers, and were not correlated with changes in either striatal dopamine concentrations or nigral GAD activity seen after such knife cuts.

In summary, dopamine agonists and antagonists do not appear to influence nigro-striatal neuronal activity primarily by actions postsynaptic to nigro-striatal neurons. Striatal rather than nigral presynaptic mechanisms appear to be more important for this function.

for Bill, with love

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INTRODUCTION

The nigro-striatal dopaminergic system is a convenient model for studying the mechanisms for regulation within a central neuronal system because its anatomy and biochemistry have been extensively studied. It is also the only group of central neurons whose biochemistry has been correlated with specific functions. Nigro-striatal dopamine-containing neurons play a role in the initiation and control of motor behavior. Damage to these cells results in specific losses of motor activity (Parkinsonism) accompanied by deficits in concentrations of the transmitter dopamine. That normal motor behavior can be at least partially restored in patients with Parkinsons disease by transmitter replacement therapy indicates that dopamine is the biochemical correlate of function in this system.

I. Anatomy

A. Nigro-striatal Neurons

The nigro-striatal system is one of several dopamine-containing neuronal groups in the CNS. Like the majority of these, it has its cell bodies of origin in the ventral midbrain (see Figure 1) (Andén et al., 1966a; Berger et al., 1974; Dahlström and Fuxe, 1964; Hökfelt et al., 1974; Lindvall et al., 1974, 1978; Ungerstedt, 1971). A smaller group, comprising the tubero-infundibular-hypophyseal and incerto-hypothalamic systems, originate in hypothalamic nuclei

Figure 1. Distribution of nigro-striatal dopaminergic neuronal systems in the rat brain. The vertical dashed lines on the sagittal section represent the approximate location of the frontal section depicting dopaminergic cell bodies in the ventral midbrain (above). cc, crus cerebri; cp, caudate-putamine; ip, interpeduncular nucleus; ml, medial lemniscus; pg, periaqueductal gray; rn, red nucleus; sn, substantia nigra. Dotted regions represent terminals of dopaminergic nerves. Modified from Ungerstedt, 1971.

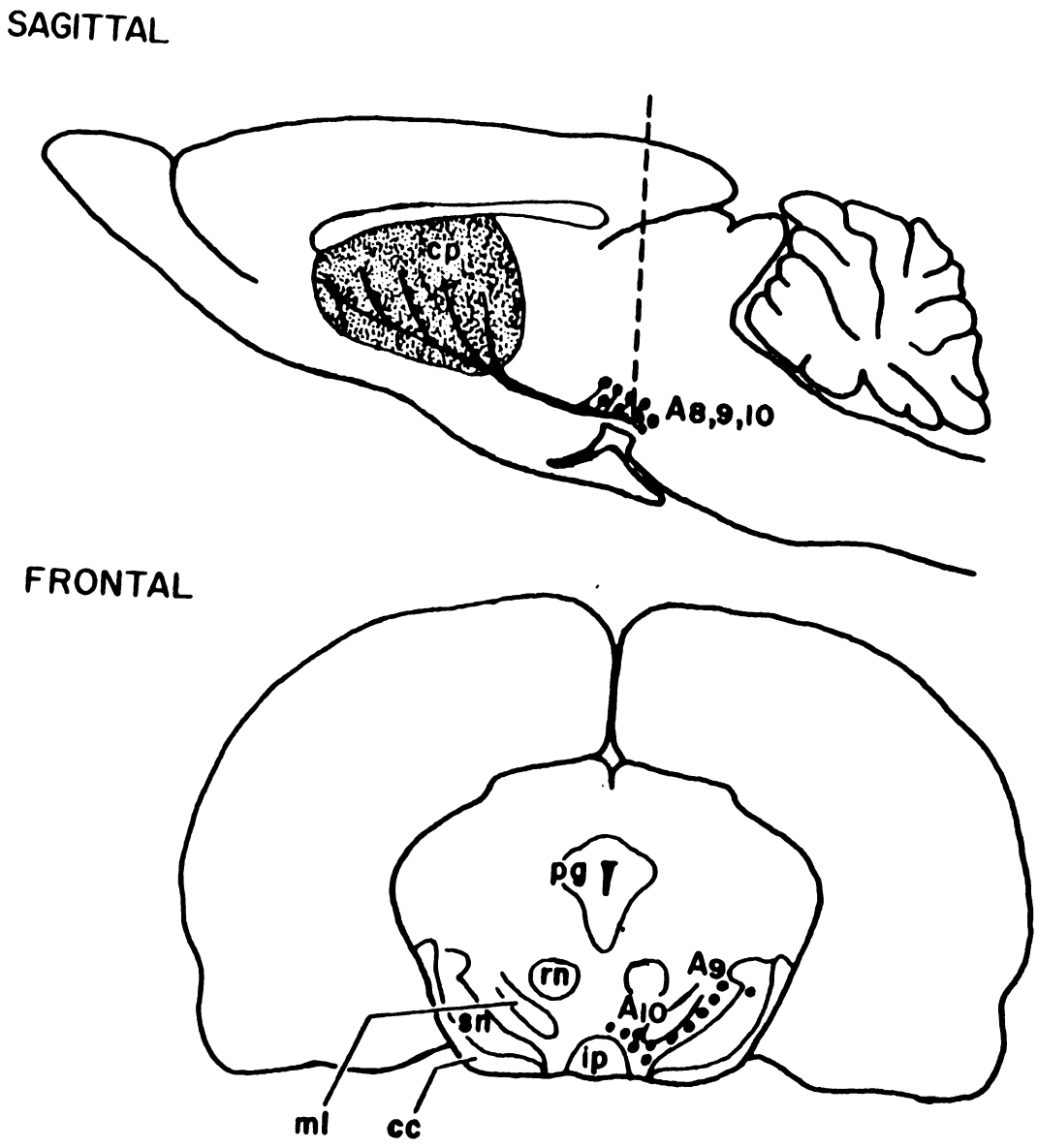

Figure 1

(Björklund et al., 1973; Fuxe, 1963; Jonsson et al., 1972; Ungerstedt, 1971). A small number of dopamine-containing cells are also found in the retina, in the medulla, periventricular and peri-aqueductal gray and in the olfactory bulb (see Moore and Bloom, 1978).

The nigro-striatal neurons are only one among many afferent groups converging on the striatum, including major projections from the thalamus (Powell and Cowan, 1956) and sensorimotor cortex (Carman et al., 1963), as well as smaller inputs from ventral tegmentum (Björklund and Lindvall, 1978) and raphé nuclei (Bobillier et al., 1976). In turn, a number of afferents project to dopaminergic cell bodies in substantia nigra, including inputs from raphé nucleus (Pasquier et al., 1977) and cerebellum (Snider et al., 1976). From the large number of connections these cells have with other neurons it appears that nigro-striatal dopaminergic neurons form only part of a very complex system, and that their activity is integrated into motor behaviors at both mesencephalic and telencephalic levels.

The area of the substantia nigra in the rat contains approximately 3500 dopaminergic cell bodies (Andén et al., 1966). Most of these are located in pars compacta and the region immediately dorsal and medial to it (Areas A8 and A9, see Figure 1), but a few are found more ventrally, in the relatively perikarya-free pars reticulata, and also in the pars lateralis. These are medium-sized multipolar cells with no remarkable ultrastructural features (Moore and Bloom, 1978). Their prominent dendrites, which extend into the pars reticulata, are unusual in that they contain and appear to release dopamine (Björklund and Lindvall, 1975).

Immediately upon leaving substantia nigra, nigro-striatal fibers from area A9 turn dorso-medially and meet those running rostrally from A8 at the level of the mesencephalic-diencephalic junction. Here they combine to form the nigro-striatal tract. These fibers, which are characteristically very fine, unmyelinated and without varicosities (Hattori et al., 1973), travel dorso-rostrally through the lateral hypothalamus and dorso-medial internal capsule to the globus pallidus, where some collaterals terminate, and into the caudate-putamen, where each axon branches widely to form a dense network of very fine terminal axons (Moore and Bloom, 1978).

A topographical relationship exists between nigral cells and the fields onto which they project (Carpenter and Peter, 1972; Lindvall and Björklund, 1974; Mettler, 1970; Moore and Bloom, 1978; Ungerstedt, 1971). Axons of cells from the dorso-caudal portions of the nigra make up the more dorsal and lateral fibers of the nigro-striatal tract. These leave the ascending fiber bundle first and enter the caudal striatum via the internal capsule; cells from the more rostral and ventral nigra send fibers through the ventral part of the ascending tract to rostral and dorsal striatum. Thus, both a rostro-caudal and reverse dorso-ventral relationship exists between the origin and termination of the system. In addition, there is also a medio-lateral topography.

Dopaminergic cells comprise the majority of those found in substantia nigra (Dahlström and Fuxe, 1964). About 20 percent of the cells, however, are non-dopaminergic, and there is evidence that these also project to the striatum (Feltz and DeChamplain, 1972; Fibiger et

al., 1972; Hattori et al., 1973; Ljungdahl et al., 1975). To date these neurons have not been identified biochemically. It is likely that these non-dopaminergic cells also influence motor behavior, since electrolytic lesions of the substantia nigra, which destroy all cells in the area, produce turning in the opposite direction to dopamine-selective lesions made with 6-hydroxydopamine (6-OHDA). Such effects have been interpreted to indicate that dopaminergic and non-dopaminergic nigro-striatal neurons function in an opposite manner (Schwartz et al., 1976).

Synaptic contacts made by ascending dopaminergic neurons have not been fully characterized, although Hattori et al. (1973) have reported synaptic contacts with striatal dendrites. Presumably dopamine is released at these sites (Portig and Vogt, 1969; Von Voigtlander and Moore, 1971). Striatal target cells may be small interneurons containing acetylcholine or γ-aminobutyric acid (GABA) (Butcher and Butcher, 1974; McGeer et al., 1971; McGeer and McGeer, 1975), although there is now evidence that most of the small neurons previously thought to be confined to this structure are actually projection neurons (Bishop et al., 1978).

B. Afferents to nigro-striatal system

With the use of horseradish peroxidase tracing techniques (Bunney and Aghajanian, 1976), it has been estimated that up to 50 percent of caudate cells project to substantia nigra in a medio-lateral, antero-posterior topographic fashion. In addition, cells in lateral and posterior globus pallidus send fibers to the nigra (Grofová, 1975).

Several groups of nigral afferents have been characterized biochemically. Two projections, originating in the striatum and globus pallidus, contain GABA and the peptide substance P (Fonnum et al., 1974; Gale et al., 1977a; Hong et al., 1977b; Kanazawa et al., 1977; Kim et al., 1971). These descending fiber groups follow a course parallel to but slightly lateral and ventral to the ascending dopaminergic projections.

Descending GABA neurons originate throughout the striatum and globus pallidus, while the majority of the substance P group arise in the anterior striatum (Gale et al., 1977a; Hong et al., 1977b). Both of these putative transmitters are found in high concentrations in substantia nigra (Brownstein et al., 1976; Fahn and Coté, 1968; Zetler, 1970). Immunochemical studies indicate that glutamic acid decarboxylase (GAD), the GABA synthesizing enzyme, is present in nigral nerve terminals (Ribak et al., 1976), and that substance P reactive particles surround dopaminergic cell bodies in pars compacta (Hökfelt et al., 1978). High concentrations of substance P are also found in pars reticulata (Brownstein et al., 1976). Substance P is associated with the synaptosomal fraction of nigral homogenates (Cleugh et al., 1964; Duffy et al., 1975), suggesting a transmitter role for this peptide. Substance P concentrations in substantia nigra are reduced by 80-90 percent by lesions of the striato-nigral pathway (Gale et al., 1977a; Hong et al., 1977b); however, maximal reductions in nigral GAD concentrations after such lesions are only about 40-50% (Kim et al., 1971; Racagni et al., 1978b). The source of the residual GAD is unknown.

There are also 5-hydroxytryptamine (5HT)-containing projections to substantia nigra. 5HT can be visualized in zona reticulata by fluorescence histochemistry after pretreatment with nialamide (Fuxe, 1965), and relatively high concentrations of this monoamine can be measured both in zona compacta and zona reticulata (Palkovits et al., 1974). 5HT can be released from the nigra in vitro by potassium, and decreases in this release are accompanied by decreased 5HT concentrations in the nigra after raphé lesions (Reubi and Emson, 1978). Autoradiographic studies indicate that dorsal and median raphé nuclei (Bobillier et al., 1976; Fibiger and Miller, 1977) are the source of nigral 5HT. Electrical stimulation of the median raphé evokes responses in cells of both compacta and reticulata (Dray et al., 1976b).

Snider et al. (1976) have demonstrated the existence of neuronal pathways which originate in cerebellar nuclei and project to the substantia nigra. The identity of the neurotransmitter in these paths is unknown, but electrical stimulation of the dentate nucleus does alter the release of dopamine from the striatum and substantia nigra (Glowinski et al., 1978). This neuronal link between the cerebellum and substantia nigra may be important for relaying sensory stimuli to the basal ganglia.

II. Biochemistry of Dopaminergic Neurotransmission

The biochemical mechanisms involved in dopaminergic neurotransmission, depicted in Figure 2, are thought to be as follows: The amino acid tyrosine is transported into the nerve terminal, where it is converted to dihydroxyphenylalanine, or DOPA, by the enzyme tyrosine hydroxylase. This is the rate-limiting step in dopamine synthesis.

Figure 2. Schematic diagram of a dopaminergic synapse. COMT, catecholamine-o-methyltransferase; D, dopamine; DOPAC, dihydroxyphenylacetic acid; HVA, homovanillic acid; MAO, monoamine oxidase; 3MT, 3-methoxytyramine.

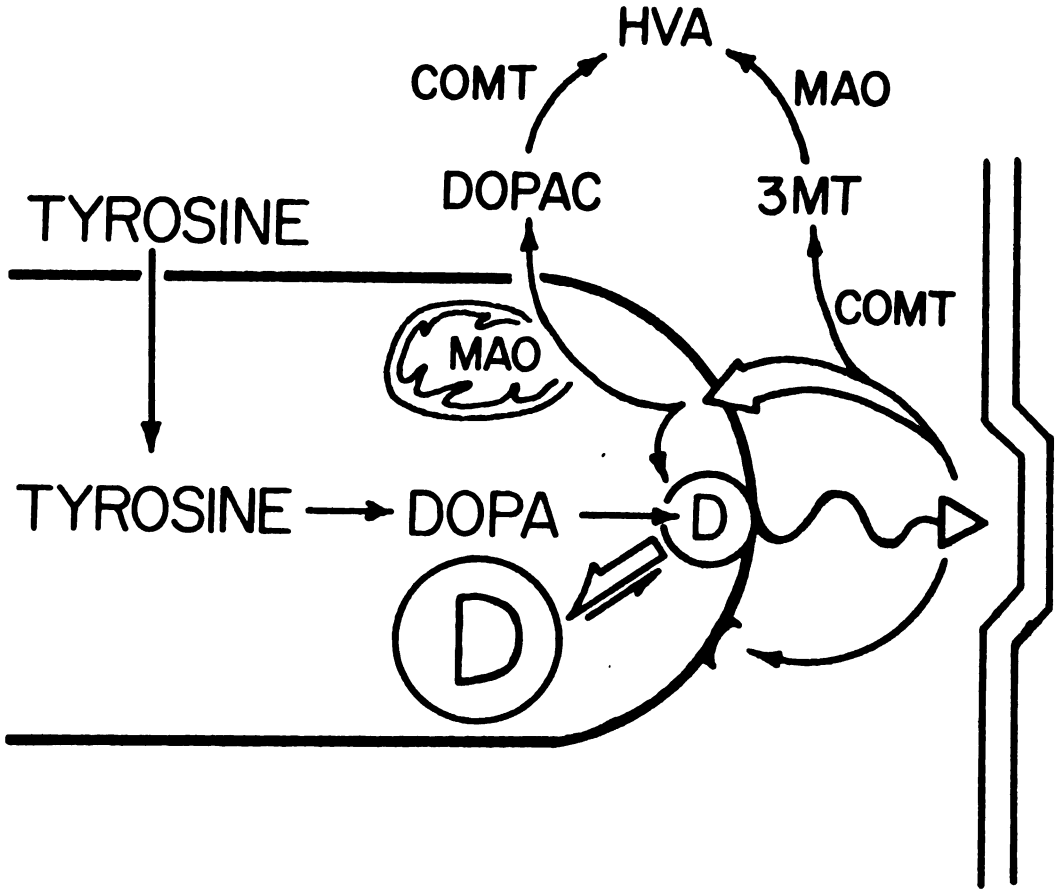

Figure 2

Aromatic L-amino acid decarboxylase then converts DOPA to the end-product dopamine, which can be stored in synaptic vesicles within the nerve terminal, or enter a much smaller "readily releasable" transmitter pool. The location of this pool, though intraneuronal, is not known. Following a nerve action potential, dopamine is released from the terminal and diffuses across the synaptic cleft to activate specific dopamine receptors located on postsynaptic and possibly presynaptic cell membranes. The biochemical identity of these receptors has not been unequivocally demonstrated. Binding of dopamine agonists and antagonists to nerve membrane preparations (Burt et al., 1976) and measurement of dopamine-sensitive adenylate cyclase activity (Horn et al., 1974) have both been used to quantify dopamine receptors, but measurements based on these techniques do not always agree (Kebabian, 1978; Garau et al., 1978). Approximately 90 percent of released dopamine is inactivated by an energy-dependent mechanism that carries the transmitter back into the presynaptic nerve terminal. Here the dopamine is either converted to the major acid metabolite dihydroxyphenylacetic acid (DOPAC) by mitochondrial monoamine oxidase (MAO), and diffuses out of the terminal, or is taken back into storage vesicles. Outside the terminal DOPAC is converted to homovanillic acid (HVA) by the extraneuronal enzyme catecholamine-o-methyltransferase (COMT). This enzyme also converts the remaining 10 percent of released dopamine to 3-methoxytyramine (3MT). This probably occurs within glial cells (Kaplan et al., 1978). 3MT is subsequently converted to HVA by extraneuronal MAO.

III. Methods for Estimating Dopaminergic Neuronal Activity

In order to study the regulation of dopaminergic neuronal activity one must be able to estimate the impulse traffic in nigro-striatal neurons under a variety of conditions. Estimations of this activity have been made utilizing both electrophysiological and biochemical techniques.

A. Electrophysiological estimates of dopaminergic nerve activity

Dopaminergic neuronal activity may be monitored by means of extracellular or intracellular electrical recordings, either directly from dopaminergic cell bodies or from elements postynaptic to these neurons. These techniques, though powerful, have a number of drawbacks. For example, estimates of dopaminergic nerve activity made from recordings from postsynaptic cells in striatum must be considered indirect, since the relationship between presynaptic stimulation and postsynaptic response may not be one to one. A postsynaptic action potential may represent the summation of a number of excitatory and inhibitory inputs from presynaptic dopaminergic or non-dopaminergic nerves as well as postsynaptic axon collaterals.

It is also difficult to record from cells with a low spontaneous firing rate, such as those in caudate. This problem has been resolved by artificially exciting the cells with iontophoretically-applied glutamate. Under these conditions, however, it is questionable whether neuronal behavior represents normal physiological activity.

