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NEUROLEPTIC RECEPTORS: THEIR CHARACTERISTICS IN THE MAMMALIAN CENTRAL NERVOUS SYSTEM AND THEIR ALTERATION IN HUMAN NEUROPSYCHIATRIC DISORDERS

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NEUROLEPTIC RECEPTORS: THEIR CHARACTERISTICS IN THE MAMMALIAN CENTRAL NERVOUS SYSTEM AND THEIR ALTERATION IN HUMAN NEUROPSYCHIATRIC DISORDERS

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I hereby recommend that this dissertation prepared under my direction

by Terry David Reisine

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be accepted as fulfilling the dissertation requirement for the Degree

of Doctor of Philosophy.

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As members of the Final Examination Committee, we certify that we have read this dissertation and agree that it may be presented for final defense.

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Final approval and acceptance of this dissertation is contingent on the candidate's adequate performance and defense thereof at the final oral examination.

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SIGNED: Terry David Reisine

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ABSTRACT

^{3}H-Spiroperidol, a butyrophenone antipsychotic agent, binds specifically and with high affinity to neuroleptic receptors in the corpus striatum and substantia nigra. Scatchard analysis of ^{3}H-spiroperidol isotherms in the rat striatum revealed a dissociation constant (K_D) of 30 pM and a total receptor density (Bmax) of 430 fmoles/mg protein. In the substantia nigra, the K_D and Bmax values were 42 pM and 41 fmoles/mg protein, respectively. Kinetic analysis of ^{3}H-spiroperidol binding to rat striatal membranes indicated that binding reached equilibrium by 20 minutes with a rate constant of association (k_1) of 2.5×10^{9} $M^{-1}min^{-1}$. The rate constant for dissociation (k_{-1}) of ^{3}H-spiroperidol binding was $5.6 \times 10^{-2} min^{-1}$. The K_D determined from kinetic analysis in the striatum was 22.4 pM. Similarly, the K_D obtained from kinetic analysis for ^{3}H-spiroperidol binding in the rat substantia nigra was 26 pM. The $t_{1/2}$ of dissociation for ^{3}H-spiroperidol binding in both the rat corpus striatum and substantia nigra was 12 minutes and 17 minutes, respectively.

A number of dopamine antagonists and agonists effectively inhibited ^{3}H-spiroperidol binding in the corpus striatum. The rank order (from highest to lowest potency) of various antipsychotic agents in displacing ^{3}H-spiroperidol binding was spiroperidol > pimozide > (+)-butaclamol > haloperidol > fluphenazine > chlorpromazine > clozapine > (-)-butaclamol. Dopamine analogs were generally more potent than any other neurotransmitter agonists in inhibiting ^{3}H-spiroperidol. A

similar drug profile exists in the substantia nigra for inhibiting ^{3}H-spiroperidol binding.

^{3}H-Spiroperidol binding has a distinct regional distribution in rat and human brain. The neostriatum has the highest binding, followed by the mesolimbic region, cerebral cortical, and hypothalamic regions. The cerebellum had one of the lowest levels of specific binding.

A series of specific brain lesions was initiated to determine the neuronal location of neuroleptic receptors in the rat basal ganglia. Thirty days after 6-hydroxydopamine lesions of the nigral-striatal dopamine pathway, an increase in the density of striatal dopamine receptors (40%) was detected without an accompanying change in affinity of the receptors for ^{3}H-spiroperidol. These same lesions lowered nigral ^{3}H-spiroperidol binding by 40%. Intrastriatal kainic acid lesions decreased the number of striatal neuroleptic receptors to 55% of control levels, 22 days after initiation of the lesions. These same lesions did not affect nigral ^{3}H-spiroperidol binding.

In the caudate nucleus of Parkinson's disease (PD) patients the density of dopamine receptors decreased (30%) compared to control values. In the putamen and globus pallidus, ^{3}H-spiroperidol binding was not significantly different between brain tissue from control and PD patients. ^{3}H-QNB binding increased in the putamen of PD patients but was unchanged in the caudate nucleus and globus pallidus as compared to control values. ^{3}H-Serotonin binding declined in the putamen of PD patients but was unchanged in the other brain regions. ^{3}H-GABA binding was no different in brain tissue from control and PD patients. Choline

acetyltransferase activity was significantly decreased in only the putamen and globus pallidus of brains from PD patients.

^{3}H-Spiroperidol binding was decreased in both the corpus striatum and frontal cortex of brains of Huntington's diseased (HD) patients. This lowered binding was associated with a diminished density of neuroleptic receptors in these brain regions with no significant difference in the affinity of these receptors for ^{3}H-spiroperidol. In the substantia nigra of HD patients ^{3}H-spiroperidol binding was similar to control values.

In brain tissue from schizophrenic patients, ^{3}H-spiroperidol binding was elevated in the neostriatum yet unaltered in the frontal cortex as compared to controls. The increased binding was associated with a greater density of dopamine receptors in the brain tissue obtained from the schizophrenic patients. The density of opiate receptors, however, was reduced in the caudate nucleus of schizophrenic patients, yet unaltered in the putamen and frontal cortex of these same brains. ^{3}H-WB-4101 and ^{3}H-flunitrazepam binding was the same in brain tissue obtained from control and schizophrenic patients.

CHAPTER 1

INTRODUCTION

Neuroleptics are compounds that can induce distinct psychic and behavioral effects in humans. One particular characteristic of these drugs is that they provide control over a number of psychiatric symptoms, such as hallucinations, mental confusion, delirium, and psychomotor agitation. These properties led Delay and Deniker (Delay and Deniker, 1952, 1969; Deniker, 1970) to introduce these drugs for the treatment of schizophrenia. Despite the widespread clinical use of neuroleptics as antipsychotic agents, little is still known regarding their specific mechanism of action. As a result, the present study as well as a great many other investigations have been initiated in order to determine how neuroleptics produce their distinct behavioral effects.

Antipsychotic drugs are very hydrophobic, highly surface active substances that readily accumulate in cell membranes (Seeman, 1972). Early biochemical studies investigating the mechanism of action of neuroleptics considered how these drugs might perturb the cell membrane in such a manner as to alter its properties. Seeman (1966) demonstrated that neuroleptics of the phenothiazine class of drugs could expand plasma membranes while at the same time causing red cells to swell in size. Subsequent studies by Davis and Brody (1966) demonstrated that phenothiazine could affect active transport into red cells by inhibiting the activity of (Na^+-K^+)-activated ATPase. The activity of numerous

other enzymes such as cholinesterase, N-methytransferase, enzymes of carbohydrate metabolism, oxidative phosphorylation and lipid synthesis, adenylate kinase, and phosphodiesterase seemed to also be influenced by the presence of phenothiazines (Bradley, 1963; Guth and Spirtes, 1964; Robinson, 1968; Janiec, Korczak-Dziuba, and Herman, 1974). However, with all of these enzymes, the concentrations of drug required to produce detectable effects was in the micromolar range, which was far above the levels needed to produce clinical activity. In addition, the potency of a series of phenothiazine analogues in altering the activity of these enzymes did not correlate with their potency in reducing psychotic symptoms. Therefore, the actions of neuroleptics on these enzymes appeared to be non-specific.

Shortly after the introduction of phenothiazines as anti-psychotic agents, it was found that reserpine could also induce similar neuroleptic actions (Davis and Cole, 1975). The widely differing chemical structures of reserpine and the phenothiazines suggested that the sites of action of these compounds were different. In fact, reserpine was shown to cause its neuroleptic effects by depleting brain catecholamine levels (Carlsson et al., 1957; Holzbauer and Vogt, 1956), a property which the phenothiazines did not share (Carlsson and Lindqvist, 1963). However, both of these types of neuroleptics could induce similar unwanted extrapyramidal side-effects in patients. These motor side-effects were strikingly similar in appearance to the symptoms inherent to patients suffering from Parkinson's disease. The neurochemical findings of Hornykiewicz (1966) of a depletion of dopamine in the basal ganglia of patients having Parkinson's disease and

the subsequent correlation of this dopamine deficit with the initiation of Parkinson's disease-like symptoms suggested that both reserpine and phenothiazines might be inducing their pharmacological effects by interrupting, although by different mechanisms, brain dopaminergic transmission.

Despite their inability to alter brain dopamine levels, phenothiazines can increase the rate of dopamine metabolism and synthesis (Sedvall, 1975). Both the phenothiazines and the butyrophenones, another class of neuroleptic drugs, can elevate the levels of 3-methoxytyramine, a dopamine metabolite (Carlsson and Lindqvist, 1963). This ability of various neuroleptics to potentiate dopamine metabolism correlated well with their antipsychotic potency (Sedvall, 1975). Antipsychotic drugs can also heighten the release of newly synthesized striatal dopamine (Seeman and Lee, 1975; Farnebo and Hamberger, 1971). However, this ability to increase release, synthesis, and metabolism of dopamine suggests that these drugs should facilitate rather than hinder brain dopaminergic transmission. A possible explanation of these seemingly contradictory properties of neuroleptics was presented by Carlsson and Lindqvist (1963). They proposed that antipsychotic drugs could block postsynaptic striatal dopamine receptors. This receptor blockade could in turn activate a feedback mechanism whose function would be to increase dopamine levels in the vicinity of the dopaminergic receptor in order to overcome the neuroleptic antagonism. The components of this feedback mechanism consisted of striatal-nigral GABAergic neurons, which have recently been found to regulate the firing of nigral dopamine neurons as well as the activity of tyrosine

hydroxylase (the rate limiting enzyme in dopamine synthesis), dopamine release, and turnover rate (Hattori et al., 1973; Kim et al., 1971; Precht and Yoshida, 1971; Tarsy et al., 1975; Gale and Guidotti, 1976). Some controversy still exists regarding the exact mechanism by which neurcleptics can alter striatal dopamine release and turnover rate. However, the concept that antipsychotic drugs block dopamine receptors appears to be widely accepted as an integral facet of their neurochemical and pharmacological properties.

A number of behavioral experiments support the hypothesis that neuroleptics block dopamine receptors (Janssen and van Bever, 1975, 1978). Phenothiazines and butyrophenones can cause catalepsy resembling parkinsonism, which can be reversed by peripheral administration of L-DOPA or intracerebral injections of apomorphine or dopamine. Apomorphine-induced stereotyped behavior in rats is accepted by most pharmacologists as involving a direct stimulation of central dopamine receptors and inhibition of these apomorphine effects is presumed to result from blockade of dopamine receptors (Janssen and van Bever, 1975). Similarly, amphetamine-induced stereotyped behavior is thought to follow from a release of dopamine onto its receptor sites and to represent an animal model of amphetamine psychosis (Janssen and van Bever, 1975). The molar potency of a number of neuroleptics to antagonize both apomorphine and amphetamine-induced stereotypy correlates well with their therapeutic efficacy in inhibiting psychotic symptoms (Janssen and van Bever, 1975). Also, neuroleptic blockade of apomorphine-induced emesis in dogs, which is presumed to involve blockade of dopamine receptors in the vicinity of the chemotrigger zone

of the brainstem, correlates closely with the antipsychotic potency of a number of phenothiazines and butyrophenones (Janssen and van Bever, 1975).

Ungerstedt (1971) developed another behavioral method for determining functional dopaminergic activity by unilaterally destroying the substantia nigra with 6-hydroxydopamine (6-OHDA). The injection of 6-OHDA selectively destroys the nigro-striatal dopamine pathway, which results in a loss of dopamine in the striatum and development of post-synaptic supersensitivity to dopamine on the lesioned side. Amine-releasing agents, such as amphetamine, produce continuous rotational behavior ipsilateral to the lesion. Direct acting dopamine receptor agonists, such as apomorphine, cause contralateral turning due to the supersensitive dopamine receptors in the ipsilateral striatum. A number of neuroleptics can suppress these rotational behaviors, probably by blocking dopamine receptors, in a rank order of potency similar to their therapeutic efficacy.

In support of the hypothesis that neuroleptics produce some of their pharmacological actions by direct blockade of dopamine receptors, Norcross and Spehlmann (1977, 1978) using extracellular recording techniques found that microiontophoretically applied neuroleptics could antagonize the effects of dopamine on striatal nerve cells that had previously been electrically stimulated. Both haloperidol and chlorpromazine when applied to striatal cells simultaneously with dopamine, competitively antagonized dopamine's ability to facilitate or inhibit the firing rate of striatal cells. This suggests that neuroleptics can interact with the same pharmacologically relevant receptor as dopamine

in the corpus striatum. Also, microiontophoretic application of antipsychotic drugs in the striatum selectively blocked the latency of action potentials elicited by stimulation of dopamine cells in the substantia nigra, yet did not affect responses elicited by stimulation of the cerebral cortex or thalamus which are believed to have striatal inputs that are non-dopaminergic in nature (Norcross and Spehlmann, 1977). Striatal dopamine application, however, was able to facilitate the responses in the striatum caused by nigral stimulation and reversed the neuroleptic antagonism. Furthermore, Aghajanian and Bunney (Aghajanian and Bunney, 1973, 1974, 1977; Bunney, Aghajanian, and Roth, 1973) found that the inhibitory effect on nigral-striatal dopamine neuronal firing rate of either systemically injected apomorphine or microiontophoretically-applied dopamine to the pars compacta of the substantia nigra could be blocked by either systemic administration of haloperidol and chlorpromazine or microiontophoretic injection of trifluoperazine, an antipsychotic drug, into nigral cells. These results suggest that antipsychotic drugs can function as dopamine antagonists by blocking dopamine receptors in the striatum and substantia nigra.

The suggestion that antipsychotic drugs interacted with dopamine receptors led many investigators to attempt to identify biochemically these membrane bound receptors. A number of hormones can initiate their tissue specific actions by activating the enzyme adenylate cyclase to form adenosine 3'5'-monophosphate (cyclic-AMP) (Robison, Butcher, and Sutherland, 1971). Similarly, neurotransmitters have also been suggested to exert their physiological effects by interacting with a

receptor that can couple with adenylate cyclase (Daly, 1977; Kebabian, 1978). Kebabian, Petzold, and Greengard (1972) have found that in the corpus striatum, a dopamine receptor is intimately involved in the regulation of adenylate cyclase activity. Dopamine is the most potent monoamine in producing maximal adenylate cyclase activation in the striatum and dopamine agonists such as apomorphine are either more potent or equipotent to dopamine in stimulating this enzyme to produce cyclic-AMP. Subcellular fractionation studies have demonstrated that the highest specific activity of this enzyme is in the submitochonrial fraction enriched with nerve endings which is consistent with its synaptic location (Clement-Cormier et al., 1975; Leysen and Laduron, 1977). The regional distribution of adenylate cyclase corresponds closely with areas of highest dopaminergic innervation. Phenothiazines such as fluphenazine and chlorpromazine are potent antagonists of dopamine stimulated adenylate cyclase and the potency of a number of phenothiazines in blocking cyclase activity correlates well with their antipsychotic efficacy as well as their ability to precipitate extra-pyramidal side-effects (Clement-Cormier et al., 1974; Miller, Horn, and Iversen, 1974; Miller, Horn, Iversen, and Pinder, 1974).

Interestingly, butyrophenones such as haloperidol or spiroperidol are relatively weak antagonists of dopamine sensitive adenylate cyclase activity (Iversen, Rogawski, and Miller, 1976), yet in in vivo animal experiments and clinical human studies these drugs are more potent than phenothiazines as dopamine antagonists (Janssen and van Bever, 1975). This discrepancy has led some investigators to suggest that there are multiple dopamine receptors in the brain (Cools,

Struyker-Boudier, and van Rossum, 1976; Cools and van Rossum, 1976). In fact, biochemical studies have revealed that the subcellular distribution of dopamine sensitive adenylate cyclase differs from that of the neuroleptic receptor (Clement-Courmier and George, 1979; Leysen and Laduron, 1977). These data support a variety of *in vivo* evidence which suggests the existence of multiple dopamine receptors (Cools and van Rossum, 1976). Thus, although the enzyme dopamine sensitve adenylate cyclase is probably coupled to a dopamine receptor in some regions of the brain, it is not the primary site of action of butyrophenone neuroleptics.

Neuroleptic Receptors

Characterization

Another way to determine the site at which antipsycotic drugs exert their pharmacological actions is through the use of neurotransmitter receptor-binding techniques. In general, this approach has been used to study the physiochemical interaction between a radioactively labeled ligand and the plasma membrane, either in the intact cell or in an isolated membrane preparation. In such studies, it is essential to satisfy a number of criteria in order to establish that the binding measurements obtained truly reflect a neurotransmitter-receptor interaction in a pharmacological sense (Yamamura, Enna, and Kuhar, 1978; Snyder, 1975; Cuatrecasas and Hollenberg, 1976).

1. The labeled ligand used as a membrane probe must be fully active biologically so as to mimic or antagonize the activity of the parent compound; it is presumed that such an analogue will be

interchangeable with the parent compound at the receptor site.

2. The binding must exhibit absolute structural and steric specificity with the known biological activity of the parent ligand, its structural analogues, and its antagonists.
3. The binding should demonstrate saturability within a concentration range than can be related meaningfully to that of agonists which elicit the known biological response in intact biological systems.
4. The binding interaction should reflect high affinity, in harmony with the sensitivity of the tissue to the physiologically active concentration range of the ligand.
5. The presence of this binding should be restricted to tissues known to be physiologically sensitive to the agonist.

For studies of receptors in the central nervous system, it is difficult to obtain an intact preparation whereby ligand binding can be compared directly with a biological response. Rather, studies of binding to membrane preparations can be performed, as have been done for ^{3}H-opiate analogues (Pert and Snyder, 1973). While absolute comparisons of ligand affinities with biological activities cannot be made directly in the same membrane preparation, it is still possible to evaluate accurately the steric and structural properties of the ligand interaction and to compare the relative potencies of known agonists and antagonists with measured membrane affinities. Thus, most of the criteria previously outlined can be met.

Initial studies used ^{3}H-haloperidol, a butyrophenone antipsychotic agent to label the neuroleptic receptor in the mammalian brain (Burt et al., 1975; Burt, Creese, and Snyder, 1976; Seeman and associates, 1975, 1976). Approximately 30% of striatal ^{3}H-haloperidol binding could be inhibited by physiological concentrations of dopamine, suggesting that it might be binding to dopamine receptors (Burt et al., 1976). Inhibition of ^{3}H-haloperidol with nonradioactive haloperidol indicated the existence of multiple components of binding, to both the specific dopamine receptor and non-dopaminergic receptors (Burt et al., 1976). Some of this non-specific binding probably occurred to alpha-adrenergic and opiate receptors since haloperidol had previously been shown to have substantial affinity for these receptors (Somoza, 1978; Creese, Feinberg, and Snyder, 1976; Peroutka et al., 1977). Specific binding to the dopamine receptor can be distinguished from such non-specific or other receptor binding by including nonradioactive drugs in the incubation medium, which interact primarily with the dopamine receptor. One such drug is butaclamol, and antipsychotic agent which exists as optical isomers, with virtually all the dopamine blocking activity residing in the (+)-isomer (Voith and Cummings, 1976). The maximum stereospecific difference between the binding of the ^{3}H-ligand in the presence of (+)-butaclamol and that in the presence of an equal concentration of (-)-butaclamol is thus a measure of stereospecific binding of the ^{3}H-ligand to the dopamine receptor. For the ^{3}H-haloperidol binding sites, (+)-butaclamol is over 1000-fold more potent than its (-)-isomer (Burt et al., 1976). Although butaclamol exhibits some degree of stereospecificity at other receptor sites the

fact that other known dopamine agonists and antagonists reduce the ^{3}H-ligand binding competitively to the same extent as the high affinity component of the displacement of (+)-butaclamol and the maximum high affinity components are not additive, indicates that these drugs are all competing for the same class of ^{3}H-ligand binding sites (Burt et al., 1976). In addition, thioxanthene neuroleptics such as flupenthixol and thiothixene possess geometrical isomers with the alpha-isomer of flupenthixol and the cis-isomer of thiothixene possessing all the antipsychotic activity. Both alpha-flupenthixol and cis-thiothixene are more than 100 times as potent as their pharmacologically inactive isomers in displacing ^{3}H-haloperidol binding (Burt et al., 1976). These results suggested that ^{3}H-haloperidol exhibits both structural and steric specificity for the striatal neuroleptic/dopamine receptor.

The specificity of ^{3}H-haloperidol binding to striatal dopamine receptors was further demonstrated by the greater potency of apomorphine and dopamine in inhibiting ^{3}H-haloperidol binding than epinephrine and norepinephrine (Burt et al., 1976; Creese, Burt, and Snyder, 1975). Other neurotransmitter agonists such as acetylcholine, serotonin, gamma-aminobutyric acid (GABA), glycine, enkephalin, and isoproterenol were relatively inactive in displacing ^{3}H-haloperidol binding. This strongly suggested that ^{3}H-haloperidol was indeed labeling striatal dopamine receptors.