With the use of extracellular techniques, responses from more than one cell may be recorded in sequence. Alternatively, two distinct responses may not be observed if signals are averaged over a

period of time that is longer than the first response. Studies in which extracellular recordings were taken from striatum following medial forebrain bundle stimulation have generated controversy because of this point. In a number of these studies, changes in spike rate suggesting initial depolarizations followed by hyperpolarizations were observed (see Section IVA). These can be interpreted either as direct inhibition of caudate cells or as indirect inhibition resulting from primary stimulation of interneurons or collaterals.

Biochemical identification of nerve cells is a major difficulty in electrophysiological studies. For example, in substantia nigra, cells cannot be assumed to be dopaminergic simply because histology shows that the recording electrode was lowered into zona compacta. The substantia nigra contains a significant number of non-dopaminergic cells (see Section IA). In some studies nigral cells have been identified as dopaminergic on the basis of responses to antidromic stimulation from several postulated target nuclei (Guyenet and Aghajanian, 1978). Another means of identification is through electrophysiological characteristics, which for dopaminergic neurons are thought to include conduction velocity, duration and shape of action potential, and a slow rate of spontaneous firing that increases under chloral hydrate or halothane anesthesia (Aghajanian and Bunney, 1973). Cells with such characteristics have not been located in animals pretreated with 6-OHDA (Guyenet and Aghajanian, 1978).

B. Biochemical estimates of dopaminergic nerve activity

In most studies of the peripheral and central nervous system, neuronal activity has been demonstrated to be directly linked to transmitter synthesis. That is, increased neuronal activity is accompanied by enhanced synthesis and decreased activity by lowered synthesis. As a result, the concentration of transmitter in the nerve terminal remains the same under varying conditions of neuronal activity.

Under one experimental condition nigro-striatal dopamine-containing neurons do not follow these principles. These experiments have led to controvery concerning mechanisms of nigro-striatal regulation. Procedures that completely block dopaminergic impulse flow, such as the administration of drugs (γ-butyrolactone, local anesthetics) or axotomy, are not accompanied by the expected decrease in dopamine synthesis. For up to 60 minutes after such treatment, dopamine synthesis actually increases, resulting in elevated dopamine concentrations. These observations reinforce the concept of a negative feedback control system in which dopamine synthesis, and ultimately neuronal activity is regulated by released transmitter, and that receptor activation is the important controlling mechanism in these neurons (see review by Nowycky and Roth, 1978). What is not resolved is exactly how synaptic dopamine influences synthesis and neuronal activity. Because the increases in dopamine synthesis can be reversed by systemic administration of dopamine agonists, presynaptic dopaminergic autoreceptors have been proposed to monitor synaptic dopamine concentrations. According to this hypothesis, their activation by released dopamine would trigger a compensatory decrease in

synthesis and transmitter release. Indeed, dopaminergic autoreceptor agonists can be identified by determining if these compounds prevent the increased rate of synthesis or increased concentrations of dopamine that follow procedures which cause a cessation of impulse flow (Nowycky and Roth, 1978; Gianutsos et al., 1976; Gianutsos and Moore, 1977).

Except for this special case, however, end-product inhibition appears to be the mechanism that links transmitter supply and demand. Increased synthesis results in increased intraneuronal transmitter concentrations, and these enhanced concentrations, in turn, inhibit synthetic enzymes. On the other hand, depletion of transmitter stores by enhanced neuronal activity disinhibits synthetic enzymes, so that transmitter concentrations are maintained.

Thus, synthesis, and release (and therefore degradation) are considered directly related to one another and to neuronal activity. Biochemical measures of nerve activity may therefore include measurements of transmitter metabolites, declines in transmitter concentration after inhibition of synthetic enzymes, and activity of the rate-limiting synthetic enzyme as well as direct measurements of released transmitter. A number of assumptions must be made in order to use these procedures (Weiner, 1974), but in general the different methods produce similar estimates of neuronal activity. The following section describes these methods.

1. Dopamine release

The most direct biochemical index of dopaminergic nerve activity is the actual measurement of endogenous dopamine released

into brain areas where the concentration of dopamine-containing terminals or cell bodies is high. This can be accomplished with sensitive radioenzymatic assays or by measuring ^{3}H-dopamine formed from exogenously supplied ^{3}H-tyrosine.

The product may be collected by superfusion of brain tissue (Besson et al., 1971), perfusion of cerebral ventricles (Von Voigtlander and Moore, 1973) or via the use of push-pull cannulae (Nieoullon et al., 1977, 1978; Bartholini et al., 1976). Most of these experiments have been carried out in anesthetized or spinal-sectioned animals, but chronic push-pull cannulae have also been implanted into the brains of conscious, freely moving animals (Tilson and Sparber, 1972; Gauchy et al., 1974).

2. Accumulation of dopamine metabolites

The concentrations of the dopamine metabolites 3MT, DOPAC and HVA have all been used as indices of dopaminergic nerve activity (DiGiulio et al., 1978) since they vary in direct proportion to dopamine release. Of these, 3MT is the most direct, but the most difficult to measure. Since the amount of this metabolite formed is very small, it must be measured by mass fragmentographic procedures. On the other hand, DOPAC and HVA can be measured by less sensitive fluorometric or radioenzymatic methods, but because they represent dopamine that has been released and then taken back up into the neuron, they are more indirect estimates of dopamine release.

3. Decline of dopamine concentration after synthesis inhibition

Tissue concentrations of dopamine are maintained by a rate of synthesis that is proportional to release. If tyrosine

hydroxylase, the rate-limiting synthetic enzyme, is inhibited, synthesis ceases and dopamine concentrations will decline in proportion to neuronal activity. Thus, the rate constant for dopamine decline after administration of the tyrosine hydroxylase inhibitor α-methyltyrosine is often used as an index of dopaminergic nerve activity.

4. Dopamine synthesis

Since transmitter synthesis is proportional to neuronal activity, measures of the activity of the rate-limiting synthetic enzyme, tyrosine hydroxylase, are assumed to reflect dopaminergic activity. This can be estimated *in vivo* by measuring the rate of conversion of an intravenous injection of ^{3}H-tyrosine to ^{3}H-dopamine, or by measuring the rate of accumulation of DOPA after inhibition of L-aromatic amino acid decarboxylase with drugs such as RO44602 or NSD 1015) (Hefti and Lichtensteiger, 1976). *In vitro*, neural tissue can be incubated with radioactive tyrosine, and measures of the accumulation of the radioactive by-products of tyrosine hydroxylation and dopa decarboxylation, H_2O and CO_2, can be used as indices of neuronal activity. Because increases in neuronal activity are associated with increased synthesis of dopamine, an increase in nerve activity must activate tyrosine hydroxylase. It has been demonstrated that activation of tyrosine hydroxylase is mediated by an increased affinity of the enzyme for its pteridine cofactor and a decreased affinity for the endproduct, dopamine. Thus, *in vitro* alterations in the affinity of tyrosine hydroxylase for its cofactor have been used as another index of dopaminergic nerve activity. These estimates, however, do not always agree with other biochemical measures of neuronal activity (DiChiara *et al.*, 1978).

IV. Regulation of Nigro-striatal Activity

Nigro-striatal dopaminergic neurons bridge mesencephalic and telencephalic structures. Their terminals are dispersed throughout the striatum, and they, in turn, receive a number of afferents at their cell bodies in substantia nigra. Anatomical, biochemical and electrophysiological studies leave no doubt that these neurons have reciprocal relationships with other neuronal systems at both of these sites. Thus, the activity of nigro-striatal nerves, like that of other nerves in the CNS, can be considered to be a product of the many influences acting on these cells. Superimposed upon these extrinsic influences is the apparent capacity of nigro-striatal neurons to self-regulate their own output.

In 1963, Carlsson and Lindqvist reported that systemic administration of antipsychotic drugs increased striatal concentrations of 3MT. This suggested that nigro-striatal neurons responded to dopaminergic receptor blockade with a compensatory increase in activity. These data have been confirmed and extended by many other investigators. For example, antipsychotics increase nigro-striatal firing rates (Bunney et al., 1973), increase release of dopamine from striatal terminals (Cheramy et al., 1970), increase striatal DOPAC and HVA concentrations (Andén et al., 1964) and increase synthesis of ^{14}C-dopamine from ^{14}C-tyrosine (Nyback et al., 1967; Nyback and Sedvall, 1970). Conversely, drugs which either directly or indirectly stimulate dopaminergic receptors (e.g., apomorphine, amphetamine, L-DOPA) decrease nigro-striatal firing (Bunney et al., 1973a), decrease striatal DOPAC concentration (Roos, 1969) and slow the decline of

dopamine after αMT (Andén et al., 1967). Thus, dopamine antagonists increase, while dopamine agonists decrease nigro-striatal nerve activity.

The results of these pharmacological experiments support the proposal (Carlsson and Lindqvist, 1963) that the concentration of dopamine at dopaminergic receptors controls the activity of nigro-striatal nerves. According to this hypothesis increased activity of nigro-striatal dopaminergic neurons increases the concentration of dopamine at receptor sites which, through feedback mechanisms, inhibits further neuronal activity and thereby reflexly lowers synaptic dopamine concentrations. On the other hand, blockade of dopaminergic receptors, or reductions of synaptic dopamine concentrations increases dopaminergic nerve activity. The consequent enhanced release of dopamine returns synaptic concentrations of dopamine to normal. Thus, dopamine output is maintained within limits "set" by the sensitivity of dopaminergic receptors.

The location of the dopaminergic receptors that serve to monitor neuronal activity has been the object of intensive research, and a number of hypotheses concerning their sites (see Figure 3) have been proposed. They may be located: 1) presynaptically, on dopaminergic axons or terminals in the striatum, where they monitor dopamine release; 2) on dopaminergic cell bodies or dendritic processes in substantia nigra, where dopamine release may reflect neuronal activity at striatal terminals; 3) on terminals of striato-nigral afferents, where they may influence release of GABA or substance P; 4) on post-synaptic neurons, controlling nigro-striatal activity via neuronal

Figure 3. Location of hypothetical dopaminergic receptors controlling nigro-striatal activity. These receptors may be located on: 1) dopaminergic axons or terminals in striatum (presynaptic autoreceptors), 2) dopaminergic cell bodies or dendrites in substantia nigra (nigral autoreceptors), 3) terminals of nigral afferents, and 4) postsynaptic neurons.

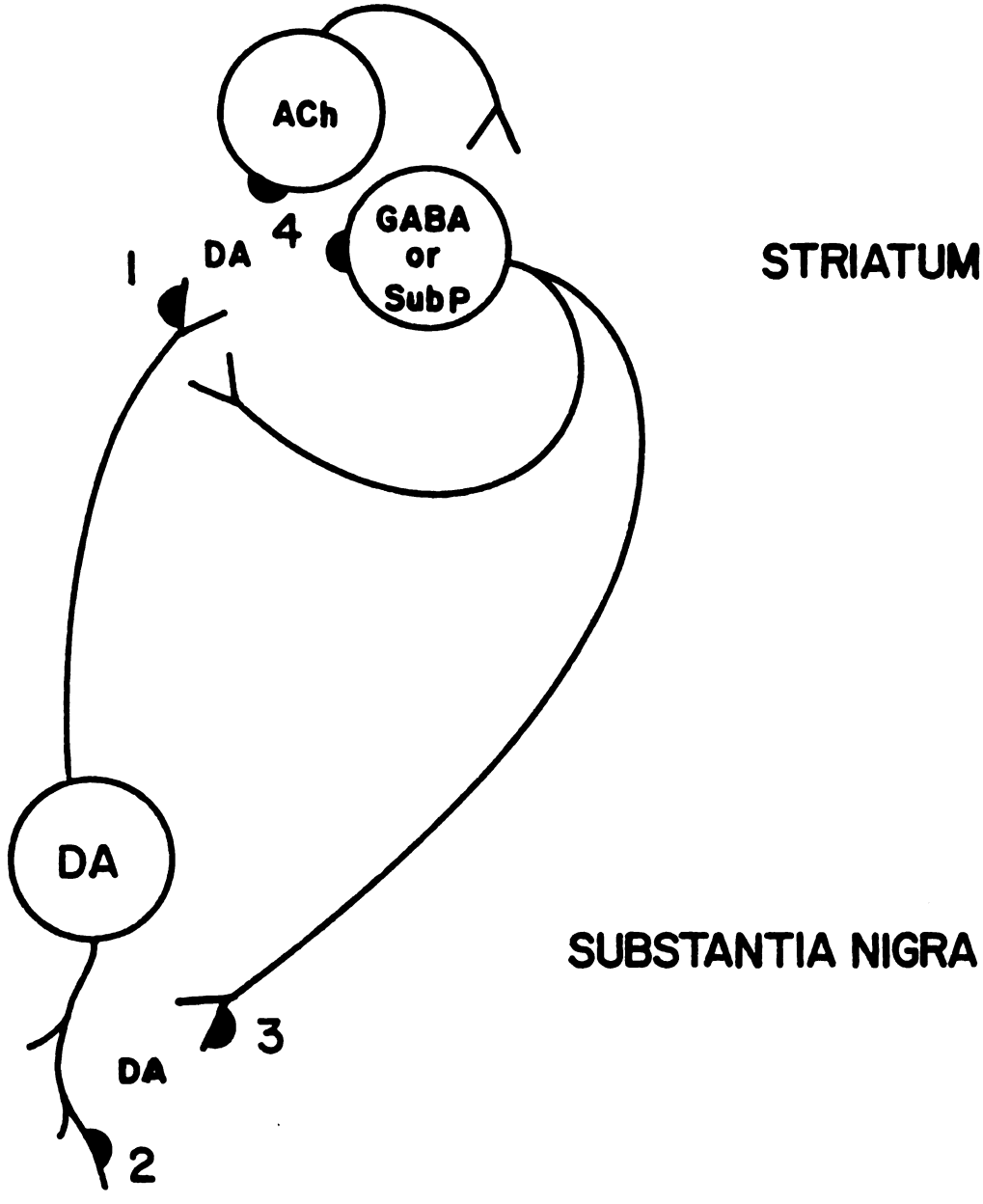

Figure 3

feedback loops within the striatum or from striatum to substantia nigra.

There is abundant evidence that the neuronal elements necessary for a basic feedback loop such as that depicted in Figure 3 exist; that is, both nigro-striatal and striato-nigral projections are well-established. The anatomy, biochemistry and electrophysiological characterization of the connections between efferent and afferent paths, however, remain unresolved. The following sections review the evidence for interactions between nigro-striatal dopaminergic neurons and other neuronal systems in striatum and substantia nigra. Those experiments indicating the site of auto-regulatory receptors are also included.

A. Neuronal regulation in the striatum.

Neuronal relationships within the striatum are exceedingly complex (see Figure 4). There is evidence to indicate that dopaminergic neurons synapse with cholinergic and possibly GABAergic or substance P-containing neurons in the striatum, and also that neurons containing 5HT, acetylcholine, enkephalin and glutamate project to the striatum and interact with dopaminergic nerve terminals there.

What influence ascending dopaminergic projections have on caudate neurons is by no means resolved. Most biochemical evidence (see Section IV.A.1) suggests an inhibitory action of dopamine; electrophysiological studies are not in agreement on this point. Extracellular recording experiments suggest that most, though not all, caudate neurons are hyperpolarized by iontophoretic application of dopamine (Connor, 1968; McLennan and York, 1967) or by nigral stimulation (Connor, 1970). Lesions of the ascending dopamine-containing

Figure 4. Schematic diagram of neuronal systems that may influence or receive input from nigro-striatal neurons at the level of the striatum (ACh, acetylcholine; DA, dopamine; Enk, enkephalin; GABA, γ-aminobutyric acid; Glu, glutatmate; 5HT, 5-hydroxytryptamine; Sub P, substance P.

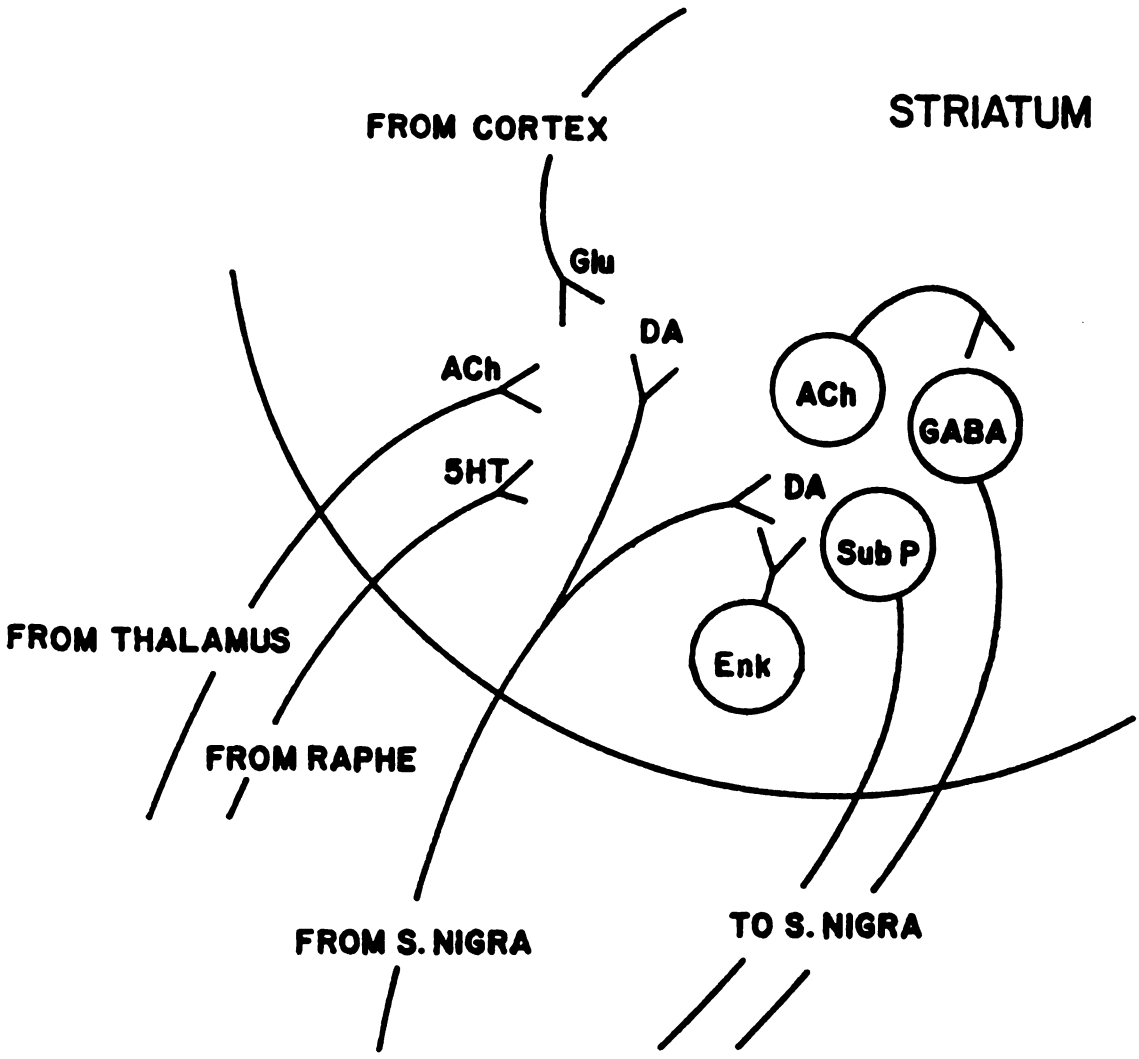
FROM CORTEX
STRIATUM
Glu
DA
ACh
ACh
GABA
5HT
DA
Sub P
Enk
FROM THALAMUS
FROM RAPHE
FROM S. NIGRA
TO S. NIGRA

Figure 4

fibers increase spontaneous activity of cells in putamen (Ohye et al., 1970), suggesting a release from tonic inhibition. Infusions of haloperidol directly into the caudate are also reported to increase firing rates of caudate cells, while similar infusions of the dopamine-releasing drug amphetamine decrease firing rates (Groves et al., 1975). These data all point to an inhibitory action. However, excitatory responses have been reported in a number of these studies, and in others (Fuller et al., 1975; Hull et al., 1970; Hull et al., 1973; Kocsis et al., 1977) as well. Furthermore, in intracellular recording studies, the depolarization of caudate neurons caused by nigral stimulation can be blocked with chlorpromazine (Kitai et al., 1976b).

Thus, while in the past there has been a general acceptance of an inhibitory action by dopamine, an excitatory effect, or both, cannot be excluded. In fact, there is now electrophysiological evidence that two dopamine-sensitive receptors exist in striatum which respond with excitation on one hand, or with inhibition on the other (Norcross and Spehlmann, 1978; Rebec and Segal, 1978). Alternatively, the existence of a non-dopaminergic nigro-striatal tract (see Section I.A) may explain why both excitatory and inhibitory responses are observed (Richardson et al., 1977).

1. Acetylcholine

The striatum contains high concentrations of acetylcholine, the source of which was believed until recently to be exclusively striatal interneurons (Butcher and Butcher, 1974; McGeer et al., 1971). Cholinergic neurons in the parafascicular neurons of the thalamus are now known to project to striatum as well (Simke and Saelens, 1977).

Apparently striatal cholinergic function is inhibited by nigro-striatal activity, since dopaminergic agonists decrease striatal acetylcholine release and increase its concentration, and dopaminergic receptor blockers enhance acetylcholine release and lower striatal concentrations of the transmitter (Guyenet et al., 1975; Stadler et al., 1973; Trabucchi et al., 1974). Furthermore, anticholinergic drugs antagonize increases in dopamine metabolites elicited by antipsychotics (O'Keefe et al., 1970). These data suggested the possibility that striatal cholinergic interneurons might complete a negative feedback loop by linking dopaminergic terminals with cells projecting to substantia nigra (see Figure 3). Anatomical studies using Golgi stain have indicated that up to 95% of caudate neurons are interneurons (Kemp and Powell, 1971).

Recent evidence, however, suggests an alternative explanation. Anatomical studies using horseradish peroxidase, a stain that demonstrates the axon more completely than Golgi stain, indicate that the interneuron population of the striatum is much smaller than originally thought and that projection neurons rather than interneurons make up the majority of caudate cells (Grofová, 1975). Bunney and Aghajanian (1976) estimated that up to 50% of caudate cells project to the nigra. It seems unlikely that a very small interneuron population could connect the diffuse dopaminergic input with the large output going to substantia nigra.

The fact that anticholinergic drugs can block the dopamine antagonist-induced increases in striatal dopamine metabolites and that cholinomimetics can increase dopamine turnover (Corrodi et

al., 1967) may also be explained by the findings that striatal cholinergic nerves have a direct action on nigro-striatal terminals. Acetylcholine releases ^{3}H-dopamine from isolated perfused striata (Besson et al., 1969), from striatal slices and from striata in vivo (Giorguieff et al., 1976) while intraventricular injections of atropine decrease striatal concentrations of HVA (Bartholini and Pletscher, 1971). Acetylcholine is thought to act directly on dopaminergic terminals, possibly via collaterals, because cholinergically-induced release of dopamine occurs in the presence of tetrodotoxin, a neurotoxin that prevents the generation of action potentials. In the presence of tetrodotoxin no interneurons could mediate the effects observed after cholinergic drug administration (Giorguieff et al., 1977b). Furthermore, Bunney and Aghajanian (1975) observed that systemically administered scopolamine had no influence on haloperidol-induced increases in firing of cells in substantia nigra. Thus, rather than participate in a feedback loop, cholinergic neurons may act at the level of the dopaminergic terminals in the striatum.

In addition, there is evidence that some nigro-striatal terminals make direct connections with cells projecting back to substantia nigra. Striatal neurons responsive to nigral stimulation have been demonstrated histologically to be output, rather than interneurons (Bishop et al., 1978); likewise, striatal cells responsive to both orthodromic and antidromic stimulation from substantia nigra have been observed (Kitai, personal communcation). Taken together, these data suggest that while a dopaminergic-cholinergic link probably exists in striatum, it is not necessarily part of a feedback loop to substantia nigra.

Interestingly, acetylcholinesterase is thought to be localized within the nigro-striatal dopaminergic neurons, since lesions of the medial forebrain bundle, which destroy dopaminergic cells in the nigra and deplete dopamine in the striatum, also cause degeneration of nigral neurons staining for this enzyme (Butcher, 1977). Intracerebral injections of 6-OHDA that decrease striatal tyrosine hydroxylase by 90% also decrease striatal and nigral acetylcholinesterase (Lehmann and Fibiger, 1978). The function of this enzyme within dopaminergic neurons remains a mystery, but it probably does not metabolize acetylcholine, since nigral lesions do not influence striatal acetylcholine concentrations or choline acetyltransferase activity.

In summary, the anatomical basis for dopaminergic-cholinergic interactions in the striatum remains unclear. Since the source of striatal acetylcholine is both striatal interneurons and projections from the thalamus, dopaminergic inhibition of striatal cholinergic function may represent either a connection with thalamo-striatal terminals, or with interneurons, or both. Likewise, acetylcholine has been shown to facilitate dopamine release directly from the striatum, but again, whether interneurons or cholinergic projections from thalamus mediate this release is not known.

2. γ-Aminobutyric Acid

The striatum contains high concentrations of the putative inhibitory neurotransmitter γ-aminobutyric acid (GABA). While some of this may represent interneurons (McGeer and McGeer, 1975) most is probably contained within neurons projecting to substantia nigra.

These cells may form the inhibitory striato-nigral feedback loop. Their functional connections in the striatum have not yet been completely described. There is little evidence that nigro-striatal dopaminergic neurons synapse directly with GABA neurons. A single two-neuron inhibitory feedback loop would require that dopamine enhance the activity of a descending inhibitory projection, but this is at variance with evidence suggesting that dopamine is inhibitory. For example, L-dopa is reported to increase nigral concentrations of GABA (Lloyd and Hornykiewicz, 1977) while dopaminergic antagonists decrease striatal and nigral GABA concentrations, and increase GABA turnover (Kim and Hassler, 1975; Costa et al., 1978). Since dopaminergic antagonists with anticholinergic properties are more potent in this respect than those with no ability to block cholinergic receptors, it has been argued (Costa et al., 1978) that dopamine only influences GABA neurons indirectly, through cholinergic interneurons. To date there is little evidence that cholinergic drugs themselves alter GABA turnover. The basis for the belief that GABA neurons form part of an inhibitory feedback loop is mainly that striatal GABA neurons project to substantia nigra and that GABA inhibits nigral cell firing.

What influence GABA neurons exert on dopaminergic nerve activity at the level of the striatum is not clear. In striatal slices, GABA increased the release of ^{3}H-dopamine synthesized from ^{3}H-tyrosine. This effect was blocked by the GABA antagonist picrotoxin, and lack of an effect in the presence of tetrodotoxin suggests that the releasing action is an indirect one (Giorgieff et al., 1978). It is difficult to assess the significance of these findings, however,

since dopaminergic neurons are probably damaged in tissue slices. On the other hand, when administered directly into the striatum, GABA first stimulates then depresses the release of dopamine (Cheramy et al., 1978). In the rat, picrotoxin and bicuculline enhanced dopamine release (Bartholini and Stadler, 1975), while GABA decreased spontaneous dopamine release and inhibited antagonist-induced release. At the present time no interpretation about GABA-dopamine interactions can be made from these conflicting results, although it is assumed that those obtained from in vivo studies are more likely to be representative of physiological conditions.

3. Enkephalins

It is well known that morphine and other narcotic analgesics increase dopamine synthesis and turnover (Lal, 1975), increase nigral cell firing (Iwatsubo and Clouet, 1977) and influence motor behavior (Rethy et al., 1971). The discovery that the striatum contains the naturally-occurring opiate enkephalin and that opiate receptors (Audigier et al., 1977; Kuhar et al., 1973) are located on dopaminergic nerve terminals in striatum (Pollard et al., 1977; Biggio et al., 1978) implies that in the striatum, and possibly in the pallidum (Hong et al., 1977a) enkephalin-containing neurons enhance dopamine release. This appears to be the case in the striatum, since intraventricular administration of D-ala-met-enkephalin also increases turnover of striatal dopamine and increases striatal concentrations of HVA and DOPAC. These increases are reversed by the narcotic antagonist naloxone (Algeri et al., 1978; Biggio et al., 1978).

Although the functional significance of an enkephalin input to striatal dopaminergic neurons is not known, it does not

appear that enkephalin-containing neurons participate in the dopaminergic response to antipsychotics because the effects of morphine and chlorpromazine on striatal concentrations of HVA are supra-additive (Kuchinsky and Hornykiewicz, 1974), and naloxone does not antagonize haloperidol-induced increases in nigral cell firing (Iwatsubo and Clouet, 1977).

4. 5-Hydroxytryptamine

The striatum contains high concentrations of 5HT. The source of this transmitter has been localized by lesion studies to the dorsal and possibly the median raphé nuclei (Lorens and Guldberg, 1974; Ternaux et al., 1977). Stimulation of the dorsal raphé releases 5HT from the caudate (Holman and Vogt, 1972; Chiueh and Moore, 1976), implying that functional connections are made at this site. Most electrophysiological studies suggest an inhibitory role for 5HT. Extracellular recordings indicate that most striatal cells, either spontaneously active or excited by glutamate, are depressed following raphé stimulation (Aghajanian et al., 1975; Davies and Tongroach, 1978; Miller et al., 1975; Olpe and Koella, 1977). The target of this inhibition may include dopaminergic terminals as well as striatal cells. The behavioral effects of amphetamine, which are thought to be mediated by the release of dopamine are enhanced by raphé lesions, by the 5HT synthesis inhibitor p-chlorophenylalanine or by destruction of 5HT neurons with intracisternal injections of 5,6 or 5,7-dihydroxytryptamine (Neill et al., 1972; Mabry and Campbell, 1973; Breese et al., 1974). Likewise, antipsychotic-induced catelepsy is reduced after raphé lesions or p-chlorophenylalanine (Kostowski et al., 1972).

The inhibitory effects of 5HT on dopamine-mediated behaviors agree in principle with the generally held concepts of the 5HT system as an inhibitor of arousal and a mediator of sleep, and the nigro-striatal dopaminergic system as an initiator of motor behaviors.

5. Glutamate

There is a substantial projection of neurons to the striatum from all areas of the cortex (Carman et al., 1963; Webster, 1961). Cortico-striatal projections may contain the excitatory amino acid and putative neurotransmitter glutamate (Kim et al., 1977). Since a high affinity glutamate uptake, considered to be a selective marker for glutaminergic nerve terminals, is reduced in striatum after neocortical lesions (Divac et al., 1977; McGeer et al., 1977), striatal glutamate may have more than a metabolic function at this site. Cortical stimulation evokes monosynaptic excitatory responses from striatal neurons (Buchwald et al., 1973; Kitai et al., 1976a), and such responses can be blocked by the glutamate antagonist glutamic acid diethyl ester (Spencer, 1976). Thus, cortico-striatal glutaminergic neurons appear to excite caudate cells.

Glutaminergic striatal afferents may also synapse with and directly influence release of dopamine from striatal nerve terminals. Application of glutamate to rat striatal slices releases dopamine, even in the presence of tetrodotoxin (Giorguieff et al., 1977a). Furthermore, Nieoullon et al. (1978) have demonstrated that endogenously synthesized dopamine is also released in vivo by stimulation of motor cortex. These results imply that nigro-striatal input

to motor control is regulated, in part, by a glutaminergic system originating in the motor cortex.

B. Neuronal regulation in substantia nigra

In addition to those neurons influencing nigro-striatal nerve activity via connections with dopaminergic terminals in striatum, a number of neuronal afferents, including those containing 5HT, GABA and substance P, as well as an unidentified projection from cerebellum appear to modulate activity of DA cell bodies in substantia nigra (see Figure 5).

1. γ-Aminobutyric acid

GABA neurons are widely held to constitute the descending limb of an inhibitory feedback loop projecting from striatum to substantia nigra. The role of GABA as a transmitter in this pathway (see section I.B), and as an inhibitor of nigro-striatal neurons is well-established; direct application of GABA to substantia nigra increases striatal dopamine concentrations (Andén and Stock, 1973) and blocks the spontaneous firing of nigral cells (Feltz, 1971), an effect that is itself blocked by the GABA antagonist picrotoxin (Precht and Yoshida, 1971a,b). Furthermore, indirectly acting GABA-mimetics like the GABA transaminase inhibitor amino-oxyacetic acid increase nigral GABA concentrations and decrease striatal dopamine turnover (Andén, 1974). When dopaminergic neurons are released from this tonic inhibition by lesions of striato-nigral fibers, striatal concentrations of the dopamine metabolites 3MT and DOPAC (Racagni et al., 1977, 1978b) are increased. In vivo studies suggest that GABA may also inhibit dopaminergic activity in striatum (see section IV.A.2).

Figure 5. Schematic diagram of neuronal systems that may project to dopaminergic cell bodies or dendrites in substantia nigra. DA, dopamine; GABA, γ-aminobutyric acid; 5HT, 5-hydroxytryptamine; S.P., substance P; Z.C., zona compacta, Z.R., zona reticulata.

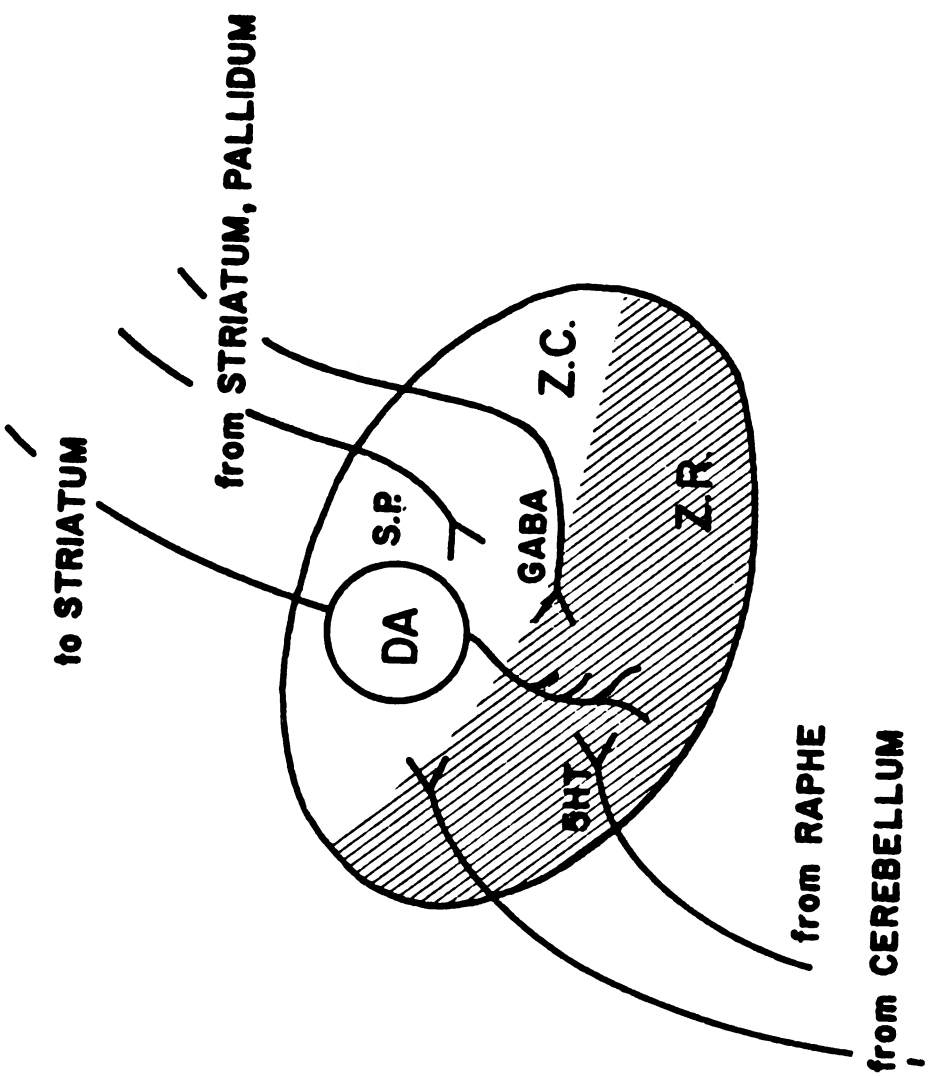
to STRIATUM
from STRIATUM, PALLIDUM
S.P.
DA
GABA
Z.C.
Z.R.
5HT
from RAPHE
from CEREBELLUM

Figure 5

Dopamine, in turn, may elicit the release of GABA at the level of substantia nigra. A dopamine-sensitive adenylate cyclase appears to be located on the terminals of descending GABA or substance P neurons in substantia nigra (Gale et al., 1977b; Phillipson et al., 1977; Nagy et al., 1978; Spano et al., 1977) since the activity of the enzyme decreases after selective lesions of the striato-nigral path, but not after 6-OHDA lesions of dopamine nerves. The implication is that dopamine released from dendrites in substantia nigra influences release of GABA or substance P. In fact, dopamine does selectively increase release of ^{3}H-GABA from substantia nigra in vitro (Reubi et al., 1977).

2. Substance P

The striato-nigral tract contains, in addition to axons from striatal GABA neurons, axons of substance P neurons (see section I.B). Anatomical studies suggest that there is a connection between these axons and dopaminergic cell bodies in zona compacta (Hökfelt et al., 1978). Excitatory nigral responses to caudate stimulation have been observed (Dray et al., 1976a; Frigyesi and Purpura, 1967). Since iontophoretic administration of substance P depolarizes nigral cells (Davies and Dray, 1976; Walker et al., 1976) these excitatory responses are generally attributed to the release of substance P by caudate stimulation. Certain experimental results of Groves et al. (1975) might be explained by the existence of such excitatory afferents to substantia nigra. They reported increases of nigral cell activity following intrastriatal injections of the dopamine-releasing drug amphetamine, and decreases in nigral cell firing after similar injections of haloperidol. This would not be predicted from inhibitory

feedback loop models, and in fact it was concluded that a positive feedback loop exists. Substance P rather than GABA neurons may mediate such effects.

3. 5-Hydroxytryptamine

5HT-containing neurons from the dorsal and possibly median raphé nuclei send fibers to substantia nigra, where they form axodendritic connections (Parizek et al., 1971; Conrad et al., 1974; Pasquier et al., 1977). The function of 5HT at this site, as in the striatum, may be inhibitory since microiontophoretically applied 5HT depresses zona compacta cells (Aghajanian and Bunney, 1975). The inhibitory effect of iontophoretic application of 5HT, but not dopamine, is blocked by p-chlorophenylalanine (Fibiger and Miller, 1977) and also correlates with inhibition produced by stimulation of the median raphé (Dray et al., 1976b). It appears then, that raphé neurons inhibit nigro-striatal nerve activity at least at the level of the nigra, and possibly at striatal terminals as well (see section IV.A.4.).