The regional distribution of ^{3}H-haloperidol binding seemed to correlate with areas of greatest dopaminergic innervation (Burt et al., 1976). Brain regions devoid of dopamine content, such as the cerebellum, lacked stereospecific ^{3}H-haloperidol binding.

Investigations using a large number of phenothiazines, butyrophenones, and related neuroleptic drugs have demonstrated that there is an excellent correlation between the pharmacological potencies of these agents in animals and man and their affinities for ^{3}H-haloperidol binding sites (Creese, Burt, and Snyder, 1976b). Thus, the affinities of these neuroleptics for the ^{3}H-haloperidol binding sites correlates highly with their ability to antagonize apomorphine and amphetamine-induced stereotypy and apomorphine-induced emesis in dogs (Creese, Burt, and Snyder, 1976b). Furthermore, the clinical potency of these drugs as antipsychotic agents in man, simply determined by the average daily oral effective dose, also correlates well with competiton for ^{3}H-haloperidol binding (Creese, Burt, and Snyder, 1976a; Seeman et al., 1976). The affinity of these drugs for the ^{3}H-haloperidol binding site is also in line with the plasma concentrations of these drugs at therapeutic dose levels as measured by the neuroleptic radioreceptor assay and other methods (Creese, Burt, and Snyder, 1976b). This indicates that the affinity of a drug for the striatal ^{3}H-haloperidol binding site of dopamine receptors is a strong indicator of *in vivo* dopamine receptor antagonism and antipsychotic activity.

Despite its usefulness in identifying neuroleptic/dopamine receptors in the brain, a relatively small percentage of total ^{3}H-haloperidol binding was specific for striatal dopamine receptors. Also, stereospecific ^{3}H-haloperidol binding sites--(+)-butaclamol being much more potent at inhibiting ^{3}H-haloperidol binding than (-)-butaclamol--were not detectable in the substantia nigra or hypothalamus, brain regions rich in dopamine content, or the frontal cortex which was

recently shown to receive a substantial dopaminergic innervation (Thierry et al., 1973). These limitations reveal the general insensitivity of the ^{3}H-haloperidol binding assay for measuring dopamine receptors in the central nervous system.

Spiroperidol (Figure 1) is a butyrophenone antipsychotic agent that is over ten-fold more potent than haloperidol as a dopamine antagonist in in vivo animal studies as well as in inhibiting ^{3}H-haloperidol binding (Burt et al., 1976; Janssen and van Bever, 1978). This higher affinity of spiroperidol for the neuroleptic receptor suggested to us that this drug might be a more specific ligand for measuring the neuroleptic receptor than haloperidol and as such might greatly increase the sensitivity of the dopamine-receptor binding assay. Therefore, in the present study, binding of ^{3}H-spiroperidol in the basal ganglia was characterized to demonstrate that it labels a pharmacologically relevant dopamine receptor.

Localization

The localization of neurotransmitter receptors to various neuronal elements in a particular brain region can reveal valuable information pertaining to how various drugs act to cause their specific pharmacological responses. In addition, a better understanding of the neurochemical circuitry responsible for neurotransmission in the brain can be obtained. In the corpus striatum, it has been suggested that neuroleptics interact with postsynaptic dopamine receptors to block dopaminergic transmission (Carlsson and Lindqvist, 1963; van Rossum,

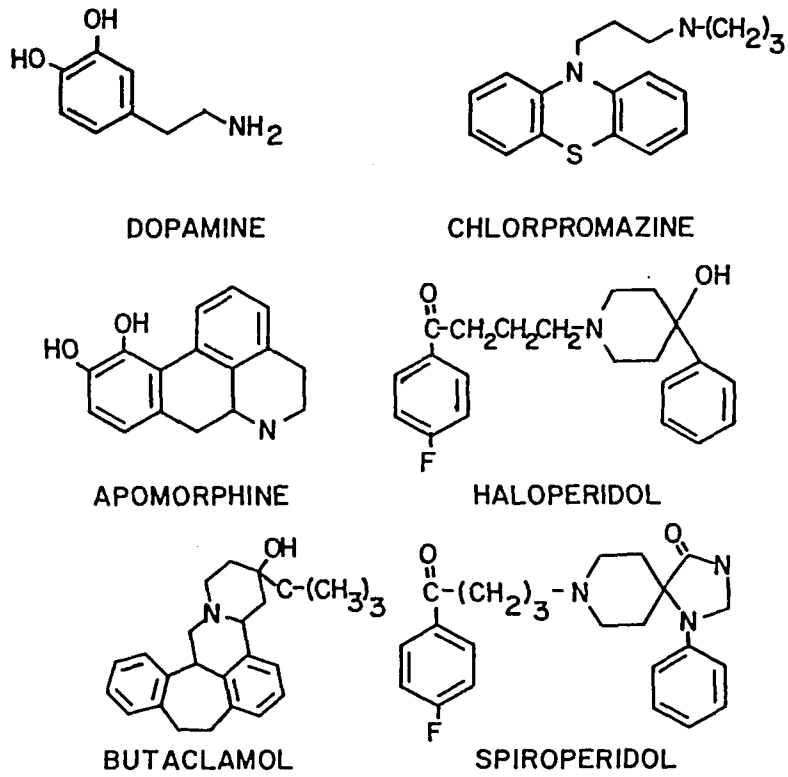

Figure 1. The structural formula of a number of dopamine agonists and antagonists, including spiroperidol.

1966). However, Seeman and Lee (1975) have proposed that neuroleptics might act on presynaptic nigral-striatal dopaminergic neurons in order to regulate striatal dopamine release. They found that in in vitro striatal slice preparations, that dopamine and apomorphine could inhibit potassium-evoked release of dopamine and that neuroleptics could reverse this action. Furthermore, a number of investigators have found that if impulse flow in nigral-striatal dopamine neurons is prevented by either lesions or injection of local anesthetics into this neuronal pathway, that there is a marked elevation in dopamine synthesis and levels in the striatum (Faull and Laverty, 1969; Anden et al., 1971; Carlsson et al., 1972). This increase in striatal dopamine levels and synthesis could be prevented by administration of apomorphine or amphetamine (Roth, Walters, and Aghajanian, 1973). The actions of these drugs can be nullified by either haloperidol or chlorpromazine (Walters and Roth, 1976). Since the increase in dopamine levels and synthesis occurred under circumstances in which there could be no further modulation of impulse flow, a feedback mechanism based upon a neuronal circuit, as originally proposed by Carlsson and Lindqvist (1963), appears to be ruled out. It has been postulated, however, that the initial diminished release of dopamine into the synaptic cleft resulting from an interruption of impulse flow leads to a depletion of dopamine acting upon receptor sites located on the presynaptic as well as the postsynaptic junctional membrane (Carlsson et al., 1972). The lowering of presynaptic dopamine receptor activation may then cause a local compensatory increase in dopamine synthesis and an eventual increase in the rate of dopamine release.

The differential location of dopamine receptors in the striatum suggests a multiplicity of sites by which neuroleptics might carry out their pharmacological actions. Thus, a precise localization of neuroleptic receptors in the striatum would seem necessary in order to define the specific mechanism of action of these drugs.

The ability of dopamine neurons in the zona compacta of the substantia nigra to respond to the microiontophoretic application of dopamine agonists and antagonists suggests the presence of dopamine receptors in this brain region (Aghajanian and Bunney, 1973, 1974, 1977). Paradoxically, there is no dopaminergic innervation to the substantia nigra. However, biochemical and electrophysiological studies have indicated that the substrate for these receptors is derived from the release of dopamine from nigral dendrites (Geffen et al., 1976; Korf, Zieleman, and Westernik, 1976). The localization of dopamine receptors in the substantia nigra is not completely understood. Gale, Guidotti, and Costa (1977) as well as others (Phillipson and Horn, 1976) have found a dopamine sensitive adenylate cyclase in the substantia nigra with similar characteristics to that present in the corpus striatum. Destruction of the striatal neuronal inputs to the substantia nigra by hemitransection resulted in an almost total loss of adenylate cyclase activity in the substantia nigra (Gale et al., 1977; Phillipson et al., 1977). Depletion of dopamine cell bodies in the zona compacta by intranigral injection of 6-OHDA caused no appreciable change in nigral dopamine stimulated cyclase activity (Phillipson et al., 1977). From these results, Gale et al. (1977) and others (Phillipson et al., 1977) have proposed that in the substantia nigra, there are dopamine receptors

coupled to adenylate cyclase molecules, located on the terminals of striatal neuronal inputs to the substantia nigra, that might function to regulate the release of a number of neurotransmitters such as GABA or substance P. Since nigral application of GABA can inhibit (Fonnum et al., 1970; Kim et al., 1971) whereas substance P (Carlsson et al., 1977; Walker et al., 1976) can potentiate nigral dopamine neuronal firings, Gale et al.'s (1977) hypothesis suggests that dopamine, through indirect mechanisms, regulates the activity of dopamine neurons.

An opposing view regarding the location of nigral dopamine receptors has been presented by Aghajanian and Bunney (1973, 1977). Using single cell extracellular recording and microiontophoretic techniques, they found that both dopamine and apomorphine could diminish the firing rate of nigral dopamine neurons. This effect could be blocked by simultaneous injection of antipsychotic drugs, yet was insensitive to alpha- or beta-adrenergic antagonists. Their results indicate that there are dopaminergic sensitive receptors on the some of nigral dopamine neurons.

In the present study, in order to localize neuroleptic receptors to specific neuronal elements in both the striatum and substantia nigra, lesions of the nigral-striatal dopamine pathway were made with the brain catecholamine depleting neurotoxin, 6-OHDA (Ungerstedt, 1968) and the density of neuroleptic receptors in the corpus striatum and substantia nigra were monitored by use of the ^{3}H-spiroperidol binding assay (Fields, Reisine, and Yamamura, 1977; Fields et al., 1979). The specific loss of only dopaminergic neuronal elements in these brain regions with or without an accompanying

decrease in ^{3}H-spiroperidol binding in the striatum or substantia nigra would suggest either the presence or absence of dopamine receptors in nigral-striatal dopamine neurons. In addition, kainic acid, a cyclic analogue of L-glutamic acid, with neurotoxic actions primarily to cell bodies and dendrites near the site of injection (Coyle and Schwarcz, 1976) was administered intrastriatally in order to destroy striatal perikarya and striatal neuronal inputs to the substantia nigra. These kainate lesions allowed us to determine whether neuroleptic receptors are located postsynaptic to dopamine neurons in either the corpus striatum or substantia nigra.

Alterations in Neuropsychiatric Disorders

Parkinson's Disease. Parkinson's disease comprises a group of symptoms which occur together with sufficient frequency to constitute a distinctive clinical picture. It is a syndrome of neurological deficits caused by a variety of infective and degenerative processes.

The most striking clinical features of Parkinson's disease are tremor, rigidity, and poverty of movement-hypokinesia (Parkinson, 1817). Posture and balance are affected in Parkinson's disease with impairment of righting reflexes also contributing to the disability (Martin, 1967). Respiratory movements are also impaired (decreased vital capacity) and control of extraocular muscles deteriorates (saccadic movements, paresis of convergence and often limitation of conjugate upward gaze) (Nugent et al., 1958). In addition, a number of disturbances of smooth muscle occur, causing dysphagia, constipation, and dysuria. The difficulties with swallowing and micturation experienced in this disorder

can lead to grave complications (aspiration pneumonia and recurrent urinary tract infections). Psychiatric changes seen in Parkinson's disease include depression and dementia (Pollack and Hornabrook, 1966). However, these symptoms are sometimes confused with the toxic side-effects of anticholinergic drug therapy.

Parkinson's disease is a relatively common neurological disorder with a prevalence of 1 in 1000 people in the United States (Pollack and Hornabrook, 1966). For most patients with Parkinson's disease, the cause of the disorder is unknown. A small group with post-encephalitic Parkinson's disease have symptoms which probably developed years after encephalitic infection. Most of these patients were victims of the epidemic of encephalitis after the First World War. The onset of idiopathic Parkinson's disease usually occurs in the fifth or sixth decade of life (Hoehn and Yahr, 1967) with tremor the most obvious physical symptom. On the average, Parkinson's disease patients die 9 years after the appearance of the symptoms with the most common cause of death being vascular disease, bronchopneumonia, or neoplasia.

It is generally accepted that the loss of substantia nigra dopamine neurons and dopamine deficiency in the corpus striatum play an essential role in the pathophysiology of Parkinson's disease. This was established by the pioneering studies of Ehringer and Hornykiewicz (1960) which showed abnormally low levels of dopamine and its metabolite, homovanillic acid in the basal ganglia of Parkinson's diseased patients. Furthermore, the activities of the dopamine synthesizing enzymes, tyrosine hydroxylase (Lloyd, Davidson, and Hornykiewicz, 1975; P. McGeer and E. McGeer, 1976) and dopa decarboxylase (Lloyd and

Hornykiewicz, 1972) were found decreased in the corpus striatum and substantia nigra of Parkinson's diseased patients. Despite the relatively low activity of these enzymes in the basal ganglia of Parkinson's diseased patients, the degree of cell loss in the substantia nigra is far greater (Forno, 1966). These findings have led some to suggest that the surviving nigral-striatal dopamine neurons may be hyperactive in order to compensate for the striatal dopamine deficiency (Hornykiewicz, 1966). This proposal is further supported by the finding of a greater ratio of homovanillic acid to dopamine in the basal ganglia of Parkinson's diseased patients than in controls (Rinne, Sonninen, and Hyyppa, 1971; Lloyd and Hornykiewicz, 1972). Finally, in Parkinson's disease there is a good correlation between the severity of signs and symptoms, loss of pigmented cells in the substantia nigra, decrease in nigro-striatal dopa decarboxylase activity, and decrease in endogenous striatal presynaptic dopamine content and turnover (Poirier and Sourkes, 1965; Pakhenberg and Brody, 1965; Goldstein, Anagnoste, and Battists, 1969; Fahn, Libsel, and Catter, 1971; Bernheimer, Birkmayer, and Hornykiewicz, 1973). This suggests that a loss of dopamine in the striatum is a direct cause for the initiation of the symptoms associated with Parkinson's disease.

Other neurochemical studies have found a depletion of dopamine and homovanillic acid in the hypothalamus of postmortem brains obtained from Parkinson patients, suggesting that there may be a more general brain deficit than just the loss of substantia nigral neurons (Rinne and Sonninen, 1973; Rinne et al., 1974). Furthermore, it has been shown that the mesolimbic dopamine neurons are affected in Parkinson's

disease. Farley, Price, and Hornykiewicz (1977) found a decrease in dopamine levels in the paraolfactory gyrus and nucleus accumbens of Parkinson's diseased patients. The functional significance of these changes is unknown, although it is possible that the dopaminergic innervation to the nucleus accumbens may be involved in the pathophysiology of hypokinesia (Farley et al., 1977).

The demonstration of a dopamine deficiency in the basal ganglia of Parkinson's diseased patients led immediately to attempts to treat the disorder by replacement thereapy (Birkmayer and Hornykiewicz, 1961; Cotzias, Van Woert, and Schiffer, 1967). Dopamine itself does not readily enter into the brain. However, the immediate precursor of dopamine, L-3,4-dihydroxyphenylalanine (L-DOPA) does enter the brain and striatal neurons where it is decarboxylated to dopamine, the deficient neurotransmitter. A number of clinical investigations have established that L-DOPA has powerful therapeutic effects in a large proportion of parkinsonian patients (Rinne, 1978). Presumably, when administered in high enough doses or simultaneously with a peripheral decarboxylase inhibitor (Carbidopa), a sufficient amount of L-DOPA can be taken up into surviving nigral-striatal dopamine neurons and converted to dopamine. The elevated levels of dopamine probably serve to rectify the abnormal activity of striatal neurons in the parkinsonian brain.

Despite the replenishment of striatal dopamine levels after L-DOPA administration, striatal dopaminergic function is not completely normalized. The underlying disease remains unaffected and continues to progress, resulting in increasingly severe Parkinson's disease 2 to

5 years after onset of treatment. The increase in Parkinson's disease is frequently associated with sudden fluctuations of response (the "on-off" phenomenon) and dementia (Marsden and Parkes, 1977; Fahn and Calne, 1978). Although many of the symptoms may result from L-DOPA, the increasing disability is attributed primarily to progression of the disease. Management at this point is either to increase L-DOPA to the maximum dosage tolerated, or to lower it to the point of disappearance of abnormal reactions. Neither approach has been satisfactory, and attention has focused on the progressive abnormalities of the disease. As Parkinson's disease progresses, decarboxylase activity, localized in the presynaptic nigro-striatal nerve terminals, may become so low that L-DOPA cannot be converted to dopamine in quantities sufficient to maintain adequate stimulation of the striatum (Hornykiewicz, 1974). Whereas such low presynaptic decarboxylase activity has been demonstrated in patients with advanced disease, it is not clear that they cannot convert L-DOPA to dopamine. Thus, postencephalitic Parkinson patients have a much greater loss of nigro-striatal neurons (and presumably lower levels of presynaptic decarboxylase activity) than patients with idiopathic Parkinson's disease, and yet these patients respond to low doses of L-DOPA (Hornykiewicz, 1974). Additionally, it has been shown that animals with virtual total loss of all nigro-striatal neurons still incorporate exogenous L-DOPA into the brain and convert it to dopamine (Poirier, Singh, and Sourkes, 1967). Finally, DOPA decarboxylase is a nonspecific enzyme and can be found in blood vessels and other monoamine-containing neurons in the brain and theoretically, L-DOPA could be converted to dopamine at sites other

than the presynaptic nigro-striatal nerve terminals (de la Torre, 1973). Thus, although it has not been established that patients with advanced Parkinson's disease cannot convert L-DOPA to dopamine in quantities sufficient to stimulate the striatum, the decreased response of these patients to L-DOPA does suggest the possibility that striatal postsynaptic neuronal elements might be dysfunctional in this disease.

Several mechanisms have been offered to explain the loss of effectiveness of L-DOPA therapy. One of these is a desensitization of brain postsynaptic dopamine receptors due to chronically high dopamine levels in L-DOPA treated Parkinson patients (Fahn, 1974). This possibility is interesting since one of the critical factors compensating for the loss of dopamine neurons in Parkinson's disease may be the development of denervation supersensitivity of striatal dopamine receptors. Recently, direct evidence for such dopaminergic supersensitivity has been found (Lee, Seeman, Rajput, et al., 1978). Parkinson patients who never received L-DOPA have a significantly higher level of dopamine receptor binding (as measured by the ^{3}H-haloperidol binding assay) in the putamen than those treated with L-DOPA or neurological controls. In addition, animals with either electrolytic or chemical lesions of the nigral-striatal dopamine pathway show decreased levels of striatal dopamine and homovanillic acid and lowered activity of striatal tyrosine hydroxylase (similar to the neurochemical finding found in the basal ganglia of Parkinson's diseased patients), but elevated striatal dopamine receptor sensitivity (Ungerstedt, 1971; Creese and Iversen, 1975). However, rats treated with α-methyl-ρ-tyrosine, a drug that depletes the brain of catecholamines and induces

striatal dopamine receptor supersensitivity (as measured by apomorphine induced stereotypy or hyperlocomotion), can have that receptor supersensitivity nullified by concurrent administration of L-DOPA (Guldelsky, Thornburg, and Moore, 1975). Also, rats treated with haloperidol in order to elevate the number of ^{3}H-neuroleptic binding sites in the striatum can have this dopamine receptor alteration prevented by concurrent treatment with dopamine agonists such as bromocryptine or L-DOPA (List and Seeman, 1979). Thus, the sensitivity of striatal dopamine receptors can be effectively modulated by the level of dopamine agonists or antagonists in the vicinity of the dopamine receptor. Therefore, these data suggest that in fact the loss of effectiveness of chronic L-DOPA therapy in Parkinson patients may be a defect in postsynaptic striatal dopamine receptors.

Understanding the nature of the brain neurochemical alterations causing the loss of responsiveness of Parkinson's diseased patient's to L-DOPA is an important factor in the development of more effective treatments of Parkinson's disease. Therefore, we chose to determine whether the density of postsynaptic dopamine receptors (as labeled by ^{3}H-spiroperidol) are altered in postmortem striatal brain tissue obtained from Parkinson's diseased patients who had been treated with L-DOPA for long periods of time as compared to neurological controls.