4. Acetylcholine

Acetylcholine, its synthesizing and metabolizing enzymes, choline acetyltransferase and acetylcholinesterase, as well as acetylcholine receptors are present in substantia nigra (Fonnum et al., 1974; Oliver et al., 1970; Dray and Straughn, 1976). The origin of nigral acetylcholine is not known, but lesion studies (McGeer et al., 1973) have ruled out striatum and structures anterior to it as the source.

Intranigral injections of cholinomimetics reduce the release and increase the concentration of dopamine in the striatum (Javoy et al., 1974), indicating that acetylcholine inhibits nigro-striatal neurons when applied to substantia nigra. On the other hand, electrophysiological experiments suggest that acetylcholine can excite cells in both zona reticulata (Aghajanian and Bunney, 1975) and zona compacta (Dray and Straughn, 1976). James and Massey (1978) have offered an interesting hypothesis that may explain these conflicting results. They observed that intranigrally administered cholinergic drugs decreased HVA concentrations in the striatum; conversely, intranigrally administered cholinergic blocking drugs increased striatal HVA, effects just opposite to what would have been predicted from the results of Aghajanian and Bunney. Interestingly, these effects were blocked by the simultaneous nigral injection of α-flupenthixol, a dopaminergic antagonist, suggesting that acetylcholine inhibits nigro-striatal nerve activity indirectly via a dopaminergic mechanism. That is, acetylcholine may release dopamine from zona compacta cells. This could result in excitatory electrophysiological responses of nigral cells to cholinergic drugs, but the released dopamine could inhibit further cell firing by activating autoreceptors (see section IV.C.2).

C. Autoreceptor Regulation

Recently, the hypothesis that regulation of nigro-striatal nerve activity is controlled by neuronal feedback loops has been called into question. Chemical (Di Chiara et al., 1977; Wuerthele and Moore, 1979), electrolytic or mechanical (Bedard and LaRochelle, 1973;

Garcia-Munoz et al., 1977) lesions of the striato-nigral pathway, which destroy such feedback projections, do not attenuate alterations in striatal dopamine metabolism observed after administration of dopaminergic agonists or antagonists. These experiments do not eliminate the possibility that feedback loops exist, but they suggest that such loops are not essential for autoregulation in the face of an agonist or antagonist challenge. One alternative explanation is that nigro-striatal dopaminergic neuronal activity is regulated, in part, via activation of presynaptic receptors.

1. Striatum

A number of studies have demonstrated that procedures which eliminate impulse traffic in nigro-striatal nerves increase the affinity of tyrosine hydroxylase for its cofactors, decrease its affinity for the end-product inhibitor, dopamine (Roth et al., 1975), increase DOPA accumulation after decarboxylase inhibition (Carlsson et al., 1972; Kehr et al., 1972) and increase ^{3}H-dopamine formation from ^{3}H-tyrosine (Walters et al., 1973). These effects were blocked by dopaminergic agonists and this agonist-induced blockade was, in turn, reversed by haloperidol (Kehr et al., 1972; Roth et al., 1973, 1974). Such observations have been interpreted to mean that presynaptic dopaminergic receptors located on nerve terminals in the striatum monitor synaptic concentrations of dopamine, and that stimulation of these receptors inhibits dopamine synthesis (Figure 3; see also review by Nowycky and Roth, 1978). Because intrastriatal injections of dopaminergic antagonists increase striatal DOPAC concentrations (Racagni et al. 1978a; Wuerthele and Moore, unpublished observations) presynaptic receptors may influence dopamine release as well.

According to some investigators postsynaptic receptors are more important than presynaptic receptors for the control of dopamine synthesis, since haloperidol-induced increases in the affinity of tyrosine hydroxylase for the pteridine cofactor are blocked by lesions of striato-nigral fibers (Gale et al., 1978). It should be pointed out, however, that measurement of the affinity of tyrosine hydroxylase for its cofactors has recently been questioned as an index of nigro-striatal dopaminergic nerve activity (Di Chiara et al., 1978).

It has also been shown that in animals with lesions of the medial forebrain bundle, which destroy the nigro-striatal fibers, haloperidol had no effect on the disappearance of dopamine fluorescence after αMT (Andén et al., 1971). Furthermore, neuroleptics had no effect on the manufacture and release of ^{3}H-dopamine from isolated striata (Cheramy et al., 1970). In order for a dopaminergic antagonist to have any effect it must compete with released dopamine for the receptor. In these preparations dopamine is not released and dopaminergic antagonists should therefore not have an effect. This has been shown to be the case. For example, when striatal release of endogenously synthsized dopamine was measured directly in vivo, dopaminergic antagonists increased release, but this release was blocked by prior treatment with γ-butyrolactone, a drug that blocks impulse flow in dopaminergic neurons (Bartholini et al., 1976; Cheramy et al., 1975). Likewise, tyrosine hydroxylase activity in striatal synaptosomes is not influenced by haloperidol, but can be decreased by the dopaminergic agonist apomorphine. This decrease can then be dose-dependently blocked by haloperidol (Christiansen and Squires, 1974). Such studies

also rule out the possibility that postsynaptic axon collaterals regulate dopamine synthesis.

At the present time it appears that presynaptic receptors may play a role in the regulation of dopamine synthesis under conditions of very low or high impulse flow, and may also influence dopamine release. Interestingly, dopaminergic antagonists increase, and dopaminergic agonists decrease HVA formation in the retina, (Westerink and Korf, 1976) indicating that these neurons respond to these drugs in a manner similar to nigro-striatal neurons, and implying that similar mechanisms may be common to a number of dopaminergic systems.

2. Substantia nigra

In 1973 Bunney and Aghajanian hypothesized that autoregulation of nigro-striatal nerves might occur through presynaptic inhibitory receptors on dopaminergic cell bodies or dendrites in substantia nigra. Several lines of evidence support this hypothesis. Dopamine has been observed in nigral dendrites with the use of histochemical techniques (Björklund and Lindvall, 1975). Dopamine can be synthesized in the nigra from tyrosine (Nieoullon et al., 1977b) and a dopamine uptake mechanism is also present there (Parizek et al., 1971). Antidromic stimulation of the medial forebrain bundle increases nigral concentrations of DOPAC and HVA (Korf et al., 1977) and systemic administration of haloperidol increases, while apomorphine decreases nigral concentrations of DOPAC (Wuerthele et al., 1979). These results suggest that DA may be released from the nigra during neuronal activity. Although a dopamine-sensitive adenylate cyclase

appears to be associated with striato-nigral terminals (Phillipson et al., 1977), 6-OHDA lesions of the nigro-striatal nerves eliminate ^{3}H-apomorphine binding in the nigra (Nagy et al., 1978) suggesting that the binding sites (receptors) are located on dopaminergic neurons or their processes. Finally, local application of dopamine or dopamine agonists inhibited, while antagonists enhanced nigral cell firing (Aghajanian and Bunney, 1973; Groves et al., 1975) and dopamine release (Nieoullon et al., 1977b). Taken together these data imply that nigro-striatal neuronal activity is associated with dopamine release both from striatal terminals and from nigral cell bodies or dendrites. Dopamine released in the nigra would then stimulate inhibitory autoreceptors located on cell bodies or on the dendrites themselves. Blockade of these receptors could disinhibit the neurons and increase nigral cell firing.

There are some reservations concerning this rather attractive hypothesis. The first is that nigral release of dopamine is remarkably different from the phenomenon observed at striatal terminals. Tetrodotoxin, which blocks striatal release, not only fails to block, but is reported to enhance nigral release of dopamine (Nieoullon et al., 1977b). Since tetrodotoxin blocks axonal conduction without depolarizing neurons (Kao, 1966) this suggests that dopamine release in the nigra is not dependent on or coupled with increases in neuronal firing, an unlikely situation if dopamine release has an autoinhibitory function. Secondly, a number of investigators (Glick and Crane, 1978; Kelly and Moore, 1978; Racagni et al., 1978a; Wuerthele and Moore, 1979) have applied both dopamine agonists and antagonists to substantia nigra without observing behavioral or

biochemical evidence for alterations in nigro-striatal nerve activity.

If the release of dopamine from cell bodies or dendrites in substantia nigra is a normal physiological phenomenon it may serve some function other than inhibition of nigro-striatal activity. For example, dendro-dendritic synapses have been observed in substantia nigra (Hajdu et al., 1973). Perhaps rather than altering the level of neuronal activity, dopamine released from these sites may serve to coordinate neuronal activity.

STATEMENT OF PURPOSE

Although the nigro-striatal neurons have been extensively studied, the mechanisms governing their activity are not known. Since nigro-striatal activity is altered by dopamine agonists and antagonists, dopamine receptors appear to have an important regulatory function. The purpose of these studies is to test three hypotheses concerning the location of the dopamine receptors that influence nigro-striatal neuronal activity: that they are located on neuronal feedback loop neurons, on dopamine dendrites or cell bodies in the substantia nigra, or on the terminals of nigro-striatal dopaminergic neurons.

MATERIALS AND METHODS

I. Materials

A. Animals

Male Sprague-Dawley rats (200-300 g, Spartan Research Animals, Haslett, MI) were used. These were kept 3 per cage at constant temperature (25°) on a 12 hour light cycle. Commercial rat chow and water were available ad libitum. In some cases following intracranial injections of kainic acid, a palatable mixture of powdered lab chow and a chocolate diet drink (Sego) was also provided.

B. Drugs

The following drugs were dissolved in saline: baclofen (intranigral injections; Dr. R. Robson, Ciba-Geigy, Summit, NJ, chloral hydrate (Sigma Chemical Co., St. Louis, MO), α- and β-flupenthixol (Dr. I. Møller-Nielsen, Lundbeck and Co., Copenhagen, Denmark), m-hydroxybenzylhydrazine dihydrochloride (NSD 1015; Sigma Chemical Co.), kainic acid (Sigma Chemical Co.), D,L-α-methyltyrosine methylester HCl (Regis Chemical Co., Morton Grove, IL), piribedil mesylate (Dr. Derome-Tremblay, Les Laboratoires Servier, Neiully, France) and sulpiride (Ravizza Research Laboratories, Milan, Italy). Apomorphine HCl (Eli Lilly Co., Indianapolis, IN) was dissolved in 0.1 percent sodium metabisulfite, baclofen for systemic injections was dissolved in warm water; clozapine and thioridazine HCl (Ms. K.D. Roskaz,

Sandoz, Inc., East Hanover, NJ) were dissolved in 1.5% tartaric acid, and haloperidol (Dr. J. Kleis, McNeil Laboratories, Ft. Washington, PA) was dissolved in 0.3% tartaric acid. An injectable form of haloperidol (HALDOL^{R}) and its accompanying vehicle were supplied for intracranial injections by Dr. J. Plostnieks, McNeil Labs. The anesthetic Equithesin was prepared from chloral hydrate, pentobarbital, $MgSO_4$, propylene glycol and ethanol: 4.2%, 0.9%, 2.1%, 44% and 11.5%, respectively. ^{3}H-S-adenosyl methionine and ^{14}C-acetyl CoA were purchased from New England Nuclear (Boston, MA) and ^{14}C-glutamic acid was purchased from Amersham Corporation (Arlington Hts, IL).

II. Methods

A. Surgery

Stereotaxic procedures were performed on 250-300 g rats. Animals were anesthetized with Equithesin (3 ml/kg, i.p.) unless otherwise stated.

1. Intracranial injections

a. Kainic acid injections

Animals were anesthetized and placed in a stereotaxic apparatus. The scalp was opened and a small hole was drilled 2.0 mm anterior to Bregma and 3.0 mm lateral from the midline. A 30 gauge stainless steel cannula was lowered through the hole into the striatum 5.3 mm ventral to the dura (Pellegrino and Cushman, 1969). Drug or vehicle was injected over 2 minutes from a 5 µl syringe mounted on an infusion pump and connected to the cannula by a short piece of polyethylene tubing (PE-10). Following the injection the cannula was left in place one minute before it was removed. The

incision was then closed with a wound clip and the animal allowed to recover.

b. Intracranial injections through permanent cannula guides

Animals were anesthetized and placed in a stereotaxic apparatus. The scalp was opened and holes were drilled through the skull 3.0 mm posterior to bregma and ±2.0 mm lateral from the midline (nigral injections) or 2.0 mm anterior to bregma and ±3.0 mm lateral from the midline (striatal injections). Four stainless steel screws were inserted into but not through the skull at points forming a rectangle around the site of implantation. Two 23 gauge, 14 mm stainless steel cannula guides stereotaxically mounted on parallel wires were lowered through the skull holes and into the brain -7.5 mm (nigral injections) or -4.3 mm (striatal injections) ventral to the dura. Liquid dental acrylic (NuWeld) was poured over the area bounded by the screws and allowed to harden. Parallel wires were removed and the implanted cannula guides fitted with 30 gauge 14 mm occluder wires (see Figure 6).

Injections were made as described above for acute injections through the chronically implanted cannulae in freely moving, fully conscious rats: Occluder wires were then removed, and 15 mm, 30 gauge injector cannulae were inserted into each implanted cannula. This length allows the injector cannula to extend 1.0 mm below the outer cannula tip. Occluder pins were replaced following the injection.

Figure 6. Sagittal view of the rat brain implanted with permanent cannula guide for injection into substantia nigra. A, skull; B, hardened dental acrylic; c, stainless steel screw; D, cannula guide; E, occluder pin; F, injection target, located 1 mm ventral to the tip of the guide.

2. Knife cuts

Rats were anesthetized and mounted in a stereotaxic apparatus. The scalp was opened and a hole was drilled in the skull 4.4 mm anterior to the intra-aural line (Konig and Klippel, 1963) and either 1.8 mm lateral to the midline (knife cuts into striato-nigral pathway) or from the midline to 5.0 mm lateral from midline (hemi-transsections). The dura was opened with a pair of microscissors, and a 1.2 mm wide stainless steel knife, cut from the beveled edge of a razor blade, was lowered through the skull hole to the base of the brain. In the case of the hemitranssections, this knife was slowly moved 4.5 mm lateral from the midline. The knife was then removed, the scalp closed with a wound clip and the animals allowed to recover.

B. Dissections

Animals were killed by decapitation and brains rapidly removed and placed on a thermoelectric cold plate (approximately 10°C). For fluorometric assays, the anterior commissure was cut at the midline and the cortex was removed. Whole striata were dissected out with microscissors. For radioenzymatic assays coronal cuts were made through the brain at approximately the level of the infundibulum and at the center of the medulla. Pieces containing the striatum and the substantia nigra were placed on numbered glass slides on a slab of dry ice. When frozen, these tissue blocks were mounted on a sliding microtome fitted with a freezing stage. A 1 mm (striatum) or three 500 μ (nigra) sections were sliced, rostral to caudal, beginning at the level where the corpus callosum crosses the midline (striatum) or at the rostral edge of the substantia nigra. These slices were placed

on numbered slides on dry ice. Appropriate regions were dissected from them on a thermoelectric cold plate with the aid of a stereoscope, using microscissors (striatum) or a 1x1.5 mm stainless steel punch (nigra).

C. Histology

Animals were anesthetized with Equithesin (3 ml/kg, i.p.) and perfused via cardiac puncture with 10% neutral buffered formalin. Brains were postfixed in 10% buffered formalin and dehydrated and embedded in paraffin. From 10 μ sections, every fiftieth section was stained with hematoxylin and eosin. Sections from selected areas were stained with Luxol fast blue-cresyl violet.

D. Biochemical procedures

1. Dihydroxyphenylacetic acid (DOPAC) concentrations

Striatal DOPAC concentrations were assayed using a modification of the fluorometric method of Westerink and Korf (1976). Striata were weighed and homogenized in 1 ml ice cold 0.4 N $HClO_4$. Two drops of 10 N KOH-formic acid solution (1:4) was added to each sample or to standards in 0.4 N $HClO_4$ 20 min prior to centrifugation at 10,000 x g. Supernatants were poured over 7 cm Sephadex G-10 columns prepared with 3 ml of 0.01 N NH_4OH and 3 ml 0.01 N formic acid. Samples were eluted with 1.5 ml 0.005 M phosphate buffer, pH 8.5. One ml of the eluate was oxidized by the addition of 100 μl 10% $K_2Fe_3(CN)_6$, and 500 μl ethylene diamine reagent. These were heated at 72°C for 14 min, and fluorescence was read at 410-450 nm. Concentrations were expressed as μg DOPAC per g wet weight of tissue. Sensitivity of the assay was 50 ng.

Striatal and nigral concentrations of DOPAC were also measured by a radioenzymatic method (Umezu and Moore, 1979). Tissue was homogenized in 0.4 N $HClO_4$ with 0.01 % EGTA. Homogenates were centrifuged and the pellet assayed for protein by the method of Lowry et al. (1951). Twenty µl of supernatant or standards in 0.4 N $HClO_4$ were incubated for 1 hour at 37°C with 50 µl of mix containing 3.3 µl 20 mM EGTA-sodium, excess catechol-o-methyltransferase (COMT), 10 µl 250 µCi/ml ^{3}H-S-adenosylmethionine (11.0 Ci/mmol), 3.3 µl of 8 mg/ml pargyline in 10% mercaptoethanol and 21.8 µl 1 M Tris Base with 3 mM $MgCl_2$. Following incubation 50 µl of the incubation mixture was assayed for the tritiated DOPAC metabolite homovanillic acid, and 20 µl of the mixture for the tritiated dopamine metabolite 3-methoxy-tyramine (see below). Homovanillic acid was isolated by organic extraction. Twenty µl of 1 N $HClO_4$-carrier solution (1:1) and 220 µl ethyl acetate were added to the incubation mixture, and vortexed. One-hundred-sixty µl of the organic phase was transferred to another tube containing 300 µl 200 mM Tris-HCl and 300 µl ethyl acetate. After an ethyl acetate wash, 100 µl water-saturated ethyl acetate and 30 µl 3 N HCl were added to the tube. Sixty µl of the organic layer were spotted on 250 µ silica gel TLC plates (Whatman LK6D). The plates were developed in an isopropanol:ammonium hydroxide:water (8:1:1) solvent, and the product visualized with Folin-phenol:water (1:1) reagent. The resulting spots were scraped into scintillation vials containing 0.5 ml ethyl acetate-acetic acid-water (3:3:1). After elution of the product in this solution for 30 min, 10 ml scintillation cocktail (toluene:95% ethanol :: 7:3, PPO 0.5%) was

added and radioactivity was measured in a Beckman LS-100 scintillation counter with an efficiency of 30%. Concentrations were expressed as ng DOPAC/mg protein. Sensitivity of the assay was 0.1 ng.