Huntington's Disease. Huntington's disease (HD) is a dyskinesia characterized by a continuous flow of randomly disturbed and irregularly timed muscle jerks that move from one portion of the body to another (Barbeau, Chase, and Poulson, 1973). The features of the HD patient are

continuously distorted by these brief movements, which occur at rest and interrupt normal activity. Speech, use of the hands, and walking are all affected and in severe cases the patient presents a grotesque picture of abnormal movements.

The disorder is a rare illness, affecting 1 in 20,000 people (Barbeau et al., 1973). Huntington's disease is inherited as an autosomal dominant trait with full penetrance, so that 50% of the children of the sufferers inevitably are affected (Shoulson and Chase, 1975; Barbeau et al., 1973). New mutations are rare and nearly all cases have affected relatives, although the familial nature of the disease is often concealed.

Symptoms usually appear in middle life, between the ages of 30 and 50 years, although onset in childhood and late life may occur (Barbeau et al., 1973). The disease is progressive, leading to death on average about 16 years from onset (Myrianthropoulos, 1966). Either chorea or mental deterioration may be the presenting feature although subtle changes in personality and behavior are nearly always the earliest sign of illness (Barbeau et al., 1973). As the disease progresses, dementia becomes more pronounced and chorea more grotesque. No cure is available, although the chorea may be somewhat controlled by phenothiazines or butyrophenones.

At postmortem examination, brains obtained from HD patients are shrunken and atrophic. Histologically the cerebral cortex shows widespread loss of neurons, particulary in the 3rd, 5th, and 6th layers and diffuse loss of nerve fibers (Alzheimer, 1911; McMenemy, 1963; Bruyn, 1968). Characteristically, the caudate nucleus and putamen are severely

affected with loss of small interneurons, particularly of the Golgi type II population (Alzheimer, 1911; Hunt, 1917). As a result, there is atrophy of the striato-nigral nerve fiber bundles. Less marked changes are found in other structures, but by and large, the substantia nigra and other brainstem nuclei are preserved. A reactive gliosis is apparent in affected areas, but there is no evidence of inflammatory changes (Shoulson and Chase, 1975).

Thus, the brunt of the disease is associated with the cerebral cortex and corpus striatum. Changes in the former are believed to cause dementia, while the striatal cell loss is held to be responsible for the chorea (Klawans, 1970).

The clinical contrast between poverty of movement, one of the cardinal features of Parkinson's disease, and the hyperkinesis and excessive movements of HD, is readily observed. In Parkinson's disease the conspicuous and consistent site of neuropathy is the substantia nigra (Forno, 1966). The dopamine-rich cells of the substantia nigra show marked degenerative changes, while the neurons of the striatum are relatively intact. In HD the distribution of the pathologic changes is quite different. The dopaminergic cells of the substantia nigra are not particularly altered, but the neurons of the striatum which normally receive the dopamine input are markedly involved. In marked contract to Parkinson's disease, in HD the dopamine content of the striatum is normal or near normal (Ehringer and Hornykiewicz, 1960), as is the CSF homovanillic acid concentration (Klawans, 1970). In Parkinson's disease, the dopamine input to the striatum is abnormal,

while the cells of the striatum are well preserved. In HD, the dopamine input is normal while the striatal cells are abnormal.

If chorea results from altered responsiveness or dysfunction of diseased striatal neurons to normal concentrations of dopamine within the striatum, then pharmacologic agents which alter either the concentration of dopamine within the striatum or which alter the ease of access of dopamine in reaching striatal dopamine receptors might conceivably alter the abnormal involuntary movements. Historically, the first agent reported to be of use in the treatment of chorea was reserpine (Weber, 1975). The observation that reserpine both improves chorea and depletes cerebral dopamine is consistent with the concept that altering striatal dopamine concentrations affects chorea. Reserpine exerts its pharmacologic effect by blocking the re-uptake of biogenic amines into storage granules (Berti and Shore, 1967), so that it produces depletion of serotonin and norepinephrine as well as dopamine, in the central nervous system. Under such circumstances it is not possible to make a definitive statement regarding which of these three biologically active agents, when decreased in concentration within the striatum, is responsible for a decrease in chorea.

The most widely used agents in the treatment of HD are the phenothiazines. Of these, chlorpromazine has been used most extensively. Reduction of chorea with chlorpromazine has been reported by a number of observers (Vaughn, Liberman, and Cook, 1955; Riser et al., 1959). Similar beneficial results have been reported with a wide variety of phenothiazine drugs, including perphenazine and trifluoperazine (Cohen, 1962; Merskey, Rice, and Troupe, 1961).

The basis of the central activity of chlorpromazine, as well as the other phenothiazines, is thought to be related to their direct blockade of dopamine receptors (van Rossum, 1966). Although these agents have some effect in blocking acetylcholine and norepinephrine peripherally (Courvoisier et al., 1953), the blockade of central dopamine receptors is accepted to be of primary importance in explaining the central activity of these agents. Thus, while the effectiveness of reserpine in alleviating chorea could be related to either norepinephrine, serotonin, or dopamine, the effectiveness of chlorpromazine could only be related to dopamine. This suggests that dopaminergic mechanisms might well be of primary importance in the initiation of such movements.

Haloperidol, a butyrophenone, has been used in a number of patients with HD. Divry and associates (1956) and Vaisberg and Saunders (1963) reported definite improvement in chorea. The mechanism of action of haloperidol is identical to that of chlorpromazine, that is, central dopamine receptor blockade (van Rossum, 1966). Thus a review of the effects of reserpine, the phenothiazines, and the butyrophenones suggests that dopamine is of primary importance in the initiation of chorea. Blocking the action of dopamine at the striatal dopamine receptors or decreasing the striatal content of dopamine improves chorea despite the fact that the striatal dopamine content is normal in this disease. These observations support the hypothesis that the defect producing chorea may involve dysfunction of the striatal neurons in their response to normal concentrations of striatal neurotransmitters.

Since drugs which decrease the striatal content of dopamine improve chorea, increasing the amount of dopamine which reaches the

same receptors should worsen chorea. L-DOPA markedly increases the striatal concentration of dopamine, has little effect on the striatal level of norepinephrine, and decreases the cerebral concentration of serotonin (Bertler and Rosengren, 1959; Garattini and Valzelli, 1965). Since L-DOPA increases striatal dopamine levels, it may increase the amount of dopamine reaching the receptors and thereby heighten chorea. This phenomenon was reported by Gerstenbrand, Patelsky, and Prosenz (1963), and later by Bruck and associates (1965). It is fairly clear that long-term oral administration of L-DOPA usually increases the hyperkinesia in HD. Thus, the role of dopaminergic influences in the HD striatum suggests that decreasing the concentration of dopamine within the striatum or blocking striatal dopamine receptors results in a decrease in choreiform movements, whereas increasing the concentration of dopamine results in increased chorea.

Despite the ability of L-DOPA to reduce the symptoms of Parkinson's disease, its effectiveness is limited by the progression of the disease processes. Similarly, HD patients become unresponsive to antipsychotic drugs probably as a direct result of the destruction of striatal tissue. Striatal dopamine levels and tyrosine hydroxylase activity are normal or near normal in HD which suggests that presynaptic dopaminergic terminals in the HD striatum are intact (Bird, 1976). However, the postsynaptic neuronal elements at the dopaminergic synapse are conceivably abnormal in this disorder, especially if one considers the severe loss of neuronal parikarya and dendrites in the HD striatum. Therefore, we chose to determine whether there might be dopaminergic receptor alterations in the basal ganglia of HD human brains.

Schizophrenia. Schizophrenia is considered to be a disorder consisting of disturbances in thinking and feeling and including withdrawal, detachment from reality, disruption of interpersonal communication, and often hallucinations and delusions (Redlich and Freedman, 1966). The etiology, pathogenesis, and pathophysiology of the symptoms of schizophrenia are unknown. Any relationship between the occurrence of schizophrenic symptoms and any demonstrable alteration of structure or function of the central nervous system is also unknown. Schizophrenia is thought by some to be a functional disease without alterations in neuronal structure or physiology within the central nervous system. No clear evidence has been reported as to which neuronal structures might be involved in the pathophysiology of this disease. However, several lines of evidence suggest that the dopaminergic system in the neostriatum may be involved in the genesis of the behavioral manifestations of schizophrenia.

The predominant biological hypothesis for a neurochemical defect in schizophrenia is the so-called "dopamine hypothesis" (Meltzer and Stahl, 1976). In its simplest form, this hypothesis states that schizophrenia may be related to a relative excess of dopamine-dependent neuronal activity. It is derived from pharmacological evidence that drugs that decrease dopamine activity such as the phenothiazines and the butryophenones may be antipsychotic and drugs that promote dopamine activity such as amphetamine may be psychotomimetic. The means by which dopamine overactivity is produced in schizophrenia is not yet known.

The basal ganglia have been implicated as a primary locus for abnormal activity in schizophrenia (Mettler, 1955). Mettler suggested

that the striatum functioned to enable an individual to shift attention from one type of sensory stimulus to another, and that lesions to this brain region could result in perceptual difficulties similar to those found in schizophrenics. The basal ganglia appear to be important brain centers for the initiation of a number of behavioral processes such as reward, memory, attention, and higher cognitive functions (Buchwald et al., 1976; Teuber, 1976). Each of these activities has been suggested by some to be dysfunctional in psychotic patients (Lidsky, Weinhold, and Levine, 1979). Also, the striatum is the site in animals for the development of stereotype behaviors (Fog and Pakkenberg, 1971; Ungerstedt, 1971), conditions similar to the repetitive motor acts found in a number of schizophrenic patients (Lidsky et al., 1979). In addition, the corpus striatum shares several common connections with the limbic system, another brain region reported to have abnormal activity in schizophrenia (Torrey and Peterson, 1974). Therefore, it would not be surprising to find biochemical alterations in the striatum of schizophrenic patients.

If hyperactivity of striatal dopaminergic systems is responsible for psychosis, then increasing brain dopamine levels should worsen the symptoms of schizophrenia. Several studies have demonstrated that the administration of L-DOPA to chronic schizophrenics receiving neuroleptic therapy exacerbated their psychosis and increased their paranoia and auditory hallucinations (Yaryura-Tobias, Diamond, and Merlis, 1970). Since the neuroleptics might be expected to block the effects of dopamine produced from L-DOPA, this reaction may be an indication of the schizophrenic's increased sensitivity to dopamine (Klawans, 1975).

However, it is unclear whether the increased response to dopamine observed in schizophrenics is an inherent defect in brain dopaminergic mechanisms or an induced effect by the neuroleptic therapy. In fact, acute administration of haloperidol to rats can elevate the density of striatal dopaminergic receptors and increase the ability of apomorphine to induce stereotypy in these animals (Burt, Creese, and Snyder, 1977). However, it is interesting to speculate that dopaminergic overactivity in schizophrenia might be associated with supersensitive striatal dopamine receptors, especially in light of the fact that neither striatal dopamine nor homovanillic acid levels are dramatically altered in postmortem schizophrenic brain tissue (Crow et al., 1978) nor are homovanillic acid levels altered in schizophrenic CSF samples (Rimon et al., 1971).

Some authors have suggested that amphetamine psychosis may provide a model of paranoid schizophrenia. Amphetamine psychosis is characterized by paranoid delusions, auditory and visual hallucinations, and stereotyped behavior (Kety, 1972). The stereotyped behavior seen in amphetamine psychosis often consists of lingual-facial-buccal dyskinsisas, which are analogous to dopaminergic amphetamine-induced stereotyped behavior in animals and suggest that increased activity of dopamine at striatal dopamine receptors is present in many patients with amphetamine psychosis. While amphetamine psychosis does not simulate as wide a range of schizophrenic behavior as is desirable in a model of schizophrenia, there is some comparable behavior and a considerable amount of evidence that the amphetamine psychosis involves an

overactivation of dopamine receptors in the striatum which is similar to the dopamine hypothesis of schizophrenia (Meltzer and Stahl, 1976).

Interestingly, acute administration of amphetamine to rats has been found to increase the density of striatal dopamine receptors (Howlett and Nahorski, 1979). This information as well as the lack of any evidence for any appreciable alteration in striatal dopamine turnover in postmortem schizophrenic brain tissue suggests that events at the presynaptic dopamine terminals in the striatum might be relatively normal in nature in schizophrenia. However, it is conceivable that postsynaptic striatal dopaminergic receptors might be dysfunctional in this disease.

The ability of dopamine antagonists such as the butyrophenones and phenothiazines to ameliorate the symptoms of schizophrenia would suggest that an excess of striatal dopamine is acting at dopamine receptors to cause an overstimulation of these postsynaptic sites. One might expect that dopamine hyperactivity in this case would induce desensitization of dopamine receptors as has been demonstrated for agonists acting at adrenergic receptors (Bylund, 1979). However, the worsening of psychotic symptoms caused by L-DOPA or amphetamine indicates that striatal dopamine receptors in schizophrenic brain tissue might in fact be supersensitive to dopamine's effects. Thus, both human clinical and animal studies suggest that a major defect in striatal postsynaptic dopamine receptors might be responsible for some of the symptoms of schizophrenia. We therefore propose to investigate this possible receptor alteration by measuring the density and affinity of striatal dopamine receptors in postmortem brain tissue obtained from

schizophrenics either receiving or not receiving neuroleptic drugs and from control subjects with no known psychiatric disturbances. In this manner, we hope to determine whether striatal dopamine receptors are affected by the disease processes associated with schizophrenia.

Rationale

Since dopamine is believed to be a neurotransmitter in the mammalian brain, it is reasonable to assume that membrane-bound receptors might mediate its physiological actions. A number of behavioral, electrophysiological, and biochemical approaches have been used by various investigators to study the dopamine receptor. In the present study, a simple, sensitive biochemical method is described for monitoring the characteristics of dopamine receptors in the brain.

The activity of brain dopaminergic neurons has been suggested by some to be altered in a variety of neuropsychiatric disorders. In Parkinson's disease, there is a depletion of dopamine within the basal ganglia. The loss of dopamine in this brain region has been directly related to the behavioral and motor abnormalities association with Parkinson's disease. Conversely, both Huntington's disease and schizophrenia might involve overly active brain dopamine neurons. The symptoms characteristic of these afflictions are probably either directly or indirectly caused by this dopamine imbalance. An indication of the nature of the imbalance in dopaminergic activity in the basal ganglia of patients suffering from these neurological diseases might be reflected in changes in the characteristics of dopaminergic receptors in these brain regions. Thus, in the present study, we have

investigated whether the characteristics of dopaminergic receptors are different in postmortem brain samples obtained from patients with these neurological diseases compared to brain samples obtained from neurologically and psychiatrically normal individuals.

CHAPTER 2

METHODS

^{3}H-Spiroperidol Binding Assay

Neuroleptic receptors were measured by membrane binding of ^{3}H-spiroperidol (specific activity of 26.4 Ci/mmole, NEN) (Figure 1). In the standard binding assay, aliquots of twice washed brain tissue homogenates (5 and 10 mg original tissue weight of striatum and substantia nigra, respectively) were added to two triplicate-sets of glass culture tubes. One set of tubes contained 2 ml of sodium-potassium-phosphate buffer (pH = 7.4 at 37°C), 0.1 nM ^{3}H-spiroperidol, and 0.1 μM (+)-butaclamol to displace the specifically bound ^{3}H-spiroperidol. The other tubes contained 2 ml of buffer and 0.1 nM ^{3}H-spiroperidol. Both sets of tubes were incubated at 37°C for 30 minutes. The tissues were then filtered through glass-fiber filter pads (GF/B, Whatman), positioned over a vacuum. The filter pads were then washed immediately with three consecutive 5 ml rinses of ice-cold buffer. The filter pads were air-dried and placed into liquid scintillation vials containing 8 ml of Triton-toluene omnifluor liquid scintillation cocktail. The filtered tissue was allowed to extract for 12 hours before being counted on a Nuclear-Chicago Isocap/130 liquid scintillation spectrometer that had a counting efficiency of 40%. Specific ^{3}H-spiroperidol binding (expressed as femtomoles ^{3}H-spiroperidol/mg tissue protein) was calculated by subtracting non-specific from total binding.

Variations on the Standard
^{3}H-Spiroperidol Binding Assay

Several variations of the standard binding assay were used to characterize ^{3}H-spiroperidol binding. These variations are described below.

Tissue Linearity. Tissue linearity studies were done to determine an appropriate tissue concentration for use in the binding assay. In these studies, the standard binding assay was used except that the tissue concentration was varied from 0.05 to 1.3 mg protein/ assay.

Association Rate. The standard binding assay was used except that the incubation time was varied from 0.5 minutes to 60 minutes to determine the rate of ^{3}H-spiroperidol's association to the neuroleptic receptor, as well as its association rate constant.

Dissociation Rate. This study was done to determine the rate of dissociation of ^{3}H-spiroperidol from neuroleptic receptors. Instead of adding 0.1 μM (+)-butaclamol to displace ^{3}H-spiroperidol, the tissue was equilibrated in buffer with ^{3}H-spiroperidol alone for 30 minutes. After 30 minutes, (+)-butaclamol was added to the incubating tubes at various time intervals from 30-60 minutes. The tissues were then filtered through GF/B filter pads under reduced air pressure.

Saturation Studies. To demonstrate the saturability of ^{3}H-spiroperidol binding, the concentration of ^{3}H-spiroperidol was varied from 10 to 900 pM. From these data, the apparent affinity of the

tissue for ^{3}H-spiroperidol as well as the density of neuroleptic receptors in the tissue were calculated, via Scatchard (1949) analysis.

Drug Inhibition Studies. Studies involving the displacement of ^{3}H-spiroperidol binding by various drugs were performed in triplicate-sets of culture tubes. One set contained 2 ml of buffer, 0.1 nM ^{3}H-spiroperidol, and tissue. The other sets of tubes contained buffer, 0.1 nM ^{3}H-spiroperidol, tissue, and varying concentrations of unlabeled drugs. From these results, the IC_{50} values were calculated to determine the binding affinity of the drugs for the neuroleptic receptor. All drugs were obtained from commercial sources.

Tissue Preparation. In experiments which used rat brain tissue, male adult Sprague-Dawley rats (weighing 200-250 g) were killed by decapitation and the brains were quickly removed and placed in ice-cold buffer. Either the corpus striatum or substantia nigra was dissected out of these brains and immediately frozen at -20 C. At the time of the assay, the frozen tissue was thawed, placed in 100 volumes of ice cold buffer, and homogenized by a Brinkman polytron (setting 5, for 15 sec). The homogenates were then centrifuged (Sorvall, RC2-B) at 48,000 x g for 10 minutes. The supernatants were then discarded and the tissue pellets were resuspended and subsequently centrifuged again to obtain a final pellet. This final pellet was resuspended in 100 volumes of cold buffer immediately prior to its use in the ^{3}H-spiroperidol binding assay.

Postmortem human brain samples were kindly supplied to us by Drs. Bird and Iversen of Cambridge, England. Three separate groups of

control and neurologically diseased brain tissues were obtained for these studies. In one set, the corpus striatum (caudate nucleus, putamen, and globus pallidus) was obtained from ten individuals with Parkinson's disease having a mean age of 70 years (range of 60-88) and ten control individuals with a mean age of 60 years (range of 21-88) (Reisine, Fields, Yamamura, et al., 1977). All of the Parkinson's diseased patients were receiving either L-DOPA, sinemet, or benzotropine up until the time of death. The diagnosis of Parkinson's disease was based on neuropathological and neurological examinations performed by Dr. Bird. Neuropathological examinations revealed a significant depletion of melanin-containing cell bodies in the Parkinson's diseased substantia nigra whereas the rest of the brain had no significant histological alterations. Each Parkinson patient exhibited characteristic symptoms of this disorder such as resting tremor, rigidity, and akinesia.

In the Huntington's disease study, the globus pallidus, caudate nucleus, putamen, frontal cortex, and substantia nigra were obtained from nineteen adult patients (average age = 63 ± 3 years) diagnosed as having Huntington's disease (adult-onset type) and nineteen neurologically normal patients (average age = 60 ± 5 years) (Reisine, Fields, Stern, et al., 1977; Reisine et al., 1978; Reisine, Beaumont, et al., 1979). The diagnosis of Huntington's disease was confirmed by both gross and histochemical analysis of the corpus striatum and cerebral cortex. Each Huntington's disease patient exhibited choreiform movements and dementia. All Huntington's diseased patients were receiving either phenothiazines or haloperidol up to the time of death.