2. Dopamine

Striatal and nigral concentrations of dopamine were measured by the radioenzymatic method described above for DOPAC. Following incubation, the product, tritiated 3-methoxytyramine, was isolated by organic extraction. Twenty µl of the incubation mix was transferred to tubes containing 30 µl carrier-borate buffer solution (1 vol carrier to 5 vol 0.45 M borate buffer, pH 10.0). Five-hundred-fifty µl toluene:isoamyl alcohol solution (3:2) were added and the tubes vortexed. Four-hundred µl of the organic layer were transferred to tubes containing 40 µl 0.1 N HCl and vortexed. Following a 200 µl ethyl acetate wash, the organic layer was discarded and 30 µl of the aqueous phase was spotted on 250 µ silica gel TLC plates (Whatman). These were developed in a methylamine:100% ethanol:chloroform (5:18:40) solvent system. The plates were sprayed with Folin phenol reagent-water (1:1) to visualize the products. Darkened areas on the plates corresponding to the product were scraped into scintillation vials, and the product eluted with 0.5 ml acetic acid:ethanol:water (3:3:1). Radioactivity was measured by liquid scintillation spectrometry as described above for DOPAC. Concentrations were expressed as ng dopamine per mg protein. Sensitivity of the assay was approximately 0.05 ng.

3. Dihydroxyphenylalanine (DOPA) concentrations

Striatal and nigral concentrations of DOPA were measured by a modification of the method of Hefti and Lichtensteiger (1976). Animals were sacrificed 30 minutes after systemic administration of m-hydroxybenzylhydrazine dihydrochloride (NSD 1015, 100 mg/kg, i.p.).

Tissue was homogenized in 10 volumes 0.2 N $HClO_4$ with .01 % EGTA. Homogenates were centrifuged and the pellet was analyzed for protein according to the method of Lowry et al. (1951). Ten µl of supernatant or standards in 0.2 N $HClO_4$ were incubated for 1 hour at 37° with 25 µl of mix containing 3.3 µl 20 mM EGTA-sodium, excess catechol-o-methyltransferase (COMT), 10 µl 250 µCi/ml ^{3}H-S-adenosyl methionine (11.0 Ci/mmol), 3.3 µl of 9 mg/ml o-benzylhydroxylamine in 10% mercaptoethanol and 21.8 µl 1 M Tris base with 3 mM $MgCl_2$. Incubation was stopped by the addition of 1 ml ice cold citrate buffer, 0.1 M, pH 2.0. The product, tritiated 3-o-methylDOPA, was isolated by cation exchange chromatography, adsorbtion on activated charcoal and anion exchange chromatography. The incubation mix was poured over a 5x15 mm cation exchange column (AG 50W-X4, H^+, 200-400 mesh) previously prepared with 3 ml 0.1 M phosphate buffer, pH 6.5 and 1.5 ml 0.1 M citrate buffer, pH 2.0. Columns were washed with 6 ml 0.1 M citrate buffer, pH 2.0 and the product was eluted with 2.5 ml 0.1 M citrate buffer, pH 4.5. Fifty µl of a slurry of activated charcoal was added to the eluate and the tubes vortexed. Following two washes with 0.5% acetic acid the product was eluted from the charcoal with 1 ml 5% phenol. The supernatant was transferred to another tube containing 200 µl 2 N HCl and the phenol extracted with 3 ml ethyl

acetate, 1 ml 0.5 M piperazine and another 3 ml ethyl acetate. Three ml 0.2 M piperazine, pH 10.5, was added to the samples, and they were then poured over a 5x15 mm anion exchange column (AG1-X2, 200-400 mesh, OH^- form) previously prepared with 5 ml 2 N NaOH, 5 ml H_2O and 5 ml 0.2 M piperazine, pH 10.5. Columns were then washed with 5 ml 0.2 M piperazine, and the product eluted into scintillation vials with 3 ml 0.2 M piperazine, pH 6.0. Fifteen ml ACS scintillation cocktail (Amersham/Searle, Inc.) was added to the vials and radioactivity was measured in a Beckman LS-100 scintillation counter with an efficiency of approximately 30%. Concentrations were expressed as ng DOPA/mg protein. Sensitivity of the assay was 0.2 ng.

4. Choline acetyltransferase (ChAT) activity

Water homogenates of striatal tissue were diluted to approximately 100 fold with 10 mM EDTA containing 0.5% Triton X-100, and assayed for ChAT activity by a modification of the method of Fonnum (1975). Five µl of tissue homogenate was incubated for 15 min at 37°C with 10 µl of incubation mix, containing 450 mM sodium chloride, 12 mM choline iodide, 30 mM EDTA 0.15 mM physostigmine and 0.3 mM acetyl-[1-^{14}C] coenzyme A (3.33 µCi/µmole). Following incubation tubes were rinsed with two 2 ml portions of ice cold 10 mM sodium phosphate buffer, pH 7.4 and transferred to scintillation vials. Two ml of 0.5% sodium tetraphenylboron in acetonitrile and 10 ml of 0.05% PPO in toluene were added to each vial. Thirty min afterwards radioactivity was measured in a Beckman LS-100 scintillation counter with an efficiency of approximately 80%. Radioactivity increased linearly with amount of tissue and incubation time.

5. L-glutaminc acid decarboxylase (GAD) activity

Nigral and striatal GAD activity was measured by a modification of the method of Kanazawa et al. (1976). Tissue was homogenized in water. Five µl of homogenate was incubated for 15 min at 37°C with 5 µl of a mix containing 0.1 M potassium phosphate buffer, pH 6.5, 0.5 mM dithiothreitol, 0.5 mM pyridoxyl phosphate, 25 mM sodium L-glutamate and 10 µCi/ml L-[1-^{14}C]-glutamic acid (55 µCi/µmole). The reaction tube was connected by Tygon tubing to a similar tube containing 150 µl of NCS (Amersham/Searle). The reaction was stopped by the injection of 100 µl 6N sulfuric acid into the reaction tube. The tubes were left in the incubating bath for another 30 min, and the tube containing NCS was then inverted in a scintillation vial containing 25 µl glacial acetic acid. Ten ml 0.05% PPO in toluene was added and radioactivity was determined in a Beckman LS-100 liquid scintillation counter with an efficiency of approximately 80%. Radioactivity increased linearly with amount of tissue and incubation time.

6. Statistics

Significance of the differences between groups of animals or between data obtained from different treatments on either side of the brain were tested using one way or two-way analysis of variance, respectively (Sokal and Rohlf, 1969). Tests were two-tailed unless otherwise indicated. Student's t-test was used to test the significance of the differences in the first order rate constants obtained by least squares regression analysis (Goldstein, 1971). The 0.05 level of probability was used as the criterion for significance.

RESULTS

I. Studies on Nigral Dopaminergic Autoreceptors

Systemic administration of dopamine agonists and antagonists has long been known to influence nigro-striatal nerve activity (see Section IV), suggesting that release of dopamine from nerve terminals in the striatum is maintained by some receptor-mediated control mechanism. The first hypothesis examined was that dopamine receptors on cell bodies or dendrites of nigro-striatal neurons in substantia nigra are responsible for control of nigro-striatal neuronal activity. During nerve activity dopamine may be released from cell bodies on dendrites in substantia nigra as well as at striatal terminals. Inhibitory nigral dopamine receptors monitoring this released dopamine may then control dopaminergic nerve activity.

A. Striatal and nigral concentrations of dopamine and DOPAC following systemic administration of apomorphine and haloperidol

Table 1 illustrates the effects of exogenously administered dopamine agonists and antagonists on nigro-striatal dopaminergic activity. Nigral and striatal concentrations of dopamine and DOPAC, expressed as a percent of control following systemic administration of haloperidol and apomorphine are listed. Haloperidol did not significantly alter striatal dopamine concentrations but increased striatal DOPAC concentrations by 270 percent. Presumably haloperidol increases

TABLE 1

Striatal and Nigral Concentrations of Dopamine and DOPAC Following Systemic Administration of Haloperidol and Apomorphine

Treatment	Dopamine (% of control)		DOPAC (% of control)	
	Striatum	Substantia Nigra	Striatum	Substantia Nigra
Haloperidol	99.0±3.4	115.5±8.3	269.5±30.0[a]	140.6±7.2[a]
Apomorphine	121.7±4.8[a]	93.1±3.4	46.1± 3.4[a]	64.9±4.3[a]

Animals were given i.p. injections of drug (haloperidol, 0.1 mg/kg; apomorphine, 0.4 mg/kg) or vehicle (haloperidol, 0.3% tartaric acid; apomorphine, 0.03% sodium metabisulfite), and were killed 60 (haloperidol) or 30 minutes (apomorphine) later. Values represent means ± 1 S.E. as determined from 12 to 30 samples.

[a]Indicate values significantly different from control ($p<.05$). Combined control values were 88.2±2.8 and 10.7±0.6 ng/mg protein for striatal and nigral dopamine, and 9.5±0.5 and 3.4±0.2 ng/mg protein for striatal and nigral DOPAC.

release by blocking dopamine receptors responsible for tonic inhibition of dopaminergic activity. Apomorphine, on the other hand, lowered striatal DOPAC concentrations to 46 percent of control values. Such agonists are thought to act much as released dopamine would to inhibit dopaminergic activity. The decrease in DOPAC concentration was accompanied by a small but significant increase in striatal dopamine concentration, a result also reported in other studies (Roth et al., 1974). This may be due to a combination of decrease in impulse flow and increase in dopamine synthesis.

A similar response to both the agonists and antagonists, but less marked than in striatum, was observed in the substantia nigra. The response to apomorphine in the nigra was 65% of that in the striatum, while the nigral response to haloperidol was only one half the striatal response. The biological significance of these findings is not fully understood. Both synthesis and release of dopamine (see Section IV.C) occur in substantia nigra zona reticulata, the area populated by most of the dendrites of nigro-striatal dopaminergic cells. The fact that alterations in nigral DOPAC concentrations, though small, paralleled those seen in striatum after systemic administration of dopamine agonists and antagonists supports the idea (Korf et al., 1977) that nigral dopamine release might be a functional correlate of dopaminergic nerve activity.

B. Striatal and nigral concentrations of dopamine and DOPAC following systemic administration of haloperidol and intranigral administration of baclofen

To test the hypothesis that dopamine release in substantia nigra is correlated with neuronal activity, an effort was made to

reverse the haloperidol-induced increases in both striatal and nigral DOPAC with baclofen, a drug known to block nigro-striatal activity (Davies and Dray, 1976; Olpe et al., 1977). When given systemically baclofen reversed antagonist-induced increases in striatal DOPAC (Waldmeier and Maitre, 1978). This drug appears to act via nigral mechanisms since nigral administration increased striatal dopamine concentrations (Kelly and Moore, 1978). The actions of baclofen in the nigra do not appear to be mediated through dopaminergic mechanisms, however, since its action on striatal dopamine was not reversed by haloperidol (see below). As shown in Table 2, nigral administration of baclofen reversed haloperidol-induced increases in striatal DOPAC. Baclofen also decreased nigral DOPAC and prevented haloperidol-induced increases in DOPAC at this site. These results suggest that haloperidol-induced increases in nigral DOPAC represent enhanced dopamine release associated with increased neuronal activity. Nevertheless, dopamine dynamics in these two regions of the nigro-striatal neuron are not qualitatively the same, because baclofen did not have the same effects on nigral and striatal dopamine concentrations. Baclofen increased dopamine in striatum, but decreased it in the nigra. The latter effect is inconsistent with decreased release. Likewise, GBL, another drug inhibiting nigro-striatal impulse flow, failed to increase nigral dopamine (Pericic and Walters, 1976). GBL increased the striatal (Roth et al., 1973) but reduced the nigral accumulation of DOPA after inhibition of aromatic-L-amino acid decarboxylase (Hefti et al., 1976). The hypothesis that terminals and cell bodies of nigro-striatal dopamine neurons respond differently to changes in

TABLE 2

Striatal and Nigral Concentrations of Dopamine and DOPAC Following Systemic Administration of Haloperidol and Intranigral Administration of Baclofen

	Treatment	Dopamine (ng/mg protein)		DOPAC (ng/mg protein)	
		L	R	L	R
Striatum	Vehicle	100.0±4.3	163.4±4.7[a]	10.3±1.6	11.6±1.6
	Haloperidol	102.6±3.0	180.9±9.3[a]	28.5±2.0	14.9±3.2[a]
Nigra	Vehicle	15.9±1.6	11.0±1.1[a]	3.6±0.4	2.2±0.3[a]
	Haloperidol	17.2±1.5	14.6±1.7	4.8±0.4	2.7±0.2[a]

Animals received injections of baclofen (0.1 µg) in 2.0 µl saline into the right substantia nigra (R). Two µl saline were simultaneously injected into the left substantia nigra (L). Immediately afterwards animals received i.p. injections of haloperidol (0.1 mg/kg) or vehicle (0.3% tartaric acid) and were sacrificed one hour later. Values represent the means of at least 11 separate determinations.

[a]Indicates right (baclofen-treated) side significantly different ($p<0.05$) from left side.

neuronal activity is also supported by the fact that haloperidol-induced percent increases in nigral DOPAC were only half those observed in the striatum (see Table 1). This may reflect intrinsic differences in the amount of dopamine released at cell bodies and terminals. Nieoullon et al. (1977) report reciprocal rather than parallel release of dopamine from nigral and striatal sites in vivo following unilateral sensory stimulation. In summary, these experiments indicate that striatal dopaminergic terminals and nigral dopaminergic cell bodies do not always exhibit parallel changes in synthesis and release of dopamine in response to alterations in neuronal impulse flow.

C. Striatal and nigral concentrations of dopamine and DOPAC following intranigral administration of dopamine agonists and antagonists

Despite the fact that nigral dopamine dynamics are different from those in striatum, release of dopamine in substantia nigra may have a functional role. If nigral dopaminergic receptors are important for the control of nigro-striatal activity, intranigral injection of dopamine agonists and antagonists should mimic the effects of systemic administration.

Results in Table 3 indicate that intranigrally administered apomorphine failed to mimic the effects of systemic injections of this drug. Unlike systemic injections (Table 1), intranigral injections of apomorphine did not decrease striatal DOPAC concentrations. Furthermore, though apomorphine decreased nigral DOPAC, nigral dopamine was also decreased, an effect not seen after systemic administration.

TABLE 3

Striatal and Nigral Concentrations of Dopamine and DOPAC Following Intranigral Administration of Dopamine Agonists and Antagonists

Treatment	N	Dopamine (% of control)		DOPAC (% of control)	
		Striatum	Substantia Nigra	Striatum	Substantia Nigra
Agonists:					
Apomorphine (0.1 µg)	4	86±14	61± 9	111±12	-----
Apomorphine (1 µg)	3	92± 2	68± 5	91±12	-----
Apomorphine (5 µg)	6	111± 7	73±11	104± 8	88± 7
Apomorphine (10 µg)	10	104± 4	47± 7[a]	103± 6	55± 4[a]
Antagonists:					
Haloperidol (0.1 µg)	4	100± 6	103±14	104±10	107± 8
Haloperidol (0.5 µg)	6	119± 8	71± 9	164±37	95±15
Haloperidol (1 µg)	6	122±13	64± 4	108± 6	92±10
Haloperidol (4 µg)	16	124± 6[a]	74± 6[a]	149±14[a]	101±11
Thioridazine (4 µg)	5	107± 6	80± 7[a]	128± 7[a]	92± 4
Sulpiride (10 µg)	9	137± 5[a]	43± 5[a]	184±15[a]	116±16
α-Flupenthixol (5 µg)	10	112± 9	71± 7[a]	130±17	97±10
β-Flupenthixol (5 µg)	6	102± 3	74± 9	88± 5	84± 7

Drugs were injected unilaterally in 2.0 µl vehicle over 2.0 minutes. The contralateral nigra was simultaneously injected with an equal volume of vehicle. Animals were sacrificed 15 minutes (apomorphine) or 1 hour (all other drugs) later. Values are expressed as percent of control side. Mean control values as calculated from at least 39 separate determinations are 79.6±2.7 and 11.5±0.6 ng/mg protein for striatal dopamine and DOPAC, and 10.8±0.6 and 3.8±0.2 ng/mg protein for nigral dopamine and DOPAC.

[a]Indicate values significantly different from control side ($p<0.05$).

Intranigral injections of dopamine antagonists also failed to mimic systemic administration. Intranigral injections of haloperidol increased striatal DOPAC to a much lesser extent than systemic administration (see Table 1), while at the same time no increases in nigral DOPAC were observed. Furthermore, intranigral injections of haloperidol increased striatal, and decreased nigral dopamine concentrations, effects that are not seen following systemic administration of the antagonist.

These results argue against the proposal that dopaminergic autoreceptors in substantia nigra have a major role in control of nigro-striatal activity.

II. Studies on the Striato-nigral Feedback Loop

An alternative explanation for the effects of dopamine agonists and antagonists on dopaminergic nerve activity is that a neuronal feedback loop controls nigro-striatal neurons (see Section IV). According to this hypothesis, inhibitory neurons projecting from the striatum to the substantia nigra (the "striato-nigral feedback loop") regulate nigro-striatal neuronal activity. Nigro-striatal neurons either directly or indirectly inhibit the activity of GABAergic striatal neurons whose descending axons terminate on dopaminergic cell processes in substantia nigra. Inhibitory action by the transmitter released here completes a negative feedback loop. Increased release of dopamine in the striatum therefore initiates compensatory inhibition of nigro-striatal neurons through the feedback loop. Blockade of any element within this loop causes the release of these neurons from this tonic inhibition and thereby increases their activity.

In order to test this hypothesis, challenge doses of dopamine agonists and antagonists were administered systemically to rats in which striato-nigral fibers were previously destroyed. It was expected that following such treatment any alterations in the normal response to these drugs could be used to deduce what role, if any, the neuronal feedback loop plays in regulation of nigro-striatal neuronal activity.

Two techniques were used to destroy the feedback loop. In the first, unilateral intrastriatal injections of kainic acid, a neurotoxic glutamate analog reputed to be selective for neuronal perikarya, were made. This technique was expected to destroy all striatal neurons, including striato-nigral cells, but to spare dopaminergic terminals and other axons of passage (Coyle and Schwarcz, 1976; McGeer and McGeer, 1976). Seven days were allowed to elapse after these injections to permit degeneration of the striato-nigral fibers. At this time destruction of striatal cholinergic neurons was confirmed by the assay of striatal choline acetyltransferase (ChAT). Approximately 80% reduction in striatal glutamic acid decarboxylase (GAD) activity has also been reported for similar treatments at this time (Friedle et al., 1978), indicating that striatal GABAergic neurons are also largely destroyed. Striatal dopamine concentrations were used to indicate if dopamine axons or terminals were affected.

In a second series of experiments the striato-nigral feedback loop was destroyed by stereotaxically-placed knife cuts. In animals with knife cuts, striatal dopamine concentrations were used to verify

that nigro-striatal fibers were left intact, and nigral GAD activity was used as an index of destruction of striato-nigral GABAergic fibers.

A. Striatal DOPAC concentrations following systemic administration of dopamine agonists and antagonists to rats pretreated with unilateral intrastriatal injections of kainic acid

Seven days after rats were given intrastriatal injections of kainic acid striatal dopamine concentrations were lower than, but not significantly different from normal (Friedle et al., 1978; Figure 7). Striatal ChAT activity ranged from 9.2 to 20.4 percent of that in the untreated striatum (Table 4). In saline-pretreated rats (kainic acid + vehicle) intrastriatal injections of kainic acid increased the striatal DOPAC concentration on the side of the injection but did not alter DOPAC in contralateral striata. That is, the concentration of DOPAC in the control striata was not different from that in the striata of rats that had received only a unilateral intrastriatal injection of saline (sham + vehicle). The fact that kainic acid alone increased striatal DOPAC concentrations supports the contention that the feedback loop tonically inhibits nigro-striatal activity. Destruction of the loop then, causes increased dopamine release. These results are in agreement with similar studies by DiChiara et al. (1977), but are in conflict with those of Gracia-Munoz et al. (1977), in which the feedback loop was lesioned electrolytically. In these latter experiments no increases in DOPAC were observed on the lesioned side.

Figure 7. Striatal dopamine concentrations following administration of α-methyltyrosine to rats pretreated with unilateral intrastriatal injections of kainic acid. All rats were injected unilaterally with kainic acid into the right striatum. Seven days later they were injected with haloperidol (0.5 mg/kg i.p.) and αMT (250 mg/kg i.p.) and were killed 3 hrs after receiving haloperidol and either 0, 1 or 2 hrs after receiving αMT. Circles represent means from 12 (control group), 9 (1 hour group) and 10 (2 hour group) animals. Vertical lines represent standard errors. The rate constants for decline of dopamine in control animals are 0.26 and 0.18 for treated and untreated sides, respectively, and in animals receiving haloperidol, 0.43 and 0.36 for treated and untreated sides, respectively.

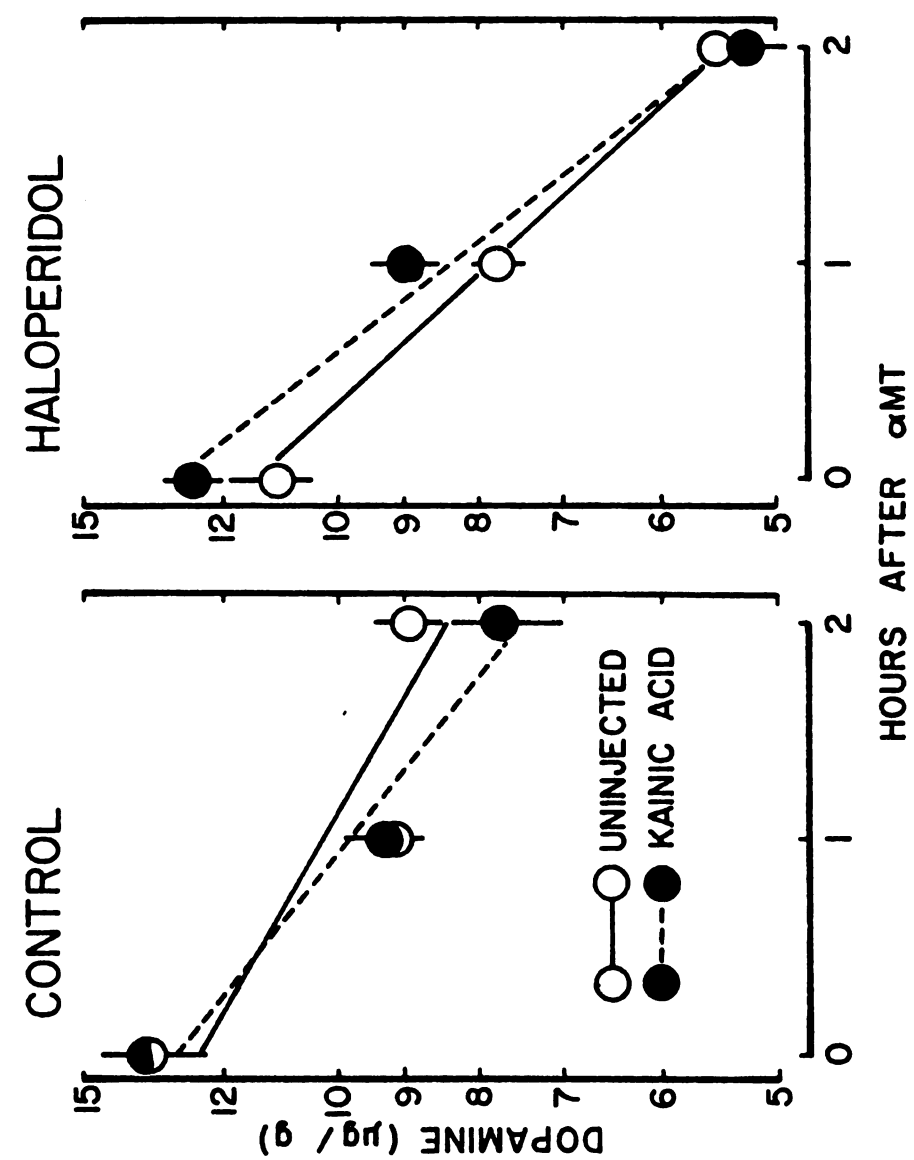
CONTROL
HALOPERIDOL
DOPAMINE (µg/ g)
15
12
10
9
8
7
6
5
0
1
2
HOURS AFTER αMT
UNINJECTED
KAINIC ACID

Figure 7

TABLE 4

Effects of Dopamine Agonists and Antagonists on Striatal DOPAC Concentrations and ChAT Activities in Control and Kainic Acid-Treated Striata

Group	N	Striatal DOPAC (μg/g)		Striatal ChAT (μmole ^{14}C-ACh/g/hr)	
		Untreated Striatum	Kainic Acid Treated Striatum	Untreated Striatum	Kainic Acid Treated Striatum
Controls					
Sham + Vehicle	15	1.54±.10	1.53±.07	22.42±1.32	23.97±1.32
Kainic + Vehicle	36	1.59±.10	2.84±.10	21.71± .80	3.37± .38
Dopamine Agonists					
Apomorphine	11	0.71±.12[b]	1.73±.14	24.87±1.38	3.02± .66
Piribedil	11	1.08±.05	2.46±.18	26.71±1.40	2.54± .45
Dopamine Antagonists					
Haloperidol	15	4.35±.18[a]	5.47±.32	21.23±1.25	3.57± .63
Clozapine	13	4.26±.33[a]	5.94±.37	23.57± .70	2.49± .51
Sulpiride	11	3.21±.22[a]	8.10±.30	26.56±1.45	3.68± .69
Thioridazine	8	3.20±.30[a]	5.33±.49	22.44±1.50	4.59± .94

Seven days after unilateral intrastriatal injections of kainic acid (2.5 μg in 2.0 μl saline injected over 2.0 minutes) or saline vehicle, rats received an injection of a dopamine agonist (apomorphine, 0.5 mg/kg s.c.; piribedil, 30 mg/kg i.p.) or dopamine antagonist (haloperidol, 0.5 mg/kg i.p.; clozapine, 40 mg/kg i.p.; sulpiride, 40 mg/kg i.p.; thioridazine, 10 mg/kg i.p.) or vehicle (apomorphine, .03% Na metabisulfite; piribedil and sulpiride, saline; haloperidol, clozapine and thioridazine, 1.5 % tartaric acid). Animals were sacrificed 3 hrs (sulpiride), 2 hrs (thioridazine, clozapine), 1 hr (haloperidol, piribedil) or 30 min (apomorphine) after injection. DOPAC concentrations were measured fluorometrically. In all kainic acid treated groups the DOPAC concentration was significantly higher ($p<.05$) in the kainic acid than in the untreated side, while the ChAT activities were significantly less than those on the untreated side. [a]indicates values significantly greater than untreated striatum ($p<.05$; 1-tail). [b]Indicates values significantly less than untreated striatum ($p<.05$; 1-tail).

Dopamine agonists and antagonists were administered to animals pretreated with kainic acid (Table 4). In these animals apomorphine reduced the concentrations of DOPAC in control striata. Unexpectedly, however, apomorphine also reduced DOPAC in kainic acid-injected striata. A similar tendency was observed following treatment with piribedil, another dopamine agonist. Conversely, haloperidol increased the DOPAC concentrations in intact striata, but caused an even greater increase on the kainic acid-treated side. Similar results were obtained with the other dopamine antagonists shown; they all increased DOPAC concentrations in intact striata, and caused an even greater increase in kainic-acid lesioned striata. These results indicate that the feedback loop is not essential for either the dopamine agonist- or antagonist-induced changes in striatal dopaminergic nerve activity.

B. Striatal dopamine concentrations following administration of α-methyltyrosine to rats pretreated with unilateral intrastriatal injections of kainic acid

Since striatal DOPAC concentrations are an indirect measure of neuronal activity, it was decided to confirm the results of the previous kainic acid experiment using another index of nigro-striatal activity. The decline of striatal dopamine after α-methyltyrosine (αMT) is depicted in Figure 7. Haloperidol significantly increased the rate of decline of dopamine in both control and kainic acid-lesioned striata. In contrast to the results of experiments in which DOPAC was measured, no differences in the αMT-induced decline of dopamine were found between lesioned and non-lesioned striata of either saline or haloperidol-treated rats.

Thus, when striatal DOPAC concentrations are used as an index of nigro-striatal activity, destruction of the feedback loop with kainic acid results in what appears to be a release of the nigro-striatal neurons from tonic inhibition. However, when the decline of dopamine following αMT administration is used as an index of dopaminergic nerve activity, no difference between values in control and kainic acid-lesioned striata can be observed. The possibility that kainic acid created increases in DOPAC through some non-selective action in the striatum was considered.

C. Nigral and striatal DOPA concentrations after administration of NSD 1015 to rats pretreated with unilateral intrastriatal injections of kainic acid

To check the results of the previous experiment and to determine if destruction of the striato-nigral pathway with kainic acid results in a real increase in nigro-striatal activity, another index of that activity was measured. Nigral and striatal DOPA concentrations following inhibition of L-aromatic amino acid decarboxylase are shown in Table 5. There were no significant differences in these values between kainic acid-treated and control striata. This agrees with results obtained by Biggio et al. (1977), and is further evidence that kainic acid-induced increases in striatal DOPAC are the results of non-selective drug action.

D. Nigral and striatal concentrations of dopamine and DOPAC in rats pretreated with unilateral intrastriatal injections of kainic acid

If kainic acid-induced destruction of the feedback loop releases nigro-striatal neurons from tonic inhibition, kainic acid treatment should increase DOPAC concentrations in substantia nigra as

TABLE 5

Nigral and Striatal DOPA Concentrations After Administration of NSD 1015 to Rats Pretreated With Unilateral Intrastriatal Injections of Kainic Acid

Striatum		Nigra	
L	R	L	R
11.21±.66	13.18±.86	6.66±.82	6.62±1.48

All rats were injected unilaterally with kainic acid in the right striatum (R). Seven days later they were injected with NSD 1015 (100 mg/kg, i.p.) and were killed 30 min later. Values represent means ± 1 S.E. from 6 animals.

well as in the striatum. This did not occur. Seven days after unilateral intrastriatal injections (Table 6) of kainic acid nigral DOPAC concentrations were measured using a radioenzymatic method (Umezu and Moore, 1979). Nigral DOPAC concentrations on the kainic acid-treated side were not significantly different from those on the contralateral side. This failure of kainic acid to increase nigral DOPAC concentrations suggests that such increases observed in the striatum (Table 4) represent a local effect of kainic acid.

E. Histology of kainic acid-induced lesions in the rat brain

In order to determine directly whether kainic acid treatments produce nonselective effects, especially on nerve terminals or axons of passage near the site of injection, a histological study of the neuropathology of kainic acid treatments was performed. The results of these studies are shown in Figures 8, 9, 10 and 11. One week after the intrastriatal injection, extensive necrosis was observed on the injected side, maximal near the level of injection (Figure 8B). At this level, the areas affected included the dorsolateral caudate-putamen, the dorsal cortex, the area lateral to the corpus callosum and the piriform cortex. These lesions were characterized by neuronal loss, severe disruption of cytoarchitecture, myelin loss in the caudate-putamen, capillary proliferation, lipid-laden macrophages and gliosis (Figure 9). In the striatum a pattern of graded damage was present, suggesting that neurons are more sensitive to the drug than other cellular elements. For example, although the center of the lesion showed complete loss of myelin and neurons, neuronal damage clearly predominated more peripherally, and fiber

TABLE 6

Nigral and Striatal Concentrations of Dopamine and DOPAC in Rats Pretreated with Kainic Acid

Region	N	DOPAC (µg/mg protein)		Dopamine (µg/mg protein)	
		Intact	Kainic Acid	Intact	Kainic Acid
Striatum	6	8.78±.8	25.41±3.2[a]	66.39±5.2	56.15±3.0
Substantia Nigra	6	3.29±.5	3.13± .5	12.10±1.8	8.36± .7

All rats were given unilateral intrastriatal injections of kainic acid (2.5 µg in 2.0 µl saline). Seven days later they were killed and nigral and striatal concentrations of DOPAC and dopamine were measured. Values represent means ± 1 S.E.

[a]Indicates values significantly greater than control ($p<.05$).

Figure 8. Tracings of frontal sections indicating location and nature of lesions 1 week after intrastriatal injection of kainic acid (2.5 μg/2.0 μl saline). Ventricles are shown in black. In some areas neurons are indicated by dashed (hippocampus, piriform cortex) or solid (dentate gyrus) lines. The injected side is shown on the right. The arrow indicates the location of the needle track, and the black circle represents the site of injection. AC, anterior commissure; CC, corpus collosum; CPU, caudate-putamen, FD, dentate gyrus; HPC, hippocampus; LOT, lateral olfactory tract; PIR piriform cortex.

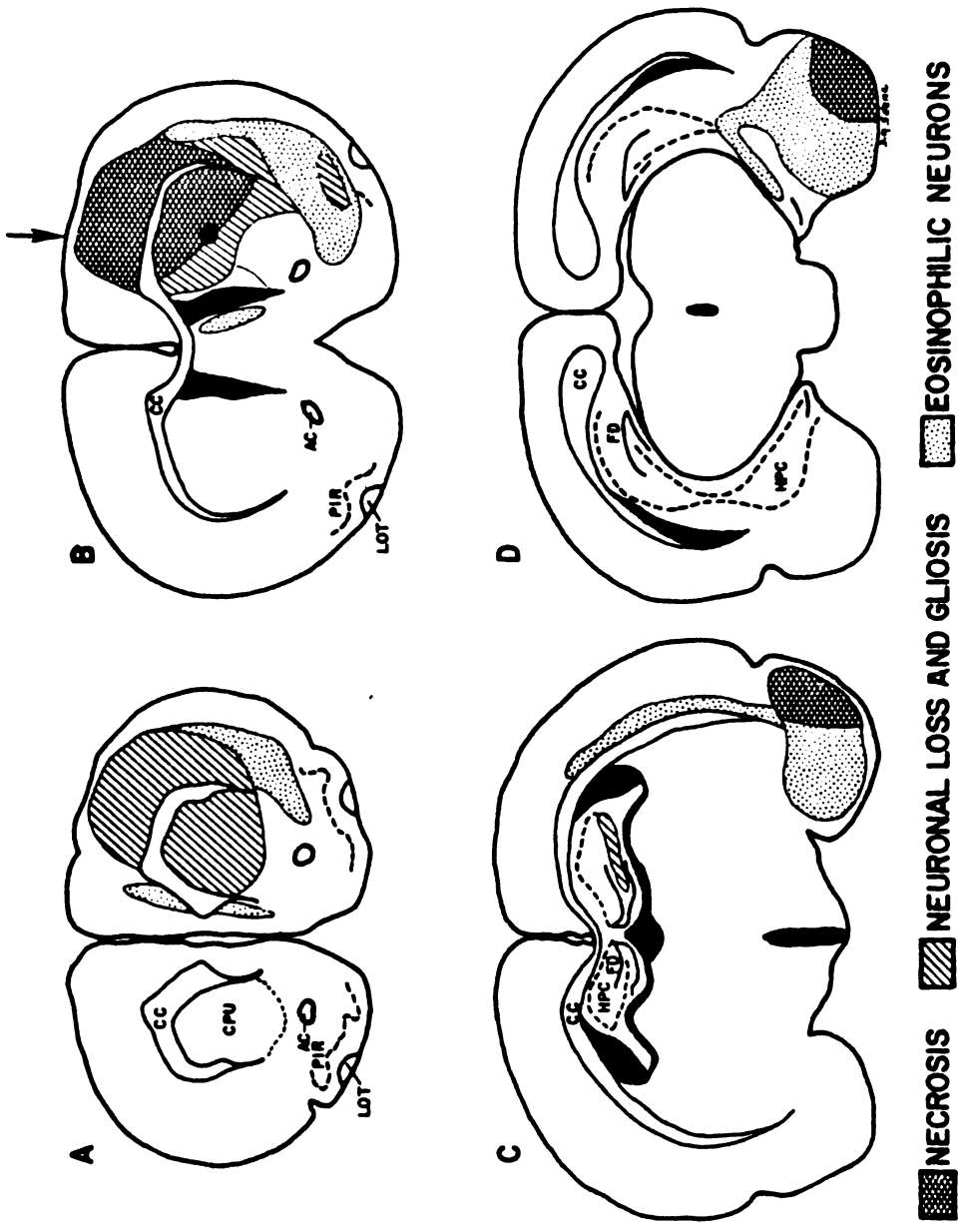
A
B
C
D
CC
CPU
PIR
LOT
HPC
FD
NECROSIS
NEURONAL LOSS AND GLIOSIS
EOSINOPHILIC NEURONS

Figure 8

Figure 9. Photomicrographs of caudate-putamen 1 week following intrastriatal injections of kainic acid (2.5 µg/2.0 µl saline). A: contralateral uninjected striatum, showing intact neurons and fiber bundles; B: injected striatum showing necrosis, neuronal loss, gliosis and demyelination. Luxol fast blue-cresyl violet. 230X.

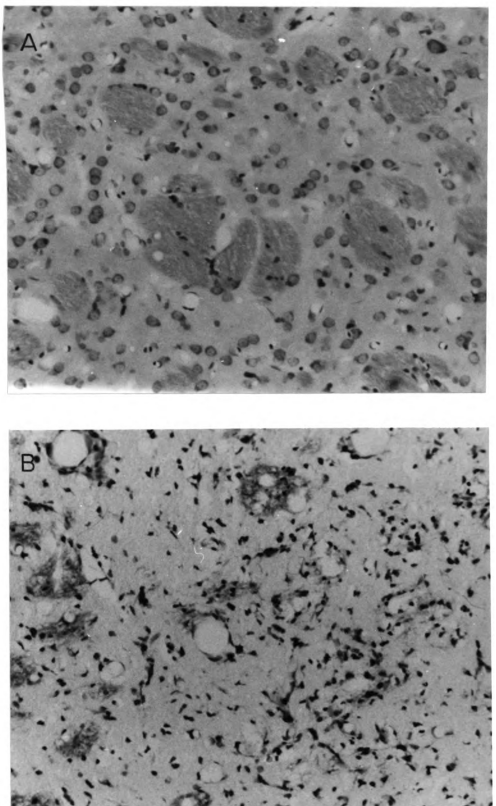

Figure 9

tracts were present, but rarefied. Immediately adjacent to the ventricles, the caudate nucleus was unaffected.

In addition to this striatal damage, other pathological changes were observed. Both lateral ventricles were symmetrically enlarged. Eosinophilic neurons were observed in the ipsilateral septal nucleus, dorsal and piriform cortices, the induseum griseum and precommissural hippocampus. The ipsilateral piriform cortex and temporal lobe, including the amygdala and entorhinal cortex, showed circumscribed areas of necrosis with neuronal loss, rarefaction, capillary proliferation and eosinophilic neurons (Figures 8C and D). Damage was less severe proceeding caudally. Hippocampal areas CA3 and CA4 (Chronister and White, 1975) were selectively affected ipsilateral to the injection (Figure 10), showing neuronal loss and eosinophilic neurons. Substantially less damage was seen 7 days after doses of 1.25 or 0.5 μg kainic acid.