In the schizophrenia study, the neostriatum and frontal cortex were obtained from eleven individuals diagnosed as schizophrenic or as having schizophrenic-like symptoms with an average age of 53 years (range of 18-85) (Reisine, Pedigo, et al., 1979; Reisine, Rossor, et al., 1979). Also, eleven psychiatrically and neurologically normal individuals with an average age of 65 years (range of 20-87) were used in this study as controls. Most of the schizophrenic subjects were receiving neuroleptic therapy (chlorpromazine, perphenazine, or fluphenazine) at the time of death. However, three patients were not receiving neuroleptics but were receiving either amytal, insulin, or electro-convulsion therapy. No noticeable neuropathological alterations were found in the schizophrenic brains. The criteria used for the diagnosis of schizophrenia in this study were (1) first psychiatric presentation under the age of 30; (2) a chronic illness with repeated hospitalization or, as in many cases, uninterrupted hospital care after the first admission; (3) the core symptoms of schizophrenic illness as included in the nuclear syndrome of the Wing symptom check list (Bird et al., 1977); and (4) the absence of significant organic illness or psychotogenic-drug abuse.

In all of these studies, no control tissue was used from individuals who suffered from an infection or malignancy of the central nervous system or who had any neurological complaints or any evidence of significant histochemical neuropathology. All control patients were unmedicated. The cause of death of control or neurologically abnormal patients was either heart failure, bronchopneumonia, or suicide. No brain tissue samples were used where there had been a delay of more

than 72 hours between death and autopsy. At autopsy, the brains were dissected and the brain regions were stored at -70 C until assay. Frozen postmortem human brain tissue was prepared for use in the ^{3}H-spiroperidol binding assay in a similar manner as previously described for rat brain tissue.

6-Hydroxydopamine (6-OHDA) Lesions. In this study, a total of 65 male Wistar rats weighing about 300 gm were used (Reisine, Beaumont, et al., 1979; Reisine, Rossor, et al., 1979; Reisine, Nagy, Fibiger, and Yamamura, 1979). All surgery was conducted in a stereotaxic apparatus (Kopf) while the animals were under pentobarbital anesthesia. Unilateral 6-OHDA lesions of the nigro-striatal dopamine pathway were achieved by injections of 2.0 µl of a physiological saline solution containing 4 µg/2µl 6-OHDA and 0.3 mg/ml ascorbic acid at the coordinates AP+ 4.4, ML+ 1.8, and DV- 2.5, according to the atlast of Konig and Klippel (1970). These animals were injected intraperitoneally with desimpramine HCl (25 mg/kg) 30 minutes before 6-OHDA injection to prevent damage to noradrenergic neurons. One month after surgery, the animals were killed by cervical fracture and the brains removed. The substantia nigra was dissected from coronal sections obtained on a freezing microtome while the striatum was dissected freehand. The brain tissue was immediately frozen (-60°C) until the time of assay.

Kainic Acid Lesions. Intrastriatal kainic acid lesions (Fields, Reisine, and Yamamura, 1978) were carried out by placing rats that were anesthetized with pentobarbital into a stereotaxic apparatus (Kopf) and slowly infusing 2 µg of kainic acid that was dissolved in 1 µl of a

saline solution into the right striatum at coordinates AP+ 8.4, ML+ 2.2, and DV+ 0.8 according to the atlas of Konig and Klippel (1970). The solution was injected over a 3 minute time span and the needle was left in place an extra 2 minutes to allow for equilibration. At various times after lesioning, animals were killed by decapitation and the left and right striata and substantia nigra were dissected out of the brain and frozen (-60°C). The striatum and nigra contralateral to the lesion served as control for each animal.

Enzyme Activity. For the determination of enzyme activities (Reisine, Beaumont, et al., 1979; Reisine, Nagy, Fibiger, and Yamamura, 1979), tissue was homogenized in 20-30 volumes of 50 mM Tris-acetate buffer (pH = 6.4 at 37°C) containing 0.2% Triton X-100. Glutamic acid decarboxylase (GAD) (Chalmers et al., 1970) and tyrosine hydroxylase (TH) (E. McGeer, Gibson, and P. McGeer, 1967) activities were assayed as previously described except that saturating concentrations of both cofactor and substrate were employed. Choline acetyltransferase (ChAC) activity was assayed according to the method of Yamamura, Gardner, and Goldberg (1972).

Regional Distribution of ^{3}H-Spiroperidol Binding. The regional distribution of neuroleptic receptors was studied in both rat and human brain tissue. After decapitation, rat brains were immediately frozen on dry ice. The tissue was cut into 300 μm coronal sections on a microtome-cryostat at -10°C. Specific regions and nuclei were removed with special hollow needles or a microknife under a stereomicroscope as described by Palkovits (1973). The tissue was quickly frozen at -20°C

until the time of assay. Each sample consisted of similar regions and nuclei pooled from 3 rats. Tissue preparation was the same as previously described for the ^{3}H-spiroperidol binding assay.

For the distribution of neuroleptic receptors in human brains, the postmortem brain tissue was obtained from psychiatrically and neurologically normal subjects at autopsy (Fields et al., 1977). The brains were dissected freehand by a faculty pathologist, Dr. P. C. Johnson, and then frozen at -20°C. Tissue preparation and binding assays were performed as previously described.

CHAPTER 3

RESULTS

Characterization of ^{3}H-Spiroperidol Binding

Tissue Linearity

Specific ^{3}H-spiroperidol binding in rat corpus striatum was linear up to 1 mg tissue protein per assay (Figure 2). The tissue linearity was similar at both low (0.1 nM) and high (0.5 nM) ^{3}H-ligand concentrations. Therefore, a tissue concentration of approximately 0.5 mg protein/assay was routinely used to insure that specific binding was always less than 10% of the total ligand concentration.

Association and Dissociation Rates

At 37°C, specific ^{3}H-spiroperidol binding to rat corpus striatum and substantia nigra reached equilibrium at about 20 minutes (Figures 3 and 4). Thus, the standard binding assays were incubated for 30 minutes to ensure that equilibrium was attained. The rate constants for formation (k_1) and dissociation (k_{-1}) of ^{3}H-spiroperidol binding to rat striatum are 2.5×10^{9} M^{-1} and 5.59×10^{-2} min^{-1}, respectively. The ratio of these rate constants (k_{-1}/k_1) yields a dissociation constant (K_D) of 22.4 pM. The half-life ($T_{1/2}$) of dissociation of ^{3}H-spiroperidol binding at 37°C is approximately 12 minutes ($T_{1/2} = .693/k_{-1}$). The k_1 and k_{-1} values for ^{3}H-spiroperidol binding to rat substantia nigra are 1.6×10^{9} $M^{-1}min^{-1}$ and $4.1 \times 10^{-2}min^{-1}$, respectively. The K_D

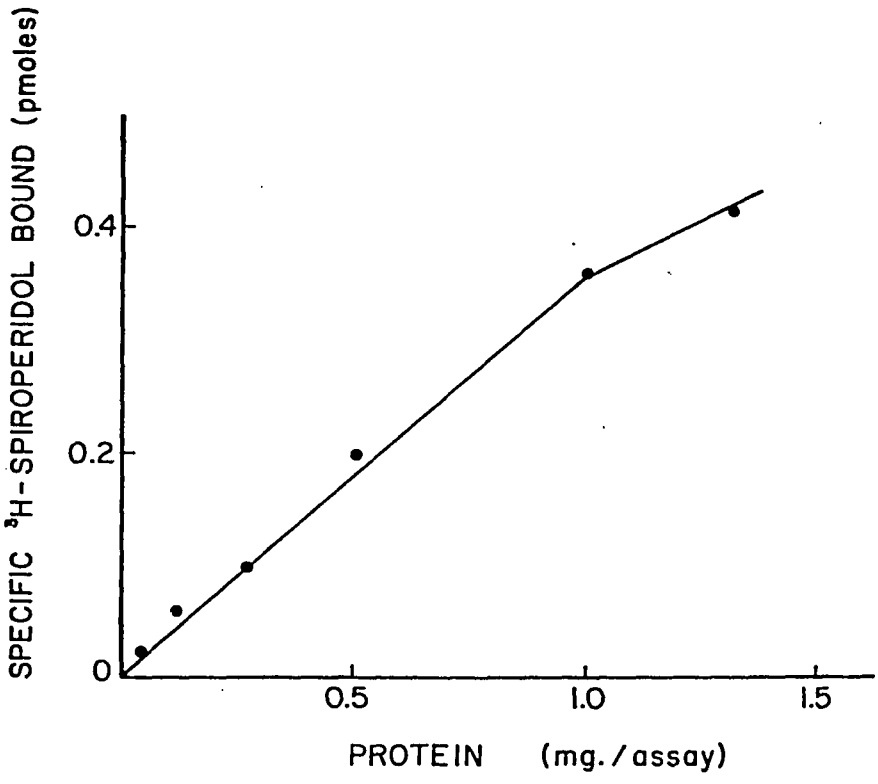

Figure 2. Tissue linearity of ^{3}H-spiroperidol binding -- Specifically bound ^{3}H-spiroperidol was determined at a concentration of 0.1 nM ^{3}H-spiroperidol. Values represent the means of triplicate determinations.

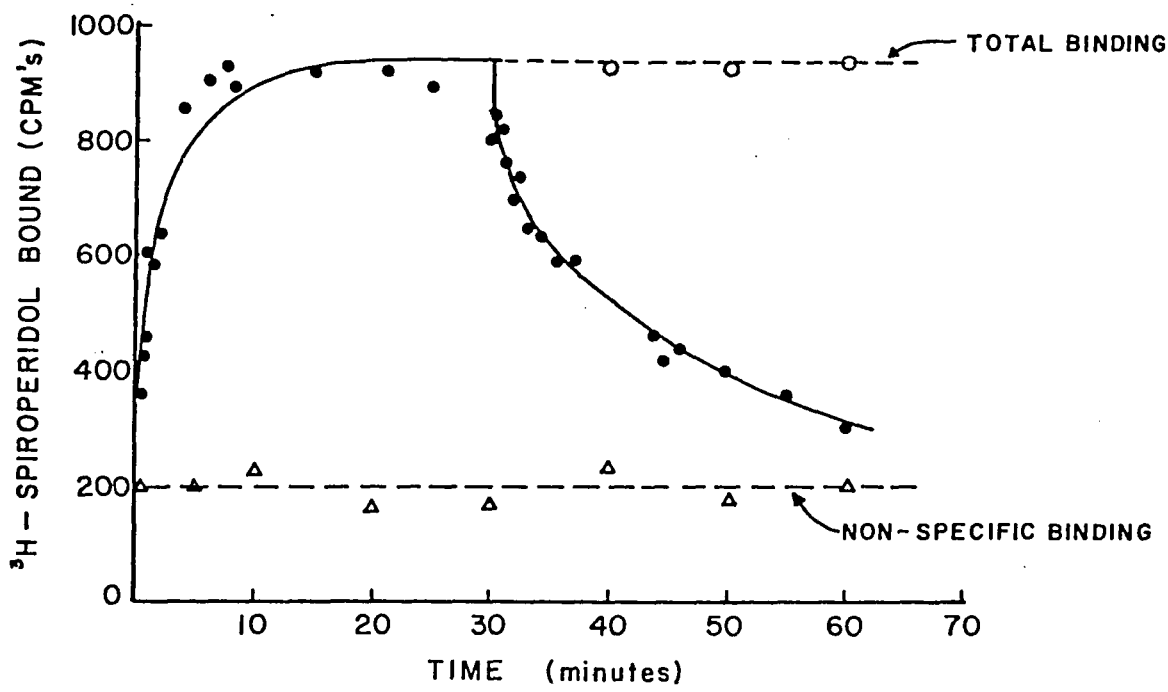

Figure 3. The rates of association and dissociation of ^{3}H-spiroperidol binding to rat corpus striatum determined at a concentration of 0.1 nM ^{3}H-spiroperidol -- After equilibration of ^{3}H-spiroperidol (30 minutes), (+)-butaclamol (0.1 μM) was added to the incubating tubes at various times from 30-60 minutes in order to observe dissociation of ^{3}H-spiroperidol binding.

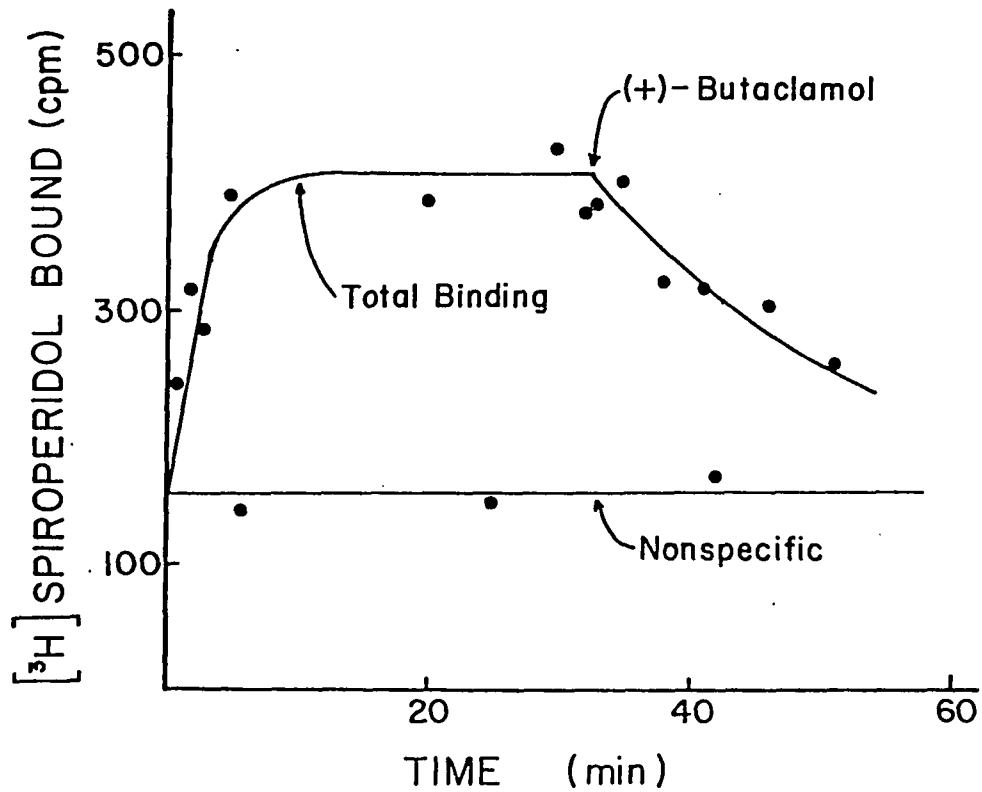

Figure 4. The rates of association and dissociation of ^{3}H-spiroperidol binding to rat substantia nigra determined at a concentration of 0.1 nM ^{3}H-spiroperidol.

is 26.0 pM and the $t_{1/2}$ of dissociation is approximately 17 minutes. At 4°C, the temperature at which the filters are routinely rinsed, there was no detectable dissociation of bound ligand from rat brain tissue.

Saturation Studies

Saturation characteristics for the rat corpus striatum and substantia nigra were measured at ^{3}H-spiroperidol concentrations between 10 and 900 pM (Figures 5 and 7). Specific ^{3}H-spiroperidol binding was saturable in both tissues whereas non-specific binding was not saturable at these ^{3}H-ligand concentrations (Figure 5). At a ^{3}H-spiroperidol concentration of 100 pM, specific binding was approximately 75% of total binding. Scatchard (1949) analysis of the saturation isotherms in both tissues revealed a single population of binding sites for the concentration range of ^{3}H-spiroperidol employed in this study (Figures 6 and 8). In the corpus striatum (Figure 6), the K_D (determined from the negative slope of the line) was 30 pM. The total receptor density (Bmax) (determined from the y-intercept) was 430 fmole/mg protein. In the substantia nigra the K_D was 42 pM and the Bmax was 41 fmole/mg protein (Figure 8). In both brain regions, the K_D's, as determined by kinetic analysis and Scatchard plot, are similar.

Inhibition of ^{3}H-Spiroperidol Binding

A number of dopamine antagonists and agonists can effectively inhibit ^{3}H-spiroperidol binding in the rat corpus striatum (Table 1). In general, dopamine antagonists are more potent than agonists in displacing ^{3}H-spiroperidol. Spiroperidol has the highest affinity of any

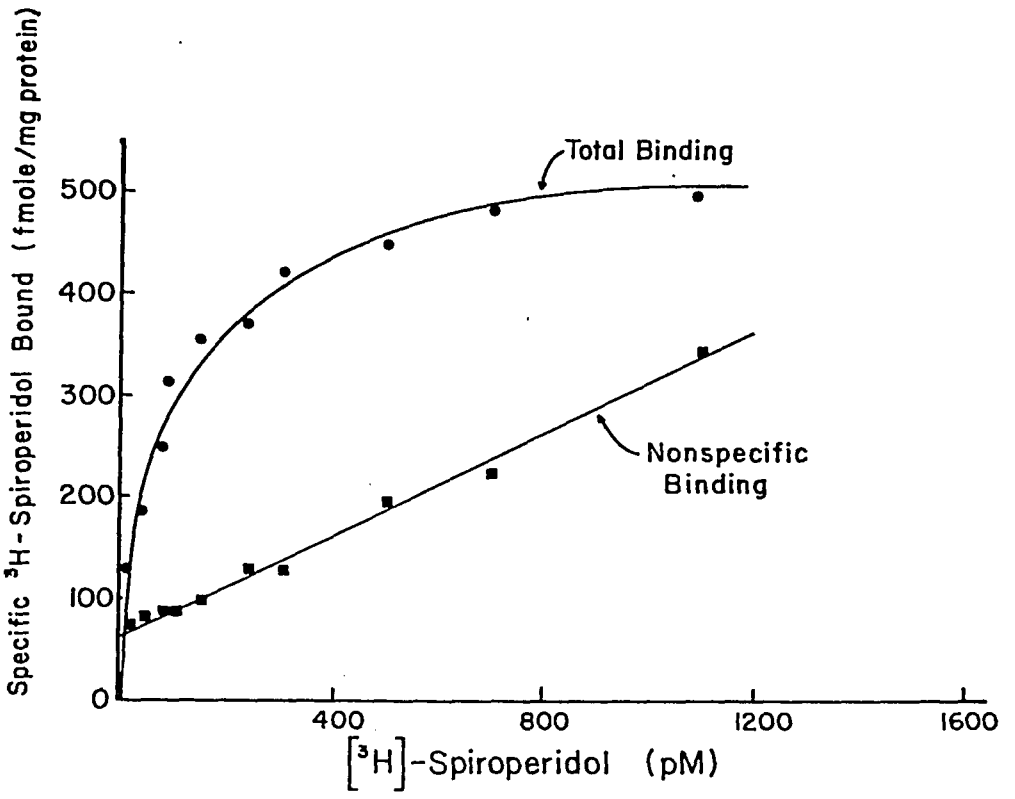

Figure 5. Saturation of specific ^{3}H-spiroperidol binding with increasing concentrations of ^{3}H-spiroperidol -- Homogenates of rat corpus striatum were incubated at 37°C for 30 minutes in $NaKPO_4$ buffer (pH = 7.4 at 37°C) containing various concentrations of ^{3}H-spiroperidol in the absence and presence of 0.1 μM (+)-butaclamol. Specific binding was saturable whereas non-specific was not.

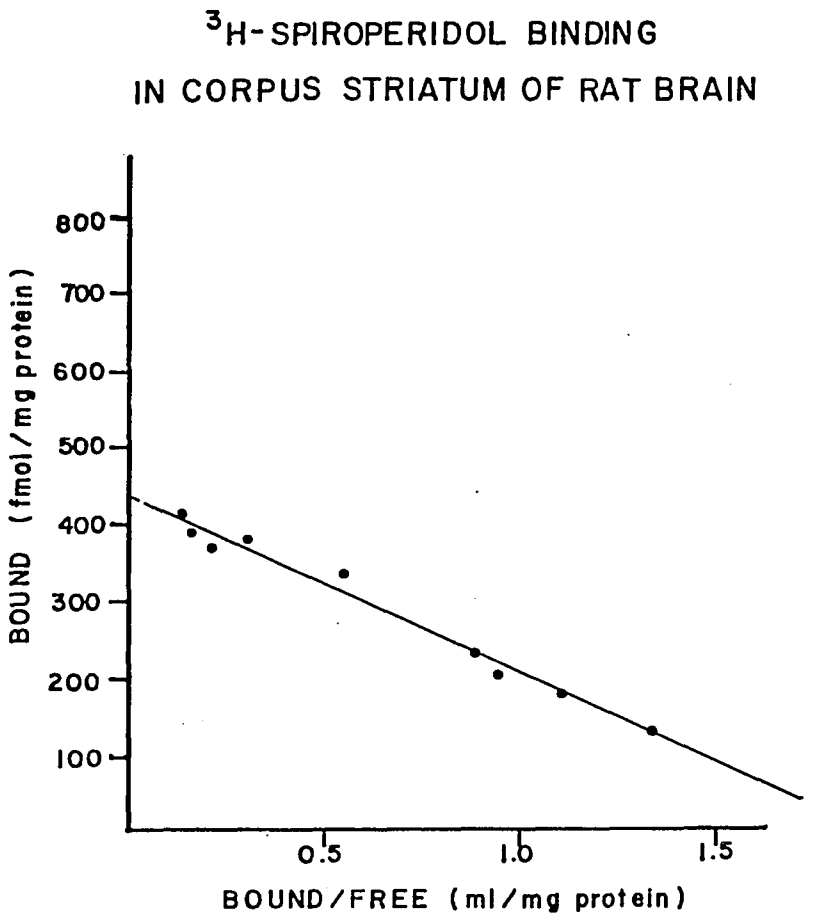

Figure 6. Scatchard plot of ^{3}H-spiroperidol binding to rat corpus striatum -- Data are transformed from Figure 5.

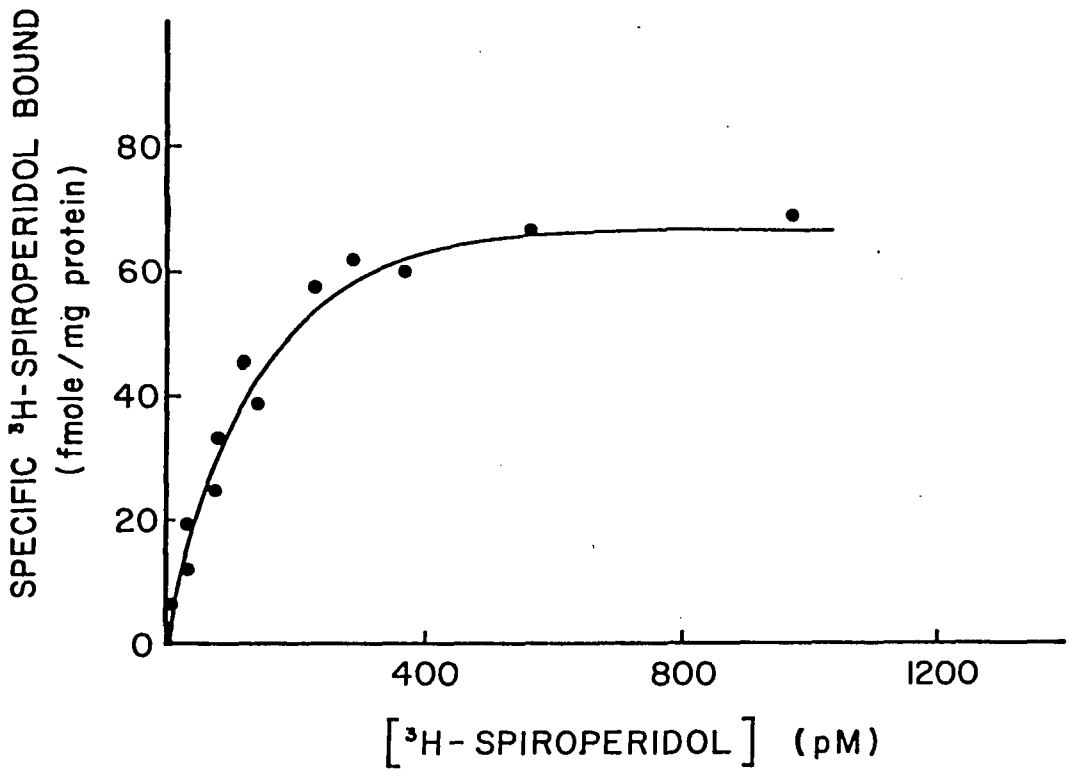

Figure 7. Saturation of specific ^{3}H-spiroperidol binding to rat substantia nigra using varying concentrations of ^{3}H-spiroperidol.

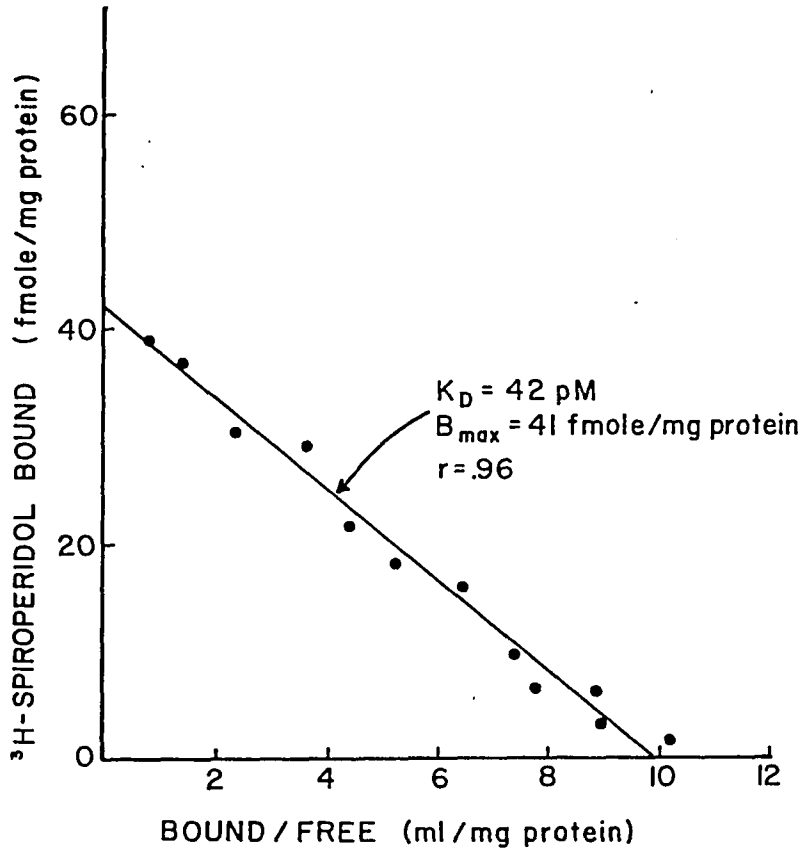

Figure 8. Scatchard plot of ^{3}H-spiroperidol binding to rat substantia nigra -- Data are transformed from Figure 7.

Table 1. Substrate specificity of ^{3}H-spiroperidol binding to rat corpus striatum -- Inhibition of specific ^{3}H-spiroperidol binding (concentration of ^{3}H-spiroperidol, of 0.1 nM) by various concentrations of the different compounds was determined by using the standard binding assay. IC_{50} values, the concentration of the drug which inhibits specific ^{3}H-spiroperidol binding 50%, were calculated by log-probit analysis.

Compound	IC_{50} (nM)	n
Spiroperidol	0.1 ± 0.04	4
Pimozide	0.2 ± 0.07	4
(+)-Butaclamol	0.6 ± 0.21	10
Haloperidol	4.8 ± 1.99	10
Fluphenazine	3.4 ± 0.9	3
Chlorpromazine	19.4 ± 3.0	3
Thioridazine	27.9 ± 5.7	3
Lenperone	37.9 ± 18.0	3
Clozapine	401.8 ± 176.0	5
(-)-Butaclamol	1754.3 ± 482.0	10
Apomorphine	180.4 ± 40.0	3
6,7-dihydroxy-2-aminotetralin (ADTN)	306.5 ± 250.0	3
Dopamine	2670.0 ± 359.0	6
Serotonin	46933.0 ± 1378.0	6
(-)-Epinephrine	28540.0 ± 600.0	3
(-)-Norepinephrine	43820.0 ± 1400.0	3

No displacement at 10^{-4} M drug concentration by clonidine, (-)-propranolol, (-)-isoproternol, atropine, acetylcholine, GABA, histamine, glycine, flunitrazepam, picrotoxin, bicuculline, d-tubocurine, and glutamate.

antipsychotic drug for the neuroleptic receptor with an IC_{50} value of 0.1 nM. This IC_{50} value is similar to the K_D values for ^{3}H-spiroperidol binding as determined by kinetic and Scatchard analysis. The rank order of various antipsychotic drugs in inhibiting ^{3}H-spiroperidol binding is similar to their relative antischizophrenic potencies and their relative efficacies in preventing apomorphine and amphetamine-induced stereotypy. Interestingly, (+)-butaclamol, a potent antipsychotic agent, is over 2000 times more potent than its inactive stereoisomer (-)-butaclamol in competing with ^{3}H-spiroperidol for the neuroleptic receptor. Clozapine, an antipsychotic drug with few extrapyramidal side-effects at clinically effective doses, has the lowest affinity for the neuroleptic receptor of any of the antipsychotic drugs tested. In contrast, spiroperidol, pimozide, (+)-butaclamol, and haloperidol, all potent antipsychotic drugs which induce severe extrapyramidal side-effects, have very high affinity for the neuroleptic receptor.

Dopamine and dopamine agonists such as apomorphine and ADTN are the most potent neurotransmitter agonists in competing for the ^{3}H-spiroperidol binding site of the drugs tested. Serotonin, (-)-epinephrine, and (-)-norepinephrine are only weakly active at the neuroleptic receptor. A number of other neurotransmitter agonists and antagonists were all impotent in competing with ^{3}H-spiroperidol for the neuroleptic receptor.

The inhibition of ^{3}H-spiroperidol binding in the substantia nigra by several drugs is revealed in Table 2. The stereospecificity of the binding was demonstrated by the over 3000-fold greater potency

Table 2. Substrate specificity of ^{3}H-spiroperidol binding to rat substantia nigra -- Inhibition of specific ^{3}H-spiroperidol binding (concentration of ^{3}H-spiroperidol of 0.1 nM) by various concentrations of different compounds was determined by using the standard binding assay. IC_{50} values, the concentration of the drug which inhibits specific ^{3}H-spiroperidol binding 50%, were calculated by log-probit analysis.

Compound	IC_{50}	n
(+)-butaclamol	0.4 ± 0.3	3
(-)-butaclamol	1580.0 ± 700.0	3
apomorphine	620.0 ± 200.0	3
dopamine	2900.0 ± 1000.0	3
6,7-dihydroxy-2-aminotetralin (ADTN)	1900.0 ± 800.0	3
Serotonin	8500.0 ± 1200.0	3

of (+)-butaclamol in competing with ^{3}H-spiroperidol for the neuroleptic receptor than (-)-butaclamol. The dopaminergic nature of the binding site was exhibited by the 3- to 16-fold greater potency of dopamine and dopamine agonists in displacing ^{3}H-spiroperidol binding than serotonin. In addition, the rank order of affinity of dopamine agonists for the nigral neuroleptic receptor is similar to their affinities for striatal neuroleptic receptors.

Regional Distribution of
^{3}H-Spiroperidol Binding

^{3}H-Spiroperidol binding has a distinct regional distribution in the rat brain (Table 3). The caudate/putamen, the brain region with the highest concentration of dopamine in the brain (Hornykiewicz, 1966), also contains the highest levels of ^{3}H-spiroperidol binding.

Table 3. The regional distribution of neuroleptic receptors in rat brain determined by using the standard binding assay -- Specific regions and nuclei were dissected from rat brain by the methods described by Palkovits (1973). Each sample (n) consisted of similar regions and nuclei pooled from 3 rats.

Region	n	fmol/mg prot
1. Caudate/putamen	4	292 ± 13
2. Nucleus accumbens	3	166 ± 32
3. Septum	3	94 ± 35
4. Globus pallidus	3	90 ± 31
5. Frontal cortex	3	75 ± 21
6. Mamillary bodies	3	53 ± 11
7. Rostral MFB	3	53 ± 16
8. Central grey	2	46 ± 9
9. Lat. geniculate	4	39 ± 11
10. Pituitary	6	37 ± 6
11. Post. amygdala	4	35 ± 11
12. Olfactory tubercle	4	37 ± 11
13. Cingulate cortex	3	32 ± 12
14. Substantia nigra	4	31 ± 11
15. Olfactory bulb	6	28 ± 5
16. Vent. hippocampus	3	26 ± 9
17. Medial thalamus	4	22 ± 4
18. Inf. colliculus	3	22 ± 4
19. Ant. amygdala	3	19 ± 6
20. Super. colliculus	3	18 ± 6
21. Retic. formation	4	17 ± 6
22. Midbrain (remainder)	4	15 ± 2
23. Dorsal hippocampus	4	15 ± 7
24. Occipital cortex	4	13 ± 3
25. Preoptic area	4	12 ± 4
26. Cerv. spinal cord	6	10 ± 1
27. Pons	6	9 ± 2
28. Medulla	6	8 ± 2
29. Cerebellar cortex	6	4 ± 1
30. Cerebellar N.	6	4 ± 1

Not detectable: Ant. vent. thalamus; Vent. thalamus; Median eminence; N. arcutus; N. ventromed.; Med. geniculate; Red nucleus; Interpeduncular nucleus; Post. medial forebrain; Ventral tegmental area.

Meso-limbic brain regions such as the nucleus accumbens and septum which contain substantial amounts of dopamine and have been implicated as important loci for the initiation of various animal behaviors dependent on dopaminergic transmission (Janssen and van Bever, 1975), contain moderately high densities of ^{3}H-spiroperidol binding. The binding of ^{3}H-spiroperidol is intermediate to high in several cerebral cortical regions. Interestingly, previous studies using ^{3}H-haloperidol as a binding ligand for the neuroleptic receptor could not detect neuroleptic receptors in the cerebral cortex (Burt et al., 1976). ^{3}H-Spiroperidol binding exhibits a micro-regional distribution in the hypothalamus. The hypothalamus taken in its entirety and the mammilary bodies contain intermediate levels of ^{3}H-spiroperidol binding, whereas hypothalamic nuclei and regions such as the median emminence, arcuate, and ventromedial nuclei have no detectable (+)-butaclamol displaceable binding. Neuroleptics have been demonstrated to elevate serum prolactin levels by actions upon cells in either the hypothalamus or pituitary (Iwasaki, Kato, and Chirhura, 1977; Ojeda, Harms, and McCann, 1974). The presence of ^{3}H-spiroperidol binding sites in the pituitary suggests that neuroleptics might act directly on pituitary cells to effect prolactin release. ^{3}H-Spiroperidol binding is detectable in the substantia nigra as well as the midbrain area taken in its entirety. The cerebellum, a brain region that contains low levels of both catecholamines and indolamines, also contains a very low degree of ^{3}H-spiroperidol binding.

The regional distribution of ^{3}H-spiroperidol binding in human brain parallels that found in rat brain (Table 4). The corpus striatum

Table 4. The regional distribution of neuroleptic receptors in human brain determined using the standard binding assay -- The human postmortem brain tissue was obtained from psychiatrically and neurologically normal subjects at autopsy. The brains were dissected freehand and then frozen at -20°C.

Region	fmol/mg prot	n	S.E.M.
Caudate	270	7	30
Putamen	239	7	49
Claustrum	237	1	--
Globus pallidus	101	7	28
Occipital cortex	92	4	44
Frontal cortex	80	7	15
Pos. hypothalamus	76	1	--
Ant. hypothalamus	56	1	--
Left hypothalamus	56	1	--
Amygdala	46	3	--
Parietal cortex	45	2	--
Midbrain tectum	38	1	--
Periventricular hypo.	22	1	--
Hippocampus	18	2	--
Thalamus	18	1	--
Ant. cerebellum (vermis)	15	2	--
Substantia nigra	14	2	--
Dentate nucleus	6	2	--

Not detectable: Pons; Inf. oliv. nucl.; Corona radiata; Pos. cerebellum; Tegmentum of medulla; Midbrain tegmentum; Corpus callosum; Optic chiasm.

contains the greatest density of ^{3}H-spiroperidol binding sites. Cerebral cortical and hypothalamic regions contain the next highest levels of binding; whereas the cerebellum contains no (+)-butaclamol displaceable ^{3}H-spiroperidol binding.

Lesions Studies

The effects of 6-OHDA lesions of the nigral-striatal dopamine pathway (NSP) and of kainic acid lesions of the striatum on tyrosine hydroxylase (TH), glutamic acid decarboxylase (GAD), and choline acetyltransferase (ChAC) activities in the striatum and substantia nigra (SN) are shown in Table 5. 6-Hydroxydopamine lesions of the NSP resulted in over a 90% decrease in both striatal and nigral TH activity, a significant increase of 19% in striatal GAD activity, while nigral ChAC and GAD activity remained unaltered. Striatal kainic acid lesions resulted in a 19% decrease in striatal TH activity while nigral TH activity remained unaltered. These same lesions decreased striatal and nigral GAD activity by 56% and 42%, respectively. Striatal ChAC activity was reduced by 70% on the lesioned compared to the contralateral control side.

Using a ^{3}H-spiroperidol concentration of 100 pM and a receptor concentration of about 10 pM, the average density of striatal neuroleptic receptors was 17.8 pmol/gm tissue. The values for the left (18.0 ± 0.7) and right (17.0 ± 0.8) striata of unlesioned rats in the ^{3}H-spiroperidol binding assay were not significantly different. Values in fresh and in frozen tissue samples were also identical.

Table 5. The effect of 6-OHDA lesions of the nigral-striatal dopamine pathway (NSP) and kainic acid lesions of the striatum on tyrosine hydroxylase (TH), glutamic acid decarboxylase (GAD), and choline acetyltransferase (CAT) activities in the striatum and substantia nigra (SN).

			Enzyme activities		
			nmoles/mg prot/hr		
Lesion	Area measured		TH	GAD	CAT
6-OHDA lesion of the NSP	Striatum	control	5.03±.19	242±12	65.6±3.0
		lesioned	0.0 ±0.0	288±14***	69.5±2.5
	SN	control	3.05±.25	930±43	--
		lesioned	0.25±.09***	912±62	--
Kainic acid lesion of the striatum	Striatum	control	5.38±.54	242±9.1	76.1±4.4
		lesioned	4.37±.30**	107±9.9*	23.0±2.9*
	SN	control	2.86±.50	862.58	--
		lesioned	3.41±.93	501±23*	--

Values represent the mean ± S.E.M. of 7 or 8 determinations of the lesioned and contralateral control side.

*p < .001.

**p < .05.

***p < .01.

NSP = Nigral-striatal dopamine pathway.

Animals were killed 30 days after the various lesions.

In preliminary experiments we found that kainic acid lesions of either the left or right striatum produce an equivalent loss of dopaminergic binding sites. Therefore all other animals were injected with kainic acid in the right striatum only. No alterations in binding were seen in the sham-operated animals nor at only one hour after lesioning.

Two days after kainic acid injection into the striatum, there was a 20%-30% decrease in ^{3}H-spiroperidol binding in the lesioned striatum compared to the contralateral, unlesioned side (Table 6) (Fields et al., 1978). This decrease reached about 50% at 21 days after the lesion. The decrease in ^{3}H-spiroperidol binding was due to a reduction in the total number of binding sites. There was no difference in the affinity of ^{3}H-spiroperidol for the dopamine receptor. The apparent dissociation constant under these conditions was aboug 2 pM in both lesioned and unlesioned sides. There was also no difference in the sensitivity of the binding assay to the pharmacologically active antipsychotic (+)-butaclamol (IC_{50} = 0.5 nM) for both sides.

In the substantia nigra, there were no significant differences in ^{3}H-spiroperidol binding at any of the time points investigated between the control and lesioned side (Table 6). Control values (contralateral nigra) for ^{3}H-spiroperidol binding in the substantia nigra averaged about 20 fmoles/mg protein.

The effectiveness of the kainic acid lesions was confirmed by several criteria. The animals turned contralateral to the lesioned side for several hours after the lesion (Schwarcz and Coyle, 1977). Histological examination indicated extensive loss of striatal perikarya and

Table 6. Intrastriatal kainic acid lesions performed as described in the Methods Section -- Various days after the lesion was induced, animals were killed by decapitation and the left and right striata and substantia nigra were dissected out of the brain and frozen (-60°C). The ^{3}H-spiroperidol binding and ChAc activity were measured in these tissues as described in the Methods Section. The striatum and nigra contralateral to the lesioned side served as control for each animal.

Brain region	Days after lesion	^{3}H-Spiroperidol binding (% of control)	ChAc activity (% of control)
Corpus Striatum	2	72*	80*
	5	75*	60*
	14	70*	35*
	20	46*	35*
	21	43*	30*
	22	55*	30*
Substantia Nigra	8	98	--
	14	120	--
	20	103	--
	30	90	--

*$p < 0.05$.

an increase in glial elements. In addition, there was a 48% decrease by 21 days in ^{3}H-QNB binding in the lesioned striatum and a 70% decrease in the activity of ChAC (Fields et al., 1978). These latter two findings are consistent with previously documented neuronal losses and neurochemical changes in the striatum after kainic acid lesions (Coyle and Schwarcz, 1976; E. McGeer and P. McGeer, 1976; Hruska et al., 1978).

As revealed in Table 7, unilateral 6-OHDA lesions of the nigral-striatal dopamine pathway (NSP) resulted in increases in the striatum of both (+)-butaclamol (18%) and ADTN (55%) displaceable ^{3}H-spiroperidol binding. Similar findings have been reported using both ^{3}H-spiroperidol and ^{3}H-haloperidol binding assays (Nagy et al., 1978). There was no significant change in the affinity of striatal tissue for (+)-butaclamol displaceable ^{3}H-spiroperidol binding after 6-OHDA lesion of the NSP (K_D of control striatum = 60 ± 12 pM, n = 3; K_D of lesioned striatum = 84 ± 11 pM, n = 3). There was, however, a significant ($p < 0.05$) increase (40%) in the total number of binding sites for (+)-butaclamol displaceable ^{3}H-spiroperidol in the lesioned striatum (Bmax of control striatum = 348 ± 20 fmol/mg protein, n = 3; Bmax of lesioned striatum = 434 ± 58 fmol/mg protein, n = 3). There were no alterations in striatal ^{3}H-5HT binding after NSP lesions. 6-Hydroxydopamine lesion of the NSP caused a decrease in (+)-butaclamol displaceable ^{3}H-spiroperidol binding (42%) in the substantia nigra (Table 7).

Table 7. The effects of brain lesions on neurotransmitter receptor binding in the corpus striatum and substantia nigra of the rat.

Lesion site	Neurotoxin	Area measured		Receptor binding (fmol/mg prot) ^{3}H-Spiro/ (+)But	^{3}H-Spiro/ ADTN	^{3}H-5HT
NSP	6-OHDA	Striatum	L	181±5.9[a]*	146±12.6*	123±15.7
			C	154±7.1	94±7.8	112±11.8
NSP	6-OHDA	SN	L	11±2.3*	--	--
			C	19±2.8	--	--

[a]Mean ± S.E.M. of 5 determinations.

L = lesion side.

C = contralateral side.

*$p < 0.05$.

NSP = nigral-striatal dopamine pathway.

The ADTN displaceable ^{3}H-spiroperidol and ^{3}H-5HT (serotonin) binding assays were described elsewhere (Reisine, Nagy, Fibiger, and Yamamura, 1979). Animals were killed 30 days following initiation of the lesion.

Parkinson's Disease

In the Parkinson's disease (PD) study, in addition to measuring ^{3}H-spiroperidol binding in the corpus striatum, ^{3}H-serotonin (^{3}H-5HT), ^{3}H-GABA, ^{3}H-QNB (a muscarinic cholinergic antagonist), and ChAC activity were measured as previously described (Reisine, Fields, Yamamura, et al., 1977). These other neurochemical assays were studied in the same tissue homogenates as the ^{3}H-spiroperidol binding assay to put into better perspective any changes in ^{3}H-spiroperidol binding that we might have detected in these tissues.

One of the major difficulties in studying autopsy material is the possibility that alterations observed are the result of postmortem changes. Thus, all tissues used in this study were collected in an identical manner and the time from death to freezing of the brain was carefully monitored. In addition, care was taken to use brains which had been stored for equal periods of time.

The stability of ChAC activity in postmortem human brain is well documented (P. McGeer and E. McGeer, 1976), as is the stability of neurotransmitter receptors (Enna et al., 1976). In addition, no correlation was found between age, sex, duration of illness, drug treatment, time from death to freezing of tissue, and receptor binding or ChAC activity in any of the brain regions studied. These findings suggest that any alterations observed in these parameters in the basal ganglia are most likely due to the disease process rather than to postmortem artifacts or some other variable.

With regard to the basal ganglia, ^{3}H-spiroperidol binding was significantly reduced (30% in the caudate nucleus of the brains

obtained from Parkinson patients (Figure 9). Saturation isotherms indicate this reduction was due to a decrease in the maximum number of binding sites rather than to a change in the affinity of the receptor for the ligand (Figure 10). GABA receptor binding in the brains from Parkinson's diseased patients, while decreased, was not significantly different from controls in the caudate nucleus, nor were receptor binding for ^{3}H-QNB and ^{3}H-5HT (Figure 9). While the mean ChAC activity was not significantly different between the two groups, there appears to be two separate populations in the Parkinson group with four patients having relatively high ChAC and six patients having much lower activities (Figure 9). Nevertheless, no correlation was found between ChAC activity and any of the receptor binding sites studied.

In contrast to the caudate nucleus, several differences were found between the putamens obtained from Parkinson patients, with ^{3}H-QNB binding significantly increased (30%) and ^{3}H-5HT binding significantly decreased (37%) in the putamen of Parkinson's diseased patients as compared to controls (Figure 11). In addition, ChAC activity was profoundly reduced (56%) in the putamen of Parkinson patients. Furthermore, there was a significant correlation ($p < 0.5$) between ^{3}H-5HT receptor binding and ChAC activity in this brain area. No correlation was found between ^{3}H-QNB and ChAC activity. Both ^{3}H-GABA and ^{3}H-spiroperidol binding were unchanged in the putamen (Figure 11).

In the globus pallidus, only ChAC activity was significantly altered, with a 62% reduction in enzyme activity in the tissue procured from the Parkinson patients (Figure 12). ^{3}H-QNB, ^{3}H-spiroperidol, and ^{3}H-5HT receptor binding were all unchanged in this brain area. Though

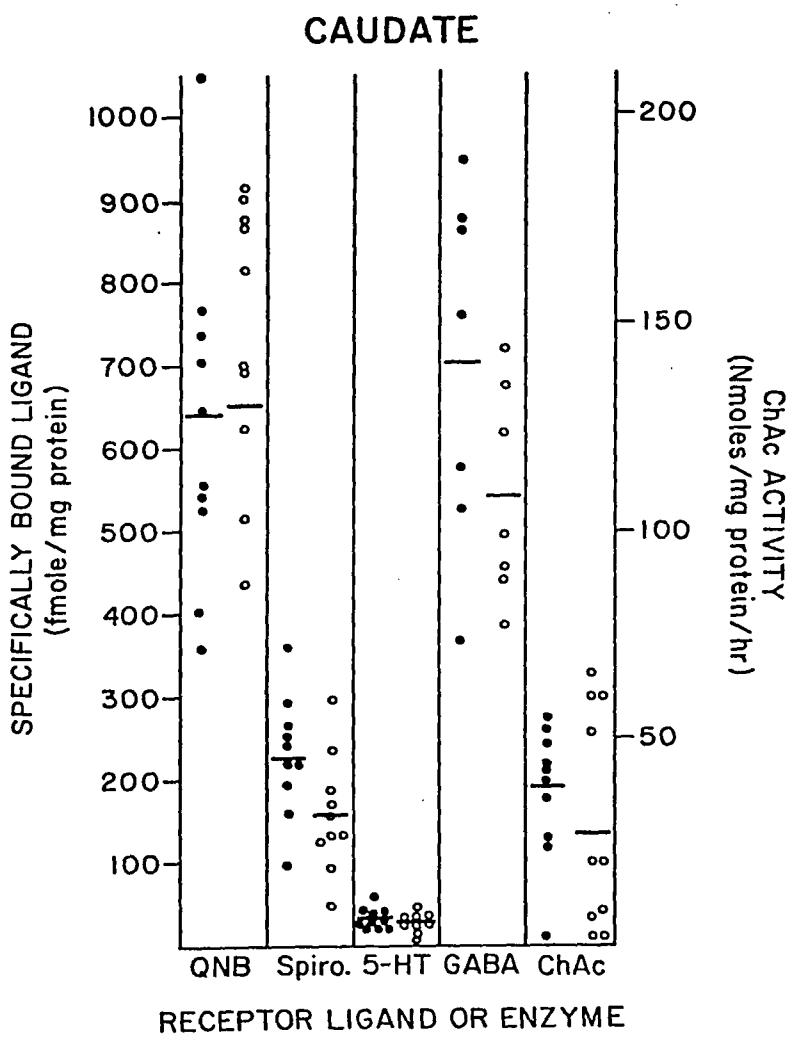

Figure 9. ^{3}H-QNB, ^{3}H-spiroperidol (Spiro), ^{3}H-Serotonin (5-HT), ^{3}H-GABA binding and ChAc activity in the caudate nuclei of brains obtained from control (●) and PD (o) patients -- Each point symbol represents neurochemical values obtained from one brain. The horizontal lines represent the mean average for each group of determinations.

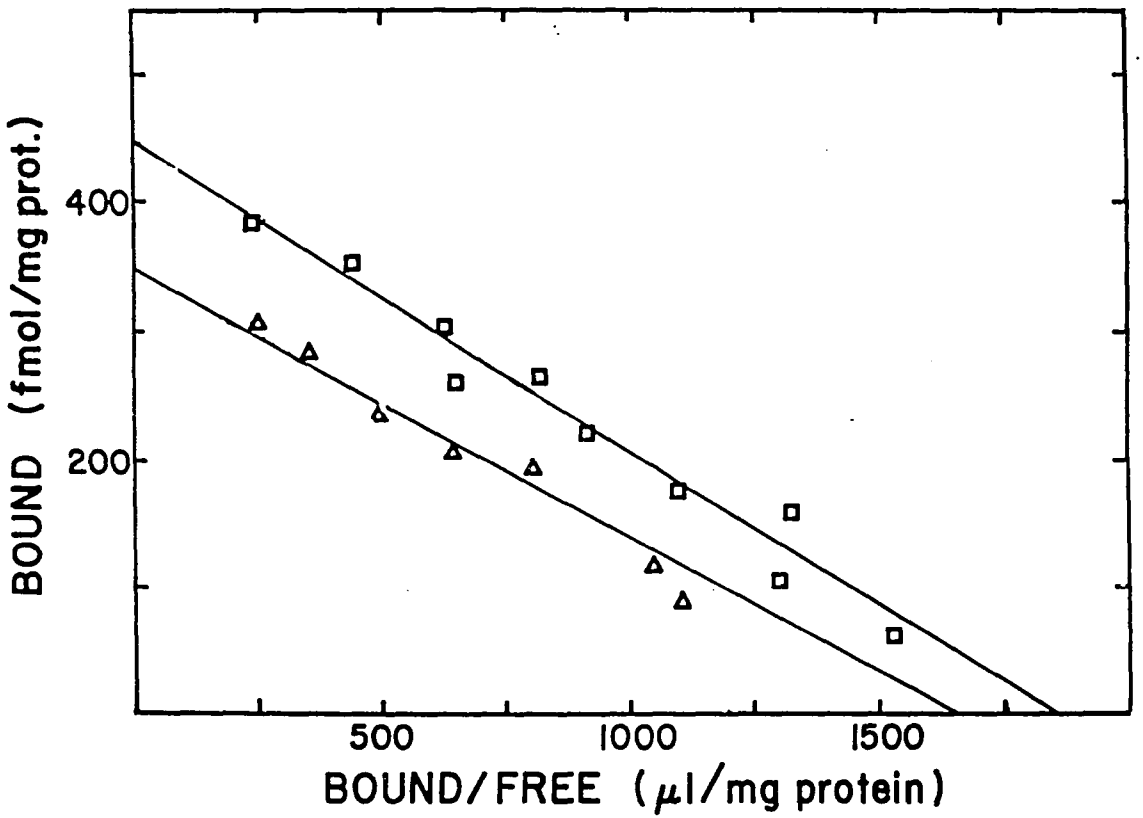

Figure 10. Scatchard plots of ^{3}H-spiroperidol binding to the caudate nucleus of brains obtained from control (□) and Parkinson (Δ) patients.

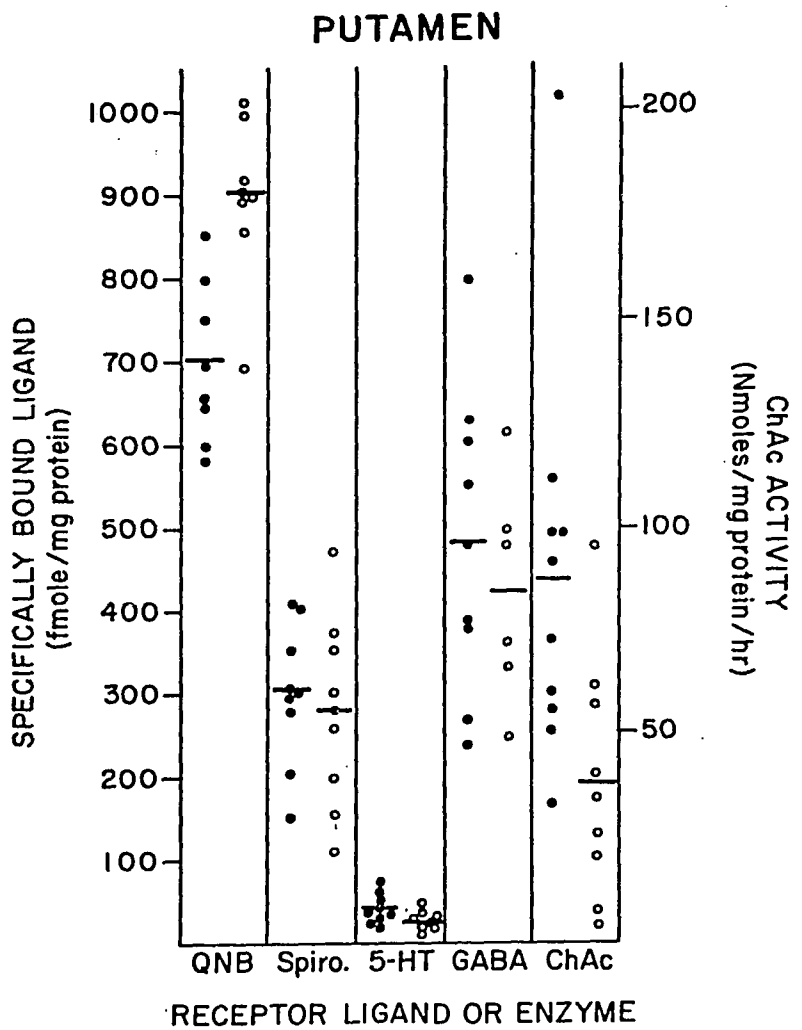

Figure 11. ^{3}H-QNB, ^{3}H-spiroperidol (Spiro), ^{3}H-serotonin (5-HT), ^{3}H-GABA binding and ChAc activity in the putamen of brains obtained from control (•) and Parkinson (o) patients -- Each point symbol represents the neurochemical observation obtained from one brain. The horizontal lines represent the mean average for each group of determinations.

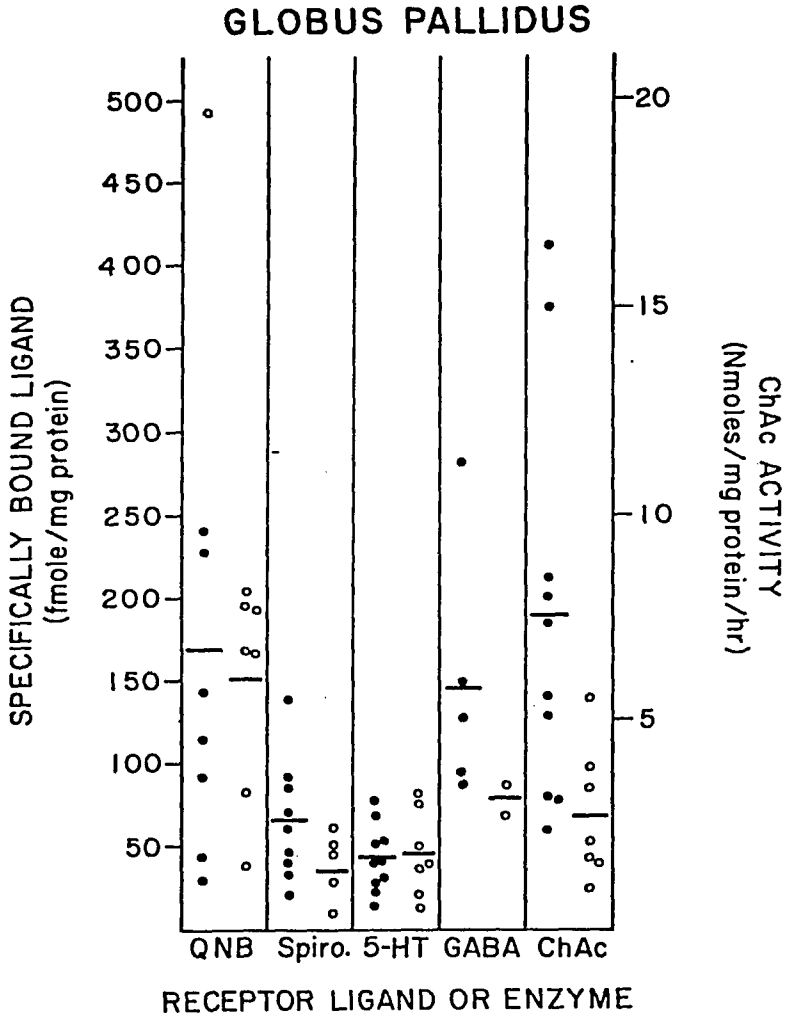

Figure 12. ^{3}H-QNB, ^{3}H-spiroperidol (Spiro), ^{3}H-serotonin (5-HT), ^{3}H-GABA binding and ChAc activity in the globus pallidus of brains obtained from control (●) and Parkinson (o) patients -- The horizontal lines represent the mean average for each group of determinations.

GABA receptor binding appeared reduced, there were too few samples to draw any firm conclusions about this receptor in the globus pallidus. No significant correlations were found between the various binding sites or between the binding sites and ChAC activity.

Huntington's Disease (HD)

Specific ^{3}H-spiroperidol binding was determined in five regions of brains obtained from control and HD patients (Table 8). Highest specific binding in control samples was found in the putamen and caudate nucleus. Intermediate binding was found in the frontal cortex and globus pallidus while lowest binding occurred in the substantia nigra. Significant decreases in binding were found in the basal ganglia and frontal cortex of HD patients. No alterations in binding were found in the substantia nigra of HD patients.

The dissociation constant and maximal binding for the ^{3}H-spiroperidol binding site in the caudate nuclei of brains procured from control and HD patients was determined by Scatchard analysis (Figure 13). The average K_D and Bmax values from four control caudate nuclei were 36 ± 9.1 pM and 190 ± 22.3 fmol/mg protein, respectively. The average K_D and Bmax values from two caudate nuclei from HD patients were 43 ± 18.5 pM and 119 ± 15.5 fmol/mg protein, respectively.

Saturation studies were also performed on the frontal cortices obtained from control and HD patients (Figure 14). The average K_D and Bmax values from two control frontal cortices were 59 ± 14.5 pM and 60 ± 7.5 fmol/mg protein, respectively. The average K_D and Bmax in

Table 8. ^{3}H-Spiroperidol binding in brains obtained from control and Huntington's diseased patients.

Brain region	Control	Huntington's Disease	% Control
		(fmol/mg protein)	
Frontal cortex	43.2± 4.1 (7)	12.1±2.5 (15)	28 (p<.001)
Globus pallidus	39.4± 4.7 (15)	24.4±4.7 (17)	62 (p<.05)
Caudate nucleus	136.6±14.5 (17)	78.4±9.1 (18)	57 (p<.005)
Putamen	158.0± 8.0 (19)	89.2±8.9 (19)	56 (p<.001)
Substantia nigra	17.6± 2.2 (11)	18.7±3.5 (6)	106 N.S.

Values are mean ± S.E.M. Values in parentheses are number of brain samples and level of significance. ^{3}H-Spiroperidol binding was determined using the standard binding assay. Tissue was prepared as described in the Methods Section.