At 21 days postinjection the cerebral hemisphere ipsilateral to the kainic acid injection showed gross atrophy when compared with the control side. Ventricular enlargement was observed bilaterally, but was greater ipsilateral to the injection. The area which was characterized at 7 days by neuronal and myelin loss showed, at 21 days, totally disrupted cytoarchitecture with vascular proliferation, gliosis, macrophage response and demyelination (Figure 11).

In summary, these studies indicate that damage to fiber tracts as well as structures remote from the site of a kainic acid injection may occur. Therefore, under certain circumstances DOPAC is not necessarily equivalent to other indices of dopaminergic nerve

Figure 10. Photomicrographs of left (A) and right (B) hippocampal formation from a rat given intrastriatal injection of kainic acid (2.5 µg in 2.0 µl saline). The dentate gyrus (FD) and fields CA1-CA4 of the hippocampal pyramidal cell layer are indicated. Areas CA3 and CA4 ipsilateral to the injection (B) show gliosis and pyramidal cell loss with only a few eosinophilic cells remaining. The contralateral hippocampus (A) is unaffected. Haematoxylin-eosin, 65X.

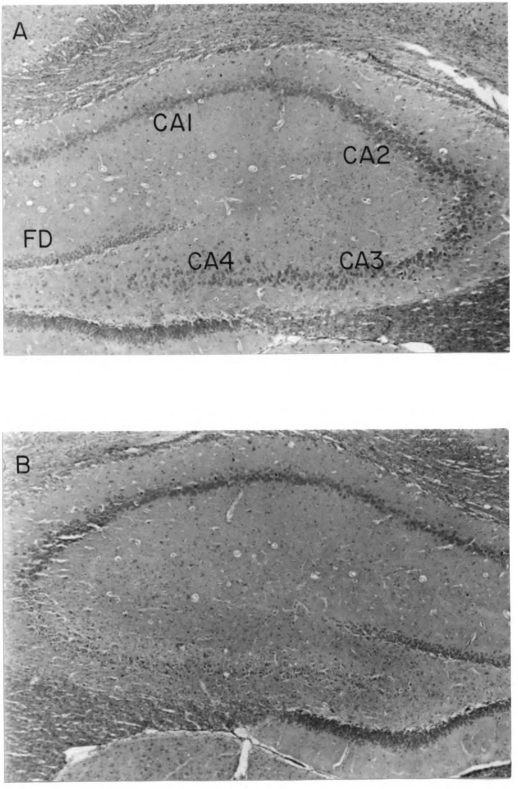

Figure 10

Figure 11. Photomicrographs of caudate-putamen and cortex 7 days (A) and 21 days (B) following intrastriatal injection of kainic acid (2.5 μg in 2.0 μl saline). Cystic degeneration and more extensive loss of architecture characterize the lesion after 3 weeks. C, cortex; CC, corpus callosum; CPU, caudate-putamen.

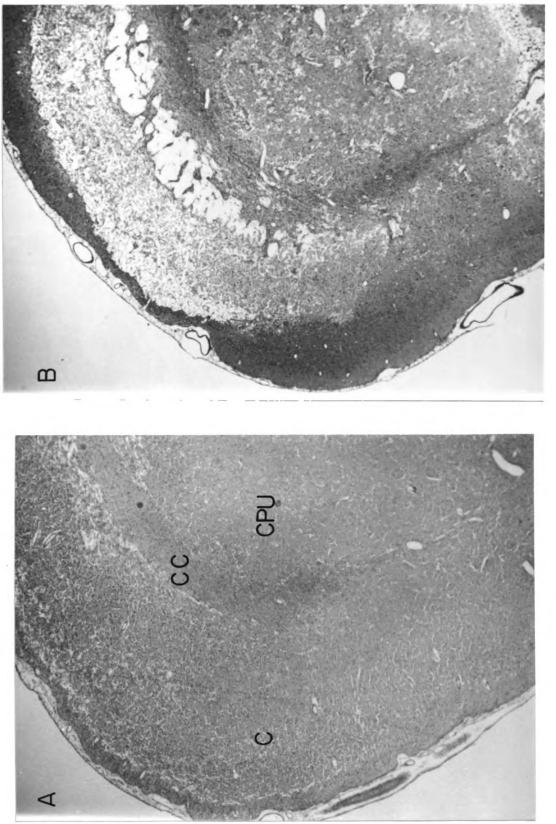

Figure 11

activity. When striatal architecture remains intact, DOPAC concentrations parallel dopaminergic nerve activity. For example, electrical stimulation of nigro-striatal neurons increases DOPAC whereas acute lesions of these nerves decreases the striatal concentration of this metabolite (Roth et al., 1976). Kainic acid causes neuronal damage near the site of its injection, including atrophy, demyelination and loss of dopamine as measured biochemically (Friedle et al., 1978) and histochemically (Meibach et al., 1978). This suggests that the increased DOPAC concentrations in kainic acid-treated striata may be due to the inability of damaged or degenerating nerve terminals to protect vesicular dopamine stores from intracellular monoamine oxidase.

F. Striatal dopamine and DOPAC concentrations and nigral GAD activity following administration of haloperidol to rats with unilateral knife cuts of the striato-nigral Pathway

Knife cuts of the striato-nigral fibers were made in order to selectively destroy the feedback loop without damaging nigro-striatal fibers or causing non-selective local effects in the striatum. Because the nigro-striatal and striato-nigral fibers are not completely separated, it is not possible to make completely selective lesions of the descending fibers. Therefore, only rats in which knife cuts produced less than 10 percent depletion of striatal dopamine compared with the contralateral striatum (indicating at least 90 percent intact nigro-striatal fibers) and approximately 50 percent depletion of nigral GAD activity were included in these studies. The effect of systemic administration of haloperidol on striatal dopamine and DOPAC concentrations and nigral GAD activity in such animals is

shown in Table 7. Hemitranssections do not completely deplete nigral GABA concentrations or GAD activity. For example, reports in the literature indicate that hemitranssections leave from 25 to 50 percent residual GAD activity in this structure (Gale et al., 1977; Kanazawa et al., 1977; Kim et al., 1971). This may originate in glial cells. In the studies summarized in Table 7, hemitranssections left an average of 17 percent residual nigral GAD actvity. Therefore, 83% represents the contribution of striato-nigral afferents. The 51 percent depletion of total activity observed after knife cuts then represents 51-17/83, or 41 percent of the nigro-striatal contribution. Therefore, these knife cuts produced approximately a 59 percent destruction of the feedback loop. Since these hemitranssections were more effective than those reported in the literature, the depletions of the nigro-striatal contribution shown in Table 7 are conservative estimates of feedback loop destruction.

In the knife cut group, nigro-striatal fibers remained intact, since no significant loss of striatal dopamine was measured. Again, in contrast to the results of experiments in which the feedback loop was destroyed with kainic acid, no significant alteration was observed in striatal DOPAC concentrations in the knife-cut striata. These results and those summarized in Figures 7-11 and in Tables 5-7 suggest that kainic acid-induced increases in striatal DOPAC are the result of nonselective action of this drug at striatal dopamine terminals.

In animals with unilateral knife cuts of the striato-nigral fibers, systemic administration of haloperidol produced increases in

TABLE 7

Striatal Dopamine and DOPAC Concentrations and Nigral GAD activity in Rats Given Hemitransections, Knife Cuts of the Striato-Nigral Pathways or Knife Cuts and Systemic Haloperidol

Group	Hemitransection	Knife Cut	Knife Cut + Systemic Haloperidol (0.1 mg/kg i.p.)
N	(7)	(6)	(7)
Nigral GAD Activity (% of intact side)	17±4%	51±5%	51±4%
Striatal DA (ng/mg protein)			
intact	98.22±10.9	91.99±3.9	93.51 ±6.8
sectioned	12.46± 4.2	83.86±5.2	120.20±14.9
% of intact	13.1%	92%	128%
Striatal DOPAC (ng/mg protein)			
intact	7.98± .29	7.95±1.0	25.98± 1.5[a]
sectioned	1.19± .33	7.13±1.1	34.65± 4.6[a]
% of intact	18.3%	90%	130%

[a] $P < .05$.

Hemitranssections or knife cuts were made at the anterior tip of the substantia nigra (+4.4 mm anterior to intraaural line, midline to 5.0 mm or 1.8 to 3.0 mm lateral to the midline, respectively. Konig and Klippel, 1963). Seven to 11 days later all animals were sacrificed. Knife-cut treated animals were given haloperidol (0.1 mg/kg, i.p.) 1 hour before sacrifice.

striatal DOPAC concentrations comparable to those shown in Table 1, both on the knife cut side and on the intact contralateral side. These data are in agreement with those of the kainic acid experiments: they indicate that a striato-nigral feedback loop is not likely to be important for drug-induced alterations in nigro-striatal activity. In agreement with the kainic acid DOPAC studies (but not the αMT experiment), the haloperidol-induced increase in DOPAC was significantly enhanced on the sectioned side.

These results suggest that the feedback loop does function in response to dopamine antagonists, but that it has little or no tonic inhibitory role. The enhanced effect of neuroleptics seen after destruction of the feedback loop suggests that it may, under periods of increased activity play an inhibitory role.

III. Studies on Striatal Dopaminergic Autoreceptors

The fact that dopaminergic agonists and antagonists still alter nigro-striatal activity after both chemical and mechanical destruction of striato-nigral fibers argues in favor of a localized control mechanism, intrinsic to the dopamine neuron. The results of experiments outlined in Section I suggest that the area of cell bodies and dendrites is not a likely site for this type of control.

A number of investigators have proposed that dopamine synthesis is controlled in the striatum (see, for example, Christensen and Squires, 1974; Nowycky and Roth, 1978) where dopaminergic activity can be directly monitored. According to this hypothesis, stimulation of dopaminergic autoreceptors, located on dopamine nerve terminals or axons in the striatum, inhibits synthesis of dopamine. Such receptors

may control dopamine release as well. Thus, in caudate slices dopamine and dopamine agonists increased stimulation-induced DA release, while dopamine antagonists blocked release (Farnebo and Hamberger, 1971; Reimann et al., 1979). If dopamine release is controlled at the level of the striatum, striatal injections of dopamine agonists and antagonists should produce changes in nigro-striatal activity at least qualitatively similar to those observed after systemic drug administration.

A. Striatal and nigral DOPAC and dopamine concentrations following intrastriatal administration of dopamine agonists and antagonists

The results of intrastriatal injections of dopamine antagonists are shown in Table 8. Striatal injections of the dopamine antagonists haloperidol and sulpiride increased striatal DOPAC concentrations 153 and 146 percent, respectively. No effect on dopamine release was observed in substantia nigra. These are similar to results obtained by Racagni et al. (1978) with another antagonist, trifluperidol. Although these responses are much smaller than those seen after systemic drug administration, they are significant and are consistently observed with both classes of antagonists tested. A tissue dilution effect may account in part for the relatively small size of the increases, since effects are presumably restricted to the area into which the drugs diffuse, and some tissue from outside this area may have been included in the sample.

To further insure that the response to local administration of neuroleptics is locally mediated, these experiments were repeated in animals whose striato-nigral fibers were destroyed.

TABLE 8

Striatal and Nigral DOPAC and Dopamine Concentrations Following Intrastriatal Administration of Dopamine Antagonists

Treatment	Striatum				Substantia Nigra			
	DOPAC (ng/mg protein)		Dopamine (ng/mg protein)		DOPAC (ng/mg protein)		Dopamine (ng/mg protein)	
	L	R	L	R	L	R	L	R
Haloperidol (5 µg) (N)	14.03± .9 (24)	21.40± .4[a] (24)	64.11±3.8 (24)	60.11±2.1 (24)	2.85±.3 (12)	2.12±.4 (12)	6.96±.5 (12)	7.93±.8 (12)
Sulpiride (10 µg) (N)	9.18±1.1 (20)	13.43±1.0[a] (20)	75.08±6.0 (19)	53.99±4.3[a] (20)	1.75±.2 (15)	1.57±.1 (15)	6.18±.7 (20)	5.33±.8 (20)

Rats were implanted bilaterally with permanent striatal cannula guides. Two to 4 days later drugs dissolved in 2.0 µl vehicle were injected into the right striatum (R) and 2.0 µl vehicle (HALDOL vehicle: haloperidol; 0.3% tartaric acid, sulpiride) was injected into the left striatum (L). Animals were killed 15 (sulpiride) or 30 minutes (haloperidol) after injection. Values represent means ± 1 S.E.

[a]Significantly different from control side, $p<.05$.

B. Striatal concentrations of dopamine and DOPAC and nigral GAD activity following intrastriatal injections of haloperidol in rats with knife cuts of the striato-nigral fibers

In animals with a significant destruction of the striato-nigral feedback loop, a significant increase in striatal DOPAC concentration was observed following intrastriatal injections of haloperidol. Because of the difficult technical nature of these experiments, these data are presented by individual animal, both as a function of striatal dopamine concentration (Table 9) and nigral GAD activity (Table 10). Arrangement of the data from highest to lowest striatal dopamine concentrations was used to rule out damage to ascending dopamine fibers as the cause of the increase in striatal DOPAC. Even when animals with less than 85% intact dopamine fibers are included, the increase in DOPAC to intrastriatal haloperidol was similar to that observed in animals that received no knife cuts (Table 8). The same results are seen if the data is arranged according to degree of feedback loop destruction, as evidenced by the amount of nigral GAD activity remaining (Table 10). Regression analysis indicates that there is no correlation between mean nigral GAD activity and the percent increase in striatal DOPAC concentrations in the groups shown ($R = .027$). Therefore, the response to intrastriatal haloperidol is independent of the degree of feedback loop destruction. These data further support the argment that dopamine release is controlled, at least in part, at the level of the striatum.

TABLE 9

Striatal Dopamine and DOPAC Concentrations and Nigral GAD Activity Following Intrastriatal Haloperidol in Rats Given Knife-Cuts of the Striato-nigral Pathway

Cumulative N	STRIATAL DOPAMINE % Control	STRIATAL DOPAMINE Cumulative Means	NIGRAL GAD ACTIVITY % Control	NIGRAL GAD ACTIVITY Cumulative Means	STRIATAL DOPAC (ng/mg protein) L	STRIATAL DOPAC (ng/mg protein) R	Cumulative %
	109		61		5.83	8.90	
	96		50		3.18	6.27	
	95		53		7.41	11.66	153±30
	93		58		7.20	5.97	
4		98±4		56±2	5.90±.97	8.20±1.33	
	89		59		9.00	8.71	
	89		52		5.58	8.09	
	86		34		6.10	10.03	145±17
7		94±3		52±3	6.32±.69	8.52± .76[a]	
	83		39		6.72	6.02	
	82		62		7.96	11.10	
	79		64		5.78	7.84	142±12
	78		25		6.70	9.96	
11		89±3		51±4	6.50±.45	8.60±.60[a]	
	67		41		5.81	9.45	
	67		33		6.34	9.92	
	63		64		7.51	5.70	142± 9
14		84±3		50±4	6.51±.36	8.54±.52[a]	

TABLE 9 (cont'd)

Data from individual animals has been placed into arbitrary groups based on percent of dopamine depletion. All rats received bilateral striatal cannula implants and knife cuts of striato-nigral fibers on the right side (R). One week later they were given intrastriatal injections of haloperidol (5 µg in 2 µl over 2 minutes) into the right striatum (R), HALDOL vehicle (2 µl over 2 minutes) into the left striatum (L), and were killed 30 minutes later. Values of striatal dopamine and nigral GAD represent the knife cut-treated side as a percent of the control side. Actual values of striatal DOPAC from both knife cut and untreated sides are listed. The N values, means and standard errors listed are cumulative values incorporating the animals from each successive group. Control dopamine concentrations are 86.38±3.54 ng/mg protein, and control GAD activity is 265±17 cpm/µg protein/15 min.

[a]Significantly greater than control, $p<.05$.

TABLE 10

Striatal Dopamine and DOPAC Concentrations and Nigral GAD Activity Following Intrastriatal Haloperidol in Rats Given Knife-Cuts of the Striato-nigral Pathway

Cumulative N	NIGRAL GAD ACTIVITY % Control	Cumulative Means	STRIATAL DOPAMINE % Control	Cumulative Means	STRIATAL DOPAC (ng/mg protein) L	R	Cumulative %
	25		78		6.70	9.96	
	33		67		6.34	9.92	
	34		86		6.10	10.03	
	39		83		6.72	6.02	
	41		67		5.81	9.45	
5		34±3		76±4	6.33±.17	9.08±.77[a]	144±14
	50		96		3.18	6.27	
	52		89		6.58	8.09	
	53		95		7.41	11.66	
	58		93		7.20	5.97	
	59		89		9.00	8.71	
10		44±4		84±3	6.40±.47	8.61±.62[a]	140±12
	61		109		5.83	8.90	
	62		82		7.96	11.10	
	64		79		5.78	7.84	
	64		63		7.51	5.70	
14		50±4		84±3	6.51±.36	8.54±.52[a]	142± 9

TABLE 10 (cont'd)

Data from individual animals has been placed into arbitrary groups based on percent of GAD depletion. All rats received bilateral striatal cannula implants and knife cuts of striato-nigral fibers on the right side (R). One week later they were given intrastriatal injections of haloperidol (5 µg in 2 µl over 2 minutes) into the right striatum (R), HALDOL vehicle (2 µl over 2 minutes) into the left striatum (L), and were killed 30 minutes later. Values of striatal dopamine and nigral GAD represent the knife cut treated side as a percent of the control side. Actual values of striatal DOPAC from both knife cut and untreated sides are listed. The N values, means and standard errors listed are cumulative values incorporating the animals from each successive group. Control dopamine concentrations are 86.38±3.54 ng/mg protein, and control GAD activity is 265±17 cpm/µg protein/15 minutes.

[a]Significantly greater than control, $p<.05$.

DISCUSSION

These experiments attempt to determine the location of dopamine receptors that control nigro-striatal neuronal activity. Neuronal activity refers to the actual release of the transmitter dopamine from nerve terminals in the striatum, in contradistinction to changes in field potentials near or intracellular action potentials from nigral cell bodies. These two indices of neuronal activity are directly related, but not necessarily identical. That is, although an action potential results in release of transmitter, the amount of transmitter released per action potential may vary under different conditions. Thus, biochemical and electrophysiological measurements of neuronal activity are intrinsically different. The experiments described in this thesis rely on biochemical indices of transmitter release to estimate nigro-striatal neuronal activity.

An assumption that has been made in these experiments is that the neuronal responses to exogenously administered drugs are an extension of the normal processes that operate when dopamine release is increased or decreased. In other words, the events induced by administration of dopamine antagonists are considered the same as those that occur during decreased dopamine release, while those caused by the administration of dopamine agonists are identical to the events brought about by increased transmitter release. It is therefore

assumed that "rules" governing neurophysiological events may be deduced from the neuronal responses to these exogenously administered compounds.