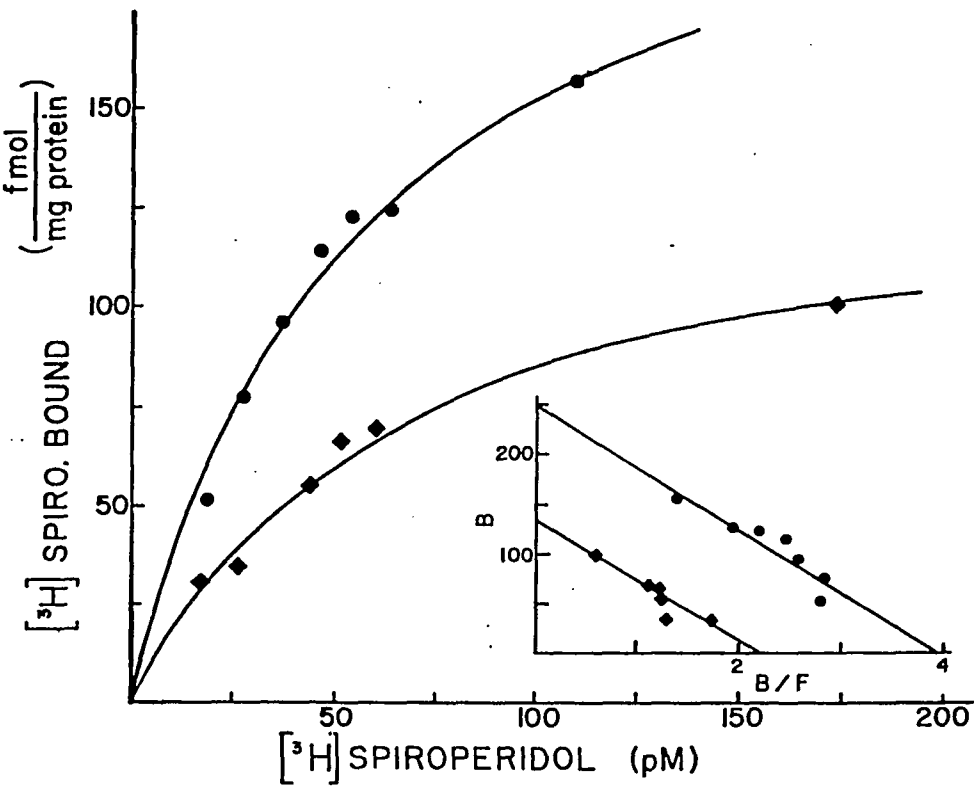

Figure 13. Saturation curve and Scatchard plots of [^{3}H]spiroperidol binding to a caudate nucleus obtained from normal (●) and HD (◆) patients -- Saturation curve of specific [^{3}H]-spiroperidol bound at receptor concentrations of 9.1 pM for normal and 8.3 pM for HD tissue. Inset. Modified Scatchard plot of saturation isotherms. The apparent K_D values (from the slope of the line) are 61 pM in both tissues. Maximum specific binding (from y-intercept) was 248 fmol/mg protein for tissue obtained from normal and 134 fmol/mg protein for HD patients. These experiments were replicated 2-4 times.

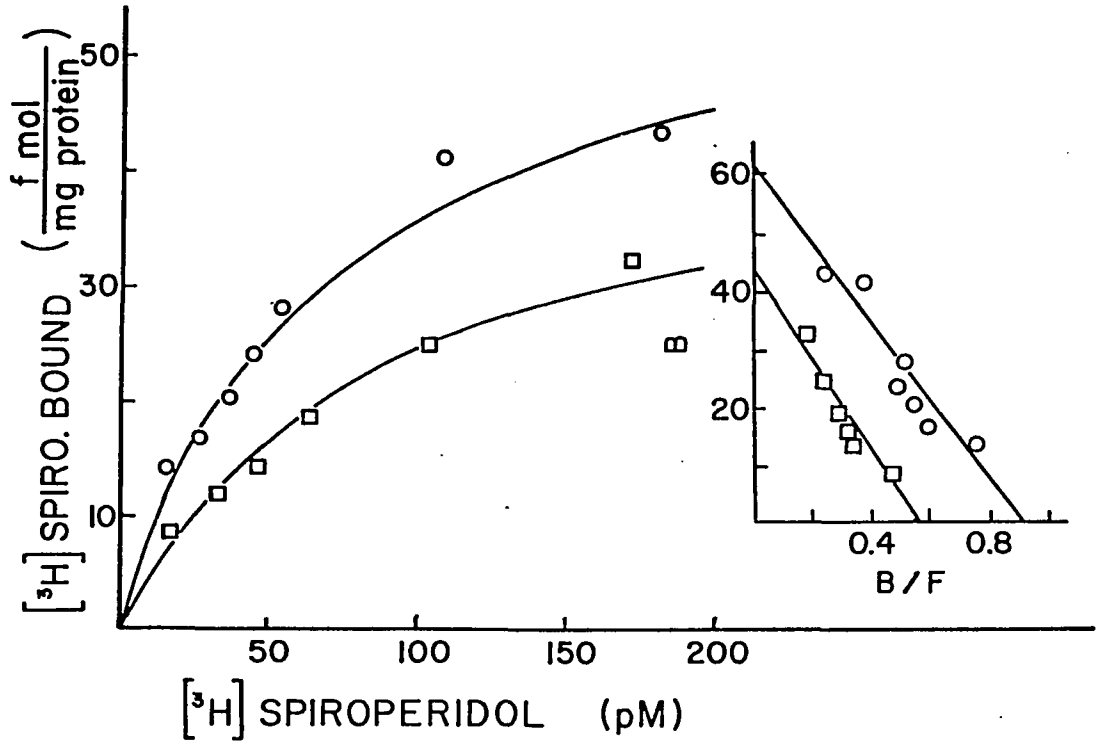

Figure 14. Saturation curve and Scatchard plot of [^{3}H]spiroperidol bound to a frontal cortex obtained from a normal (○) and a HD (□) patient -- Left side: Saturation curve of specific [^{3}H]spiroperidol bound at receptor concentrations of 4,5 pM for control and 4.7 pM for HD tissue. Right side: Modified Scatchard plot of saturation isotherms. The apparent K_D values (from the slope of the line) are 70 pM for both tissues. Maximum specific binding (from the y-intercept) was 64 fmol/mg protein for tissue obtained from normal patients and 44 fmol/mg protein for tissue obtained from HD patients. These experiments were replicated 2-3 times.

three frontal cortices obtained from HD patients was 69 ± 9.9 pM and 40 ± 13.1 fmol/mg protein, respectively.

(+)-Butaclamol was the standard inhibitor in this binding assay and its inhibition of ^{3}H-spiroperidol binding defines specific binding. (-)-Butaclamol, its stereoisomer, has much weaker pharmacological activity and is over 1000 times less potent in inhibiting ^{3}H-spiroperidol binding (Fields et al., 1977). In caudate nuclei from control and HD patients the IC_{50} value for (+)-butaclamol and (-)-butaclamol displacement of ^{3}H-spiroperidol binding was 1 nM and 1 μM, respectively (Figure 15). In frontal cortices of HD and control patients the IC_{50} value for (+)-butaclamol inhibition of ^{3}H-spiroperidol was about 10 nM. (-)-Butaclamol did not inhibit ^{3}H-spiroperidol binding at 10 μM concentrations.

Schizophrenia

In the schizophrenia study, the characteristics of neuroleptic, opiate, alpha-adrenergic, and benzodiazepine receptors were studied using ^{3}H-spiroperidol, ^{3}H-naloxone, ^{3}H-WB-4101, and ^{3}H-flunitrazepam, respectively, as the binding ligands. The latter three receptor types were investigated to determine whether they might be altered in the brains obtained from schizophrenic patients as well as to correlate any changes in these receptors with changes found in the characteristics of neuroleptic receptors.

In the present study, ^{3}H-spiroperidol binding was significantly elevated in both the caudate nucleus (39%) and putamen (51%) of brains obtained from schizophrenic patients yet was unaltered in the frontal

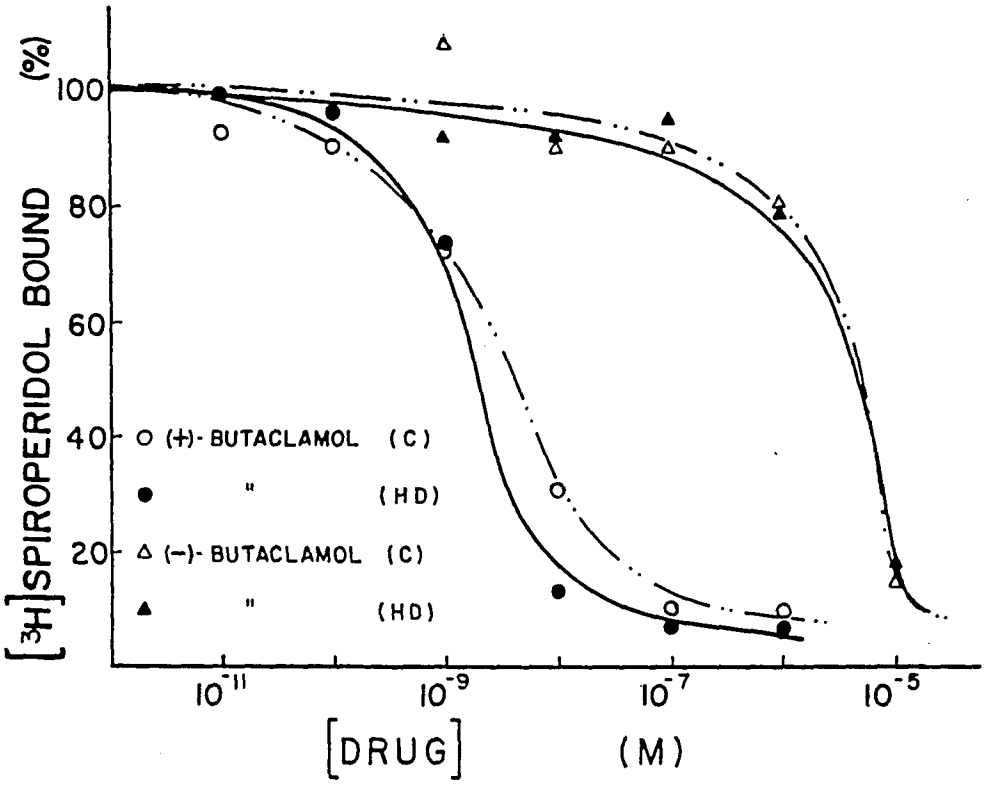

Figure 15. Representative (+) and (-)-butaclamol inhibition curves of [^{3}H]spiroperidol binding in the caudate nuclei obtained from control and HD patients -- Increasing drug concentrations plotted in relationship to % of [^{3}H]spiroperidol bound. The receptor concentrations were 12.3 pM and 14.6 pM in control and HD tissue, respectively. The [^{3}H]spiroperidol concentration was 100 pM. The IC_{50} for (+)-butaclamol inhibiting [^{3}H]spiroperidol binding was 1 nM in both tissues. IC_{50} for (-)-butaclamol inhibiting [^{3}H]spiroperidol binding was 1 μM in both tissues. Average IC_{50} values for (+) and (-)-butaclamol inhibition of [^{3}H]spiroperidol binding in three different caudate nuclei were 1 ± 0.1 nM and 1 ± 0.2 μM.

cortex (Table 9). Scatchard analysis of ^{3}H-spiroperidol binding in the putamens of brains obtained from control and schizophrenic patients revealed an increase in the density of neuroleptic receptors in the schizophrenic putamen (total receptor density in control brains = 213.2 ± 20.5 fmole/mg protein, n = 3; schizophrenic brains = 308 ± 11.1 fmole/mg protein, n = 3) whereas the affinity of the tissue for ^{3}H-spiroperidol was unaltered (control brain K_D's = 0.11 ± 0.01 nM, n = 3; schizophrenic brain K_D's = 0.11 ± 0.01 nM, n = 3). The increase in ^{3}H-spiroperidol binding in the neostriatum of schizophrenic patients was due to an increase in specific binding with no difference in non-specific binding from control values.

Opiate receptors have previously been characterized in mammalian brain using radiolabeled naloxone, an opiate antagonist (Pert and Snyder, 1973). Presumably these receptors mediate the physiological actions of endogenous brain opiates (Snyder, 1978). ^{3}H-Naloxone binding was significantly lowered in the schizophrenic caudate (43%) but unaltered in the putamen and frontal cortex (Table 9). Scatchard analysis of ^{3}H-nalaxone binding in the control and schizophrenic caudate showed a loss of opiate receptors in the schizophrenic tissue (Figure 16). The decreased ^{3}H-naloxone binding was associated with diminished specific ^{3}H-naloxone binding with no difference in non-specific binding from control tissue.

The binding of ^{3}H-WB4101, an alpha adrenergic receptor antagonist, and ^{3}H-flunitrazepam, a compound that binds to benzodiazepine receptors, were also measured in the control and schizophrenic tissue.

Table 9. Neurotransmitter receptor binding in brains obtained from control and schizophrenic patients.

Brain region	^{3}H-Ligands							
	^{3}H-WB-4101		^{3}H-Spiroperidol		^{3}H-Naloxone		^{3}H-Flunitrazepam	
	(fmole/mg protein)							
Frontal cortex								
Control	(11)	45.2±6.4	(11)	27.6± 3.9	(11)	40.3± 7.3	(11)	38.1±2.7
Schizophrenic	(11)	53.8±8.3	(11)	27.8± 4.6	(11)	42.3± 7.6	(11)	45.3±6.7
Putamen								
Control	(10)	28.0±3.4	(11)	75.4±12.6	(11)	74.2± 6.3	(11)	16.0±1.7
Schizophrenic	(11)	28.7±3.8	(11)	114.0± 7.6*	(11)	63.4± 6.2	(11)	19.2±1.9
Caudate nucleus								
Control	(11)	43.1±6.0	(11)	89.5± 9.1	(11)	80.5±11.2	(11)	32.5±4.0
Schizophrenic	(11)	38.6±3.5	(11)	124.1±11.3*	(11)	45.2± 5.8*	(11)	34.3±4.4

*$p < 0.01$.

Values in parentheses represent the number of individual patients from which brain samples were used to determine the mean ± S.E.M. for each ^{3}H-ligand binding in each tissue.

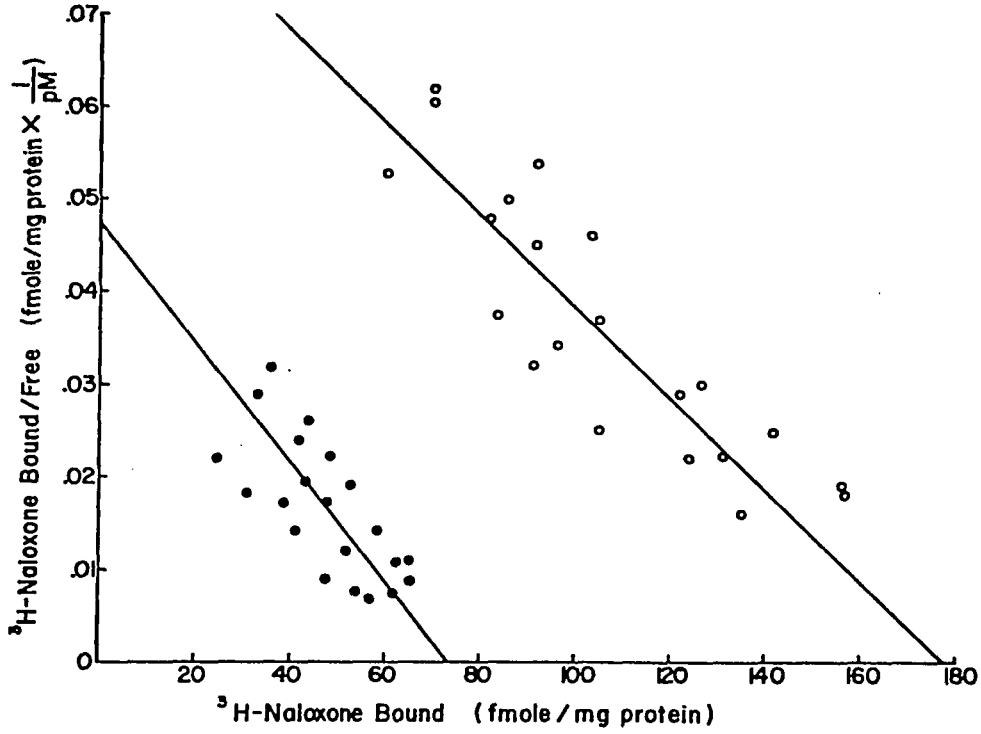

Figure 16. Scatchard plots of ^{3}H-naloxone binding to control (o) and schizophrenic (●) caudate nuclei -- Three different control and three different schizophrenic brains were used in this study. The dissociation constant (determined from the slope of the linear regression line) was 1.93 ± .1 nM for controls and 1.56 nM ± 0.1 nM for schizophrenic tissue. The total receptor density (determined from the x-intercept) was 177.4 ± 9.7 fmole/mg protein for controls and 73.3 ± 5.6 fmole/mg protein for schizophrenic tissue. The receptor densities are significantly different ($p < .001$). The r value for each linear regression line was 0.9 for each case.

None of the schizophrenic brain regions showed alterations in ^{3}H-WB-4101 or ^{3}H-flunitrazepam binding (Table 9).

The alterations in ^{3}H-spiroperidol and ^{3}H-nalaxone binding in the schizophrenic tissues were not due to the presence of either neuroleptics or alterations in the brain levels of any known or unkown compound in the tissue homogenates. This was demonstrated by diluting aliquots of tissue homogenates (10 mg tissue wet wt/ml) two-fold with distilled water, centrifuging the homogenates, discarding the tissue pellets, and applying the supernatants to ^{3}H-nalaxone or ^{3}H-spiroperidol radioreceptor assays (rat brain homogenates which were washed three times as described in the methods were used in these studies). None of the supernatants affected either ^{3}H-nalaxone or ^{3}H-spiroperidol in their respecgive binding assays.

CHAPTER 4

DISCUSSION

^{3}H-Spiroperidol binds specifically and with high affinity to brain neuroleptic receptors. Specific, (+)-butaclamol displaceable binding of ^{3}H-spiroperidol is 75-80% of total binding. This is a greater ratio of specific to total binding than was previously reported for ^{3}H-haloperidol binding (Burt et al., 1976). Kinetic analysis indicates that ^{3}H-spiroperidol dissociates from its receptor slowly, with a $T_{1/2}$ of dissociation of from 12-17 minutes. Thus, little if any ^{3}H-spiroperidol dissociates from the tissue during filter washing. This contrasts with ^{3}H-haloperidol binding which has a $T_{1/2}$ of dissociation of less than one minute (Leysen, Gommeren, and Laduron, 1978). It is possible that in the ^{3}H-haloperidol binding assay, appreciable amounts of specific binding could be lost during the washing procedure, due to the relatively rapid rate of dissociation of ^{3}H-haloperidol from the tissue. This might explain why in brain regions containing low densities of neuroleptic/dopamine receptors, such as the substantia nigra and hypothalamus, that ^{3}H-haloperidol binding is not detectable whereas ^{3}H-spiroperidol binding is easily observed. Therefore, both the high percentage of specific binding and the slow rate of dissociation of ^{3}H-spiroperidol from its receptor support the hypothesis that ^{3}H-spiroperidol binding is the most sensitive assay for monitoring neuroleptic receptors in the brain.

Scatchard analysis of ^{3}H-spiroperidol saturation isotherms reveals that ^{3}H-spiroperidol labels a homogeneous population of neuroleptic receptors in the corpus striatum and substantia nigra. The dissociation constant (30-42 pM) determined from these Scatchard plots for ^{3}H-spiroperidol binding is similar to the K_D (22-26 pM) derived from kinetic analysis. In addition, the inhibitory constant (IC_{50}) of unlabeled spiroperidol for inhibiting ^{3}H-spiroperidol binding (0.1 nM) closely approximates the K_D values obtained from the kinetic and the saturation studies. These results demonstrate that ^{3}H-spiroperidol binds with high affinity to brain neuroleptic receptors.

Recently, Pedigo and associates (1978) have observed a deviation from linearity in their Scatchard plots for ^{3}H-spiroperidol binding in the rat corpus striatum. Nonlinear Scatchard plots could represent the presence of either multiple binding sites in the corpus striatum for ^{3}H-spiroperidol or the existence of cooperative interactions between identical neuroleptic receptors in this brain region. In order to detect this phenomenon, Pedigo et al. (1978) used a wide range of ^{3}H-spiroperidol concentrations (1 pM to 3000 pM) in their saturation studies. In the present study, a concentration range for ^{3}H-spiroperidol of only 10 pM to 900 pM was used to determine saturability of ^{3}H-spiroperidol binding in the corpus striatum. The less extensive range of ^{3}H-spiroperidol concentration used in our experiments might explain the lack of any noticeable curvature in our Scatchard plots. Although the findings by Pedigo et al. (1978) might be supportive evidence for the existence of multiple types of dopamine/neuroleptic receptors in the corpus striatum, further ^{3}H-spiroperidol binding

studies must be initiated to determine whether in fact this phemonenon represents different types of dopamine receptors in the striatum or cooperative interaction between neuroleptic binding sites.

A number of antipsychotic drugs are very effective in blocking ^{3}H-spiroperidol binding in the corpus striatum. Unlabeled spiroperidol is not only the most potent compound in inhibiting ^{3}H-spiroperidol binding (IC_{50} = 0.1 nM), but also is the most potent drug in preventing ^{3}H-haloperidol binding (Burt et al., 1976). Antipsychotic agents such as pimozide (IC_{50} = 0.2 nM), (+)-butaclamol (IC_{50} = 0.6 nM), haloperidol (IC_{50} = 4.8 nM), and fluphenazine (IC_{50} = 3.4 nM) all exhibit high affinity for the ^{3}H-spiroperidol binding site. Weaker antipsychotic agents such as chlorpromazine (IC_{50} = 19 nM) and thioridazine (IC_{50} = 28 nM) are somewhat less effective in preventing ^{3}H-spiroperidol binding. The rank order of these neuroleptic drugs in inhibiting ^{3}H-spiroperidol binding is similar to their rank order of potency in displacing ^{3}H-haloperidol binding (Burt et al., 1976), in blocking apomorphine-induced emesis in dogs and apomorphine- and amphetamine-induced stereotypy (Janssen and van Bever, 1975), in preventing dopamine's inhibition of prolactin release (Meltzer, Paul, and Fang, 1977), as well as their relative clinical potencies as antipsychotic agents (Creese et al., 1976a, 1976b). These results suggest that ^{3}H-spiroperidol associates predominately with neuroleptic/dopamine receptors in the corpus striatum.

^{3}H-Spiroperidol binds stereoselectively to striatal neuroleptic receptors. (-)-Butaclamol is impotent as an antipsychotic agent and therefore hass little affinity for the neuroleptic receptors (IC_{50} =

1754 nM), as labeled by ^{3}H-spiroperidol. Its active stereoisomer, (+)-butaclamol, has over 2000 times greater affinity for neuroleptic receptors. Several other studies have further demonstrated both the structural and steric specificity of ^{3}H-spiroperidol binding to brain neuroleptic receptors (Leysen, Gommeren, and Laduron, 1978; Creese, Schneider, and Snyder, 1977; Seeman et al., 1979). This supports the hypothesis that ^{3}H-spiroperidol labels a distinct, pharmacologically relevant receptor.

In general, dopamine antagonists have greater affinity for the ^{3}H-spiroperidol binding site than do dopamine agonists. Creese et al. (1975) have suggested that striatal dopamine receptors can exist in at least two conformations. One configuration of the dopamine receptor associates tightly with dopamine antagonists and loosely with agonists, while the other prefers dopamine agonists to antagonists. Using a labeled dopamine antagonist such as ^{3}H-spiroperidol to monitor dopamine receptors shifts the equilibrium between the agonist and antagonist configurations of the dopamine receptor in favor of the antagonist state. Thus, the potency of dopamine agonists to inhibit ^{3}H-spiroperidol binding is less than for antipsychotic agents. Unfortunately, there is some controversy regarding the validity of the two-state dopamine receptor model (Nagy et al., 1978; Titeler, Tedesco, and Seeman, 1978). In fact, it has been suggested that instead of one type of dopamine receptor existing in various conformations, as proposed by Creese et al. (1975), that there are multiple types of striatal dopamine receptors with dopamine agonists having the highest affinity for one subpopulation and dopamine antagonists binding with greater

preference for another. In any case, the reason for the higher affinity of antagonists for the neuroleptic receptor than agonists remains unclear.

Dopamine and dopamine agonists have the greatest potency in displacing ^{3}H-spiroperidol from its receptor than any neurotransmitter agonists tested. Catecholamines such as (-)-epinephrine and (-)-norepinephrine and the indolamine serotonin very weakly interact with neuroleptic receptors. Cholinergic, GABAergic, histaminergic, glutaminergic, and glycinergic agents can not displace ^{3}H-spiroperidol binding even at high drug concentrations (Table 1). Thus, in the corpus striatum, ^{3}H-spiroperidol predominately binds to dopamine receptors.

A similar but somewhat less extensive drug profile for the displacement of ^{3}H-spiroperidol binding exists in the substantia nigra. Stereoselectivity of the binding site was demonstrated by (+)-butaclamol's greater potency than its pharmacologically inactive stereoisomer in blocking ^{3}H-spiroperidol binding. Dopamine agonists are of similar potency in inhibiting ^{3}H-spiroperidol in the substantia nigra as in the striatum. These agents are also more potent than serotonin in blocking ^{3}H-spiroperidol binding. Thus, it appears that ^{3}H-spiroperidol also labels dopamine receptors in the substantia nigra.

^{3}H-Spiroperidol binding has a distinct regional distribution in both rat and human brain. The caudate/putamen (neostriatum) which contains high levels of dopamine has over 400 times greater specific ^{3}H-spiroperidol binding than other brain regions which have little or no monoamine content such as the cerebellum. In fact, the distribution of ^{3}H-spiroperidol binding closely parallels the distribution of

dopamine in the brain. This would tend to support the hypothesis that neuroleptics interact mainly with brain dopamine receptors. However, intermediate to high levels of ^{3}H-spiroperidol binding were also detected in several cerebral cortical areas which contain low levels of dopamine. Both the autoradiographic studies by Klemm, Murrin, and Kuhar (1979) and the *in vivo* ^{3}H-spiroperidol studies of Laduron, Janssen, and Leysen (1978) confirm the presence of neuroleptic receptors in the cerebral cortex. Leysen, Niemegeers, Tollenaere, and Laduron (1978) have proposed that the characteristics of the cerebral cortical ^{3}H-spiroperidol binding sites are predominately serotonergic in nature. These data together with the mainly dopaminergic characteristics of ^{3}H-spiroperidol binding sites in a number of other brain regions points out the complex nature of the mechanism of action of neuroleptics and indicates that neuroleptics may interrupt different neurotransmitter systems in different brain regions to cause their pharmacological effects.

Brain Lesions

A fundamental method for characterizing neuronal circuitry in the brain has been the lesion technique. With this method, a discrete area of brain containing a group of neuronal cell bodies or a bundle of axons is destroyed by a knife cut or thermal coagulation. The lesion separates axons from their sustaining neuronal cell bodies, and the axons ultimately degenerate. This method has been widely used in neuroanatomical studies since the degenerating axons can be visualized by silver-impregnation techniques and the cell bodies of origin can be

identified by their chromolytic changes. Recently, biochemical properties associated with specific neuronal tracts have been demonstrated by monitoring the selective reduction of these markers in the region of innervation after lesioning the neuronal pathway. This method, however, suffers from an important limitation that the lesioning device not only damages the neurons of interest but any neuronal cell body or axon in its path; destruction of unrelated neurons can lead to serious problems of interpretation of the specificity of the neurochemical or histological sequelae of a lesion.

In recent years, there has been growing interest in the development of pharmacological methods for lesioning specific types of neurons. 6-Hydroxydopamine is the most widely used and best characterized example of such drugs (Sachs and Jonsson, 1975). This agent, which differs from the neurotransmitter dopamine only by the addition of an extra hydroxyl group, is extremely unstable at neutral pH and undergoes spontaneous decomposition liberating the strong oxidant, hydrogen peroxide. Catecholaminergic neurons possess avid transport processes on their neuronal membrane which take up catecholamines to terminate their synaptic action. Because of the structural similarity of 6-hydroxydopamine to norepinephrine and to dopamine, it is selectively taken up by these neurons after systemic or local injection, while noncatecholaminergic neurons, which do not possess a high affinity uptake process for catecholamines, do not accumulate the toxin.

The use of this technique along with the pretreatment of animals with desimipramine to prevent uptake of 6-hydroxydopamine into

noradrenergic neurons can cause the preferential destruction of brain dopamine neurons. Thus, we have injected minute amounts of 6-hydroxydopamine into the nigral-striatal dopamine pathway in order to destroy selectively the dopaminergic innervation to the striatum as well as the dopamine containing perikarya in the substantia nigra. Following these procedures, we have determined whether neuroleptic/dopamine receptors might be localized to nigro-striatal dopamine neurons.

Another potential site for neuronal selectivity for neurotoxins are the neurotransmitter receptors on neurons. Neurotransmitter receptors exhibit marked chemical specificity with regard to agents that activate or block them and often with affinities in the nanomolar range. Although the factors that determine the types and distribution of neurotransmitter receptors on neurons are complex, the presence of synaptic input from a neuron utilizing a particular neurotransmitter appears to play an important role. Stimulation of neurotransmitter receptors can dramatically alter the distribution of ions, the oxygen consumption, and the metabolic processes of neurons. Thus, it is conceivable that agents which interact with certain neurotransmitter receptors could have selective toxic effects on neurons bearing these receptors.

Kainic acid, a conformationally-restricted analogue of glutamate, is a potent and selective neurotoxin when injected into the brain (Coyle and Schwarcz, 1976). It causes a rapid degeneration of neurons with cell bodies near the site of infusion but spares axons of passage and of termination as well as non-neuronal constituents. Although the action of kainic acid appears to be mediated by specific

receptors (glutamate) concentrated in the region of the neuronal cell body, the synaptic input to the neurons influences its neurotoxicity (E. McGeer, P. McGeer, and Singh, 1978). In certain regions of the brain, kainate lesions can mimic human neurodegenerative disorders such as Huntington's disease (Coyle and Schwarcz, 1976). With appropriate attention to morphological analysis of the kainate lesion to characterize its extent and specificity, local injection of kainate has been found to be a useful tool for selectively ablating neuronal cell groups in the brain.

In light of its specific lesioning capabilities, we chose to inject kainic acid into the corpus striatum. The striatum contains small cholinergic interneurons as well as GABAergic neurons that innervate the substantia nigra. Intrastriatal administration of kainic acid causes destruction of striatal cholinergic and GABAergic perikarya (Coyle and Schwarcz, 1976). In addition, the striatal GABAergic input to the substantia nigra is mainly removed. However, nigral-striatal dopamine neurons are only slightly affected. Using this lesioning technique makes it possible to determine if striatal neuroleptic/dopamine receptors might be located on cholinergic or GABAergic neurons and if nigral neuroleptic receptors are direcly associated with GABAergic terminals.

Intrastriatal kainic acid lesions resulted in a gradual loss of dopamine receptors in the striatum of up to 55% of control levels, 22 days after the lesion. In addition, there were dramatic losses of both ChAC and GAD activity. These latter findings probably resulted from the dropout of cholinergic and GABAergic neurons from this brain

region following this lesion. Similar findings have been reported by Schwarcz and Coyle (1977). Recently, Schwarcz et al. (1979) have demonstrated that the dopamine receptors unaffected by intrastriatal kainic acid lesions are still functional and can mediate some of the pharmacological effects of dopaminergic agents when applied to the striatum. This indicates that those remaining dopamine receptors are probably associated with intact, viable neuronal elements.

6-Hydroxydopamine lesions of the nigro-striatal dopamine pathway do not decrease, but instead elevate, striatal ^{3}H-spiroperidol binding. The higher ^{3}H-spiroperidol binding is the result of a greater density of dopamine receptors in the striatum after these lesions. The increased ^{3}H-spiroperidol binding is in accord with the behavioral and electrophysical supersensitivity of dopamine's effects seen in the striatum after the same type of lesions (Ungerstedt, 1971; Feltz and de Champlain, 1972; Ungerstedt et al., 1975). The present findings suggest that little if any proportion of the total striatal dopamine receptor population is located presynaptic to dopamine neurons in the striatum.

Schwarcz and Coyle (1977) have found that approximately 33% of striatal dopamine receptors are directly associated with cortical-striatal afferents. These results were obtained from studies in which the cerebral cortex was ablated and 5 days later, ^{3}H-haloperidol binding was determined in the striatum. Since 45% of the striatal dopamine receptor population is localized to striatal perikarya or dendrites and 33% of striatal dopamine receptors are associated with cortical-striatal afferents, approximately 15-20% of the striatal dopamine

receptor population is of indetermined location. These receptors could be associated with striatal perikarya resistant to the neurotoxic effects of kainic acid or localized to nerve terminals of axons whose cell bodies are not of cortical origin.

There is an important network of dopaminergic dendrites both in the pars compacta and pars reticulata of the substantia nigra. This has been shown in immunohistochemical studies using antibodies against tyrosine hydroxylase and in histochemical experiments by visualizing exogenous dopamine selectively taken up into dopaminergic dendrites (Hokfelt, Fuxe, and Goldstein, 1975; Pickel et al., 1975; Bjorklund and Lindvall, 1975). Therefore, as with dopaminergic nerve terminals, the dopaminergic dendrites contain the enzyme involved in the rate limiting step of dopamine synthesis and are able to take up and to store exogenous dopamine.

Both in vitro and in vivo release of dopamine has been demonstrated in the substantia nigra (Cuello and Iversen, 1978; Geffen et al., 1976). ^{3}H-Dopamine previously taken up into nigral tissue can be released under neuronal depolarization by potassium (Nieoullon, Cheramy, and Glowinski, 1977). Drugs such as amphetamine and benzotropine, which enhance dopamine release from nerve terminals, markedly increase nigral dopamine release (Nieoullon et al., 1977). In addition, several neurotransmitters present in large amounts in the substantia nigra influence the dendritic release of dopamine. Thus, an increased release of ^{3}H-dopamine has been observed in in vivo experiments during the nigral application of acetylcholine, glycine, and serotonin (Cheramy, Nieoullon, and Glowinski, 1978; Michelot et al., 1979).

These results suggest that a number of neurotransmitter receptors might be located on dopamine neuronal elements in the nigra to both effect dopamine release and the activity of nigral-striatal dopamine neurons.

Previously, Bunney and Aghajanian (1975) suggested the presence of dopaminergic "autoreceptors" on dopaminergic cell bodies in the substantia nigra. The microiontophoretic application of dopamine in the nigra inhibited the firing of dopaminergic cells and this effect was antagonized by neuroleptics (Bunney and Aghajanian, 1975). It is possible that by acting on these receptors, dopamine released in the nigra may induce a self-inhibition or a lateral inhibition of dopaminergic neurons.

Several groups have also demonstrated that dopamine sensitive adenylate cyclase, a molecule proposed to be associated with the dopamine receptor, is located not on nigral dopamine neurons, but instead on the terminals of striatal-nigral afferents (Spano et al., 1976; Gale et al., 1977; Phillipson and Horn, 1976). By acting on the dopaminergic receptors associated with adenylate cyclase, dopamine released from dendrites could presynaptically control the release of other transmitters in the substantia nigra. Recently, it was found that dopamine could alter the release of GABA in the substantia nigra (Reubi, Iversen, and Jessel, 1978). This effect could possibly be mediated by dopamine receptors coupled to the adenylate cyclase localized to GABA nerve terminals in the nigra.

Destruction of dopamine neurons in the substantia nigra by 6-hydroxydopamine lesions of the nigro-striatal dopamine pathway caused a 40% reduction in nigral ^{3}H-spiroperidol binding. Similar findings

have been reported by Quik et al. (1978) using receptor binding techniques and by Murrin, Klemm, and Kuhar (1978) using autoradiographic techniques. In contrast, intrastriatal kainic acid lesions which significantly deplete the density of striatal-nigral neuronal inputs do not significantly alter nigral ^{3}H-spiroperidol binding. These results reveal two important characteristics about nigral dopamine receptors.

1. There are dopamine "autoreceptors" in the substantia nigra localized to dopamine neuronal elements. The "autoreceptors" could function as part of a unique regulatory system important for the normal activity of nigral-striatal dopamine neurons.
2. Neuroleptic receptors as labeled by ^{3}H-spiroperidol are not associated with the terminals of striatal-nigral afferents.

Thus, ^{3}H-spiroperidol binding sites in the nigra are of a different neuronal location than nigral dopamine sensitive adenylate cyclase activity. It is possible that the adenylate cyclase in the substantia nigra could be coupled to a dopamine receptor that is relatively insensitive to ^{3}H-spiroperidol. It has been proposed that labeled dopamine agonists bind with higher affinity to a different subpopulation of dopamine receptors than those labeled by dopamine antagonists (Titeler et al., 1978). Thus, it is possible that similar lesion studies as those presented here using labeled dopamine agonists such as ^{3}H-ADTN might reveal the presence of nigral dopamine receptors primarily associated with the same neuronal elements as dopamine sensitive adenylate cyclase. However, the present study demonstrates a differential location for ^{3}H-spiroperidol sensitive dopamine receptors and dopamine sensitive adenylate cyclase in the substantia nigra.

Interestingly, only 40% of ^{3}H-spiroperidol binding sites in the substantia nigra are related to dopamine neuronal elements. Thus a majority of ^{3}H-spiroperidol binding sites are localized to non-dopaminergic neurons in the substantia nigra.

Recent evidence suggests that nigral dopamine receptors might be associated with serotonergic nerve terminals. Thus, the main serotonergic input to the substantia nigra originates from cells of the medial and dorsal raphé nuclei (Giambalvo and Snodgrass, 1978; Ternaux et al., 1977; Palkovits, Brownstein, and Saavedra, 1974). Anatomical studies have defined these pathways using autoradiography, retrograde horseradish peroxidase (Fibiger and Miller, 1977), and histochemical techniques (Fuxe, 1965). Both stimulation of raphé neurons and direct application of serotonin to the substantia nigra causes predominantly depressive effects on nigral neuronal activity (Dray and Straughan, 1976). These actions can be reversed by methiothepin, a putative serotonin antagonist (Dray and Straughan, 1976). Application of both substance P and dopamine to nigral tissue slices enhances serotonin release (Reubi et al., 1978), However, both effects should be inhibited by α-flupenthixol, a dopamine antagonist (Miller, Horn, Iversen, and Pinder, 1974). Since substance P is known to increase nigral dopamine release (Reubi et al., 1978), it is probable that its effects on nigral serotonin release are indirect, via activation of dopamine release. These data suggest the existence of a close interaction between serotonin and dopamine neurons in the substantia nigra. This proposal is supported by the immunohistochemical studies of Pickel et al. (1975), which demonstrated a direct contact between serotonin containing

terminals and the dendrites of nigral dopamine neurons. Thus, a complex neuronal feedback system could exist in the substantia nigra with serotonin released from nigral serotonin nerve terminals, inhibiting dopamine neuronal activity, and the subsequent inhibition of nigral dopamine release preventing further serotonin release into the substantia nigra. This would suggest that nigral dopamine receptors are localized to serotonin nerve terminals. However, further biochemical studies are necessary to determine the exact relationship between dopamine receptors and serotonin nerve terminals in the nigra.

Parkinson's Disease

The results of the present study provide significant new insights into the pathology of Parkinson's disease and also yield new information about neuronal interactions which exist in the normal human brain. Thus, the finding of a significant decrease in ^{3}H-spiroperidol binding in the caudate nucleus of Parkinson patients suggests that the decreased response to L-DOPA observed after chronic treatment with this agent may be in part due to a progressive loss of dopamine receptor sites rather than solely to the degeneration of the dopamine terminals. That the caudate nucleus dopamine receptors measured in this study are not localized to dopamine terminals themselves (autoreceptors) (Westfall et al., 1976) is suggested by the fact that no change in ^{3}H-spiroperidol binding was noted in the putamen, an area which is known to be richly innervated by dopaminergic fibers and where the loss of dopamine terminals is as great or perhaps even greater than the caudate nucleus (Hornykiewicz, 1977). This finding is also interesting in light of the

fact that there was no significant correlation between ChAC activity and dopamine receptor binding in the caudate nucleus suggesting that in the normal brain there are dopamine synapses in the caudate other than the postulated dopamine-cholinergic interaction (P. McGeer, Grewaal, and E. McGeer, 1974). This conclusion is reinforced by the finding of significant decreases in ChAC activity in both the putamen and globus pallidus with no change in ^{3}H-spiroperidol binding to the dopamine receptor in these areas.

Recently, Lee, Seeman, Rajput, and Hornykiewicz (1978) have reported a trend for decreasing levels of neuroleptic receptor binding in the neostriatum of Parkinson patients who had received long-term L-DOPA therapy. In addition, they found an elevation of ^{3}H-haloperidol binding in the neostriatum of unmedicated Parkinson patients. These findings were suggested by the authors to reflect the appearance of supersensitive striatal dopamine receptors resulting from the loss of the dopaminergic neuronal input to the striatum in the Parkinson patients. Unfortunately, in their study they did not determine whether the receptor density or affinity for ^{3}H-haloperidol was altered. In addition, no other neurochemical markers, such as ChAC activity, were measured. Thus, it is hard to fully interpret Lee's findings except to say that Parkinson patients treated with L-DOPA appear to have different neurochemical alterations in the striatum than those patients that were unmedicated.

The significant decrease in ChAC activity observed in the putamen and the globus pallidus confirms previous observations that there is a decrease