I. Studies on Nigral Dopaminergic Autoreceptors

It is generally agreed that the activity of nigro-striatal neurons is normally held under a tonic inhibition by processes initiated by release of the transmitter dopamine. Thus, dopamine agonists inhibit, while dopamine receptor blockers increase dopaminergic activity. Results in Table 1 confirm these findings. They also indicate that nigral responses to these drugs are qualitatively the same as those observed in the striatum. As Korf suggested (1971), this may reflect simultaneous release of dopamine at both cell bodies and terminals, and implies that release at both of these sites is governed by the same mechanism.

Bunney and Aghajanian (1973) have postulated that nigral dopamine receptors control nigro-striatal activity. In their studies iontophoretic application of dopamine agonists decreased, while dopamine antagonists increased nigral spike rates. However, biochemical measures of nigro-striatal activity made either in the striatum or substantia nigra cannot be altered in a predictable way by intranigral administration of dopamine agonists and antagonists (Table 3).

It may be argued that intracerebral administration of microgram quantities of drugs does not mimic physiological conditions as closely as iontophoretic application. However, intranigral injections of drugs can be used to control nigro-striatal activity by non-dopaminergic mechanisms. Intranigral administration of microgram quantities

of baclofen reverses the haloperidol-induced increase in both nigral and striatal DOPAC concentrations (Table 2). Thus, it is reasonable to expect a consistent response to intranigral drug administration. That none occurred to dopamine agonists and antagonists even at drug concentrations two orders of magnitude lower than those producing nonselective effects suggests that dopamine receptors at this site do not play a major role in the control of dopamine release in either the striatum or the substantia nigra. It is interesting that intranigrally administered haloperidol increased striatal, and decreased nigral dopamine concentrations. These effects resemble the action of baclofen, and suggest that intranigral haloperidol actually inhibited neuronal activity. This inhibiting effect is probably unrelated to the ability of haloperidol to block GABA uptake (Fjalland, 1978) since other antagonists such as sulpiride, which are weak inhibitors of GABA uptake also increase striatal, and decrease nigral dopamine concentrations when injected intranigrally. Since high concentrations of neuroleptics were injected into the nigra, nonspecific effects of the drugs (e.g., local anesthetic properties) might have played a role in producing the observed effects. And, since low doses of both agonists and antagonists had no effect, these data are not consistent with the concept of nigral dopaminergic receptors acting as major regulators of nigro-striatal activity.

Nigral synthesis of dopamine does not appear to be controlled by the same mechanisms that act at terminals in the striatum. When neuronal activity is inhibited, striatal dopamine synthesis (Walters et al., 1973) and concentrations (Pericic and Walters, 1976; Table 2)

increase. Presumably this is because dopamine in the synaptic cleft activates inhibitory receptors controlling dopamine synthesis. Blockade of impulse flow lowers synaptic dopamine concentrations and releases synthesis from tonic inhibition. No such compensatory change in dopamine is observed in substantia nigra after baclofen, another drug that inhibits dopaminergic activity.

An alternative interpretation of these results is that DOPAC does not represent dopaminergic activity. Maggi and others (1978) have demonstrated that intranigral injections of apomorphine decrease striatal concentrations of 3-methoxytyramine, the o-methylated derivative of dopamine. Striatal concentrations of this metabolite (Racagni et al., 1977) but not DOPAC (Garcia-Munoz et al., 1977) are enhanced after lesions of the striato-nigral feedback loop, and 3-methoxytyramine and DOPAC do not change in parallel after enkephalin administration (Algeri et al., 1978). Recent studies by Groppetti et al. (1977) suggest that these two metabolites represent different functional compartments within the dopamine neuron. Nevertheless, the increased striatal dopamine concentrations produced by nigral administration of dopamine antagonists suggests decreased rather than enhanced neuronal activity. Furthermore, all dopamine antagonists tested mimicked the decreased nigral dopamine levels observed following baclofen, a drug known to decrease nigro-striatal activity. These data are in conflict with well-documented increases in nigro-striatal activity following systemic neuroleptic treatment, and are inconsistent with the hypothesis of inhibitory dopaminergic autoreceptors in substantia nigra. It can be argued that, whatever aspect of

dopaminergic function DOPAC represents, it is not altered identically by systemic and nigral administration of dopamine agonists and antagonists, and therefore these drugs do not act primarily through nigral mechanisms.

The experiments in section I indicate that nigral dopamine receptors do not control nigro-striatal activity. According to experiments which measure dopamine-sensitive adenylate cyclase and specific binding of radiolabelled dopamine agonists and antagonists, dopamine receptors (Phillipson et al., 1977; Nagy et al., 1978) are present in substantia nigra. It is possible that nigral release of dopamine occurs as the result of a generalized change in membrane permeability during depolarization, and that nigral dopamine receptors are nonfunctional structures. More parsimoniously, it is possible that stimulation of nigral dopamine receptors alters some electrophysiological characteristics of the neurons that cannot be measured biochemically.

II. Studies on the Striato-nigral Feedback Loop

An inhibitory striato-nigral feedback loop was originally postulated as the element controlling nigro-striatal activity (Carlsson and Lindqvist, 1963). Such a loop may not be necessary for the changes in neuronal activity observed after systemic administration of dopamine agonists and antagonists. Following chemical destruction of striatal cell bodies forming this loop, nigro-striatal neurons still respond to dopamine agonists with decreased, and to dopamine antagonists with increased neuronal activity. This is true if activity is measured as changes in striatal DOPAC concentrations (Table 4) or as

the αMT-induced decline of striatal dopamine concentration (Figure 7). Such results agree with those of DiChiara et al. (1977) and Garcia-Munoz et al. (1977).

Two additional observations from these experiments require comment. The first is that striatal DOPAC concentrations are significantly increased by kainic acid treatment alone (Table 4). Secondly, the neuronal response to dopamine antagonists is exaggerated on the kainic acid treated side. That is, the response of the kainic acid-treated striatum to neuroleptic drugs is significantly greater than the response of the control striatum.

The increases in striatal DOPAC following kainic acid treatment alone were originally interpreted to mean that the feedback loop does indeed hold nigrostriatal neurons under a tonic inhibition, and that destruction of the loop results in increased neuronal activity.

An alternative explanation for increases in striatal DOPAC concentrations is that the extraneuronal enzyme COMT might be lowered by kainic acid treatment, shunting more dopamine to intracellular metabolism and thus raising DOPAC concentrations. It has been demonstrated, however, that kainic acid treatment increases COMT activity (Schwartz and Coyle, 1977; Kelly et al., 1979). If anything, these DOPAC values are conservative estimates of dopaminergic activity. But no such increases in activity, as measured by the αMT-induced decline of dopamine (Figure 7) or DOPA accumulation after L-aromatic amino acid decarboxylase inhibition are observed (Biggio et al., 1978; Table 5). Kainic acid treatment also fails to increase nigral DOPAC concentrations (Table 6), as would be expected if neuronal

activity were increased. These results suggest that the kainic-acid-induced increases in striatal DOPAC are the result of some nonselective action of the neurotoxin at dopaminergic terminals in the striatum. Although striatal dopamine concentrations are not significantly lower than normal seven days after kainic acid treatment, they decline over time (Friedle et al., 1978; Meibach et al., 1978), suggesting degeneration of dopaminergic terminals. Direct histological examination of kainic acid-treated striata reveals extensive nonselective damage, including demyelination, in the striatum (Figures 9 and 11). Thus, the kainic acid-induced increases in striatal DOPAC concentrations can, with good probability, be attributed to toxic changes at dopaminergic terminals. If, for example, kainic acid damaged dopamine-containing synaptic vesicles, intracellular oxidation of dopamine would increase, resulting in elevated concentrations of the acid metabolite DOPAC.

The exaggerated response to haloperidol in kainic acid-treated animals might at first also be attributed to nonselective action of the neurotoxin. The increase in DOPAC baseline is approximately equal to the increase in DOPAC seen in the kainic acid, as compared with the control striata in haloperidol-treated animals. However, following sulpiride, kainic acid-treated striata exhibit an increase in DOPAC that is almost twice that of the control striata, even allowing for the increased baseline (Table 4). The fact that an exaggerated response to haloperidol is not observed when dopaminergic activity is measured by the αMT-induced decline of dopamine is probably due to the inherent insensitivity of this technique.

Following knife cuts which damage the striato-nigral path (Table 7), no significant alterations in striatal DOPAC concentrations are observed. These data are in agreement with experiments performed on animals with chemical lesions of the feedback loop in which DOPA accumulation (Table 5) and the αMT-induced decline of dopamine (Figure 7) were used as indices of neuronal activity. They support the contention that the increases in striatal DOPAC seen after kainic acid are the result of nonselective drug action, and they suggest that the feedback loop does not have a tonic inhibitory function. Likewise, since systemically administered antagonists produce significant increases in striatal DOPAC on the knife cut side, these experiments confirm the conclusion drawn from the kainic acid experiments (Table 4), that an intact striato-nigral feedback loop is not essential for the nigro-striatal response to dopamine antagonists.

Both the kainic acid and knife cut experiments are open to the criticism that these treatments may not produce complete destruction of the feedback loop and that remaining feedback loop neurons support normal function. For example, if dopaminergic receptors on remaining feedback loop neurons become supersensitive, a given dose of agonist could produce a sufficiently great response to compensate for the missing neurons. Histological studies suggest that it is unlikely that kainic acid leaves any postsynaptic cell bodies intact. If this were true, and a significantly decreased number of fibers could maintain basal function, a greatly attenuated response might be expected to a given dose of agonist, while a normal increase in neuronal activity might be expected to a given dose of antagonist.

Such a decrease in the response of the kainic acid-treated striatum to apomorphine is in fact observed. The decrease in activity on the untreated side is 33 percent, compared with 13% on the kainic acid-treated side (Table 4). Use of percent increases compensates for the increased DOPAC baseline observed after kainic acid treatment. The responses to dopamine antagonists are more complex. The percent increases in striatal DOPAC are less on the kainic acid-treated side following clozapine, thioridazine and haloperidol, but greater (288 vs. 201 percent) following sulpiride when kainic acid is used to destroy the feedback loop. When knife cuts are used to destroy the loop, the response to haloperidol is significantly greater on the kainic acid-treated side (480 percent) when compared with the untreated side (326 percent) (Table 7). In other words, with either of two types of treatment damaging the feedback loop, neuroleptics can produce an exaggerated response.

If the feedback loop is completely destroyed by these treatments, the data suggest two possibilities. One is that feedback loop neurons are not required for the response to neuroleptics and that these drugs act primarily on the dopamine neuron. The other is that the excitatory response to dopamine antagonists contains an inhibitory component mediated by the feedback loop. Since destruction of the feedback loop results in an effect that is additive with haloperidol, haloperidol may act presynaptically to increase dopaminergic nerve activity, but postsynaptically to decrease dopaminergic activity. This is contrary to the classical picture of antagonists increasing neuronal activity through the feedback loop, and is consistent in the concept of separate presynaptic receptors.

Another way to view these data is that incomplete destruction of the feedback loop results in compensatory changes in the remaining neurons. Compensatory changes do not appear to occur on the side contralateral to these treatments, since striatal DOPAC values in sham-injected control animals are not different from those on the untreated side of kainic acid animals (Table 4).

Following kainic acid or knife cuts, at least 7 days are allowed to elapse, during which damaged neurons degenerate. It is possible that during this time the remaining dopamine receptors, both pre- and postsynaptic, become supersensitive to the transmitter. Under these conditions normal transmitter release may produce sufficiently great activity in the remaining inhibitory feedback loop neurons to maintain basal function. The exaggerated response to dopamine antagonists is not so easily attributed to a small number of remaining neurons. Supersensitivity of presynaptic receptors to the lack of dopamine (i.e., to dopamine antagonists) must still be invoked to explain the exaggerated response to neuroleptics following kainic acid or knife cuts.

In summary, if kainic acid or knife cuts completely destroy the striato-nigral fibers, then a feedback loop is not required for nigro-striatal regulation, and presynaptic mechanisms must be considered essential. On the other hand, if destruction of the feedback loop is subtotal and compensatory changes maintain nigro-striatal function, the feedback loop must be considered as a possible mechanism for the residual response to dopamine agonists. The enhanced response to dopamine antagonists argues in favor of the existence of presynaptic receptors regardless of the degree of feedback loop destruction.

III. Studies on Striatal Dopaminergic Autoreceptors

Local injections of dopamine antagonists increase striatal DOPAC concentrations (Table 8), although the effects of these injections are not as great as those observed after systemic injection, and do not increase DOPAC concentrations in the substantia nigra. One explanation for these results is that haloperidol increases striatal DOPAC concentrations by enhancing dopamine reuptake and thus shunting metabolism to intraneuronal enzymes. However, two different chemical classes of dopamine antagonists had similar effects on DOPAC. Furthermore, many neuroleptics, including haloperidol, are known to be weak inhibitors of dopamine uptake into striatal synaptosomes (Horn, 1976), a property which would, if anything, result in decreased DOPAC concentrations. Therefore, despite the size of the response, these results suggest that dopamine antagonists can act locally to increase dopamine release in vivo.

Significant increases in dopamine release to such local injections are still observed if feedback loop neurons are damaged by knife cuts (Tables 9 and 10). Compensatory changes (e.g., supersensitivity) in remaining feedback loop neurons probably do not account for the increased release of dopamine observed, since these increases cannot be correlated either with a limited range of damage to ascending dopaminergic fibers (Table 9) or to feedback loop destruction (Table 10). These results suggest that dopamine antagonists act via striatal mechanisms to alter nigro-striatal activity. They do not distinguish between a short feedback loop with interneurons or an axon collateral arrangement, and presynaptic receptors. Nevertheless, two pieces of evidence argue strongly against a short feedback loop. One is that

nigro-striatal neurons still respond to agonists and antagonists after kainic acid, a treatment that appears to destroy all postsynaptic cells. The second is that because of the diffuse nature of the dopaminergic innervation to the striatum, a short feedback loop system would be expected to form many axoaxonic synapses, and these are very rare in striatum (Kemp and Powell, 1971), the predominant type being axodendritic.

The fact that the neuronal response to exogenously administered haloperidol is much greater than to local administration indicates that intrastriatal and systemic administration of this drug are not equivalent. It may be that the action of exogenously administered drugs at dopamine receptors distant from nigro-striatal neurons affects nigro-striatal activity via afferents to substantia nigra. For example, there is anatomical (Swanson and Cowan, 1975) and electrophysiological (Dray and Oakley, 1978) evidence for a neuronal input to substantia nigra from nucleus accumbens, an area with a high concentration of dopamine receptors. Such an arrangement could account for the fact that systemic, but not intrastriatal injections of haloperidol increase DOPAC concentrations in substantia nigra.

Although these experiments do indicate that some degree of control over dopaminergic activity is mediated by striatal mechanisms most likely located on the dopamine terminals themselves, they do not fully explain all the responses of these neurons to systemic administration of dopamine agonists and antagonists.

SUMMARY AND CONCLUSIONS

Three hypotheses concerning the location of dopamine receptors regulating nigro-striatal dopaminergic neuronal activity were tested: that they are located (1) postsynaptically, on feedback loop neurons, (2) on presynaptic terminals in the striatum or (3) on dopaminergic cell bodies or dendrites in substantia nigra. The effects of intracranial and systemic injections of dopamine agonists and antagonists on biochemical indices of dopaminergic activity were compared. Pre- and postsynaptic effects of these drugs were differentiated by chemical or mechanical destruction of neuronal elements postsynaptic to nigro-striatal neurons.

Dopamine appears to be released both from terminals in the striatum and from cell bodies or dendrites in substantia nigra, since systemically administered haloperidol increased, while apomorphine decreased DOPAC concentrations in striatum, and to a lesser extent, in substantia nigra.

Dopamine dynamics at neuronal terminals and cell bodies are not the same. Intranigral administration of baclofen, a drug which inhibits nigro-striatal neuronal activity, attenuated the haloperidol-induced increases in striatal and nigral DOPAC. At the same time baclofen increased striatal but decreased nigral dopamine concentrations.

To test the hypothesis that nigral dopamine receptors control nigro-striatal activity, dopamine agonists and antagonists were administered intranigrally. These procedures failed to mimic changes in striatal and nigral DOPAC and dopamine concentrations observed after systemic drug administration. Intranigral administration of high doses of apomorphine decreased nigral dopamine and DOPAC concentrations. These injections had no effect on striatal concentrations of DOPAC or dopamine. Intranigral haloperidol increased both DOPAC and dopamine in striatum at very high doses, but had no effect at lower doses. Similar results were obtained with other dopamine antagonists. Nonselective drug effects rather than specific action at nigral dopamine receptors appear to best account for these results.

To test the hypothesis that a neuronal feedback loop controls nigro-striatal activity, intrastriatal injections of kainic acid or knife cuts of the striato-nigral fibers were made to isolate nigro-striatal neurons. Seven days after intrastriatal injections of kainic acid, significant reductions in striatal ChAT activity, but no differences in striatal dopamine concentration were observed when compared with the uninjected side. Striatal DOPAC concentrations were significantly increased by kainic acid treatment.

Systemic administration of dopamine agonists and antagonists to kainic acid-pretreated rats produced decreases and increases, respectively, in striatal DOPAC, both on the sham-injected and the kainic acid-injected sides. The percent increases on the kainic acid-injected side were in some cases significantly greater than those on the control side. Similar results were obtained with haloperidol when nigro-striatal

activity was measured as a function of the αMT-induced decline of dopamine. These results argue against a postsynaptic striato-nigral feedback loop as the primary mechanism regulating nigro-striatal activity. The kainic acid-induced increases in striatal DOPAC concentrations imply a tonic inhibition of nigro-striatal activity by feedback loop neurons. However, when activity was measured as a function of the rate of αMT-induced decline of striatal dopamine or as a function of striatal DOPA accumulation after NSD 1015, no differences in kainic acid and sham-injected striata were observed. This suggests that kainic acid may alter striatal DOPAC concentrations by some nonselective action at nigro-striatal terminals. This hypothesis is supported by the fact that striatal, but not nigral DOPAC concentrations increased following kainic acid treatment. Direct histological examination of kainic acid-treated striata revealed widespread neuronal destruction as well as evidence of damage to axons of passage.

When the striato-nigral feedback loop was partially destroyed with mechanical knife cuts, no increases in striatal DOPAC concentrations were observed. This agrees with the hypothesis that kainic acid treatment has nonselective action on striatal DOPAC concentrations, and that the feedback loop is not tonically active.

Furthermore, dopaminergic neurons on the knife cut-treated side of the brain responded to systemic haloperidol with significant increases in striatal DOPAC. These increases were also greater than those on the untreated side of the brain. Like the results of the kainic acid experiments, these data suggest that the feedback loop is not necessary for drug-induced changes in nigro-striatal activity.

Dopamine antagonists can act locally to increase dopamine release in the striatum. Local injections of dopamine antagonists produced small but significant increases in striatal DOPAC concentrations, without changing dopamine dynamics in substantia nigra. Similar increases in striatal DOPAC were also observed when haloperidol was injected into the striata of animals in which the striato-nigral feedback loop neurons were cut. These increases were independent of the degree of damage to feedback loop fibers or to ascending dopamine neurons.

In conclusion, the striato-nigral feedback loop does not appear to have a major role in mediating dopamine agonist- or antagonist-induced alterations in nigro-striatal activity. Striatal rather than nigral dopamine receptors appear to be more important for this function.

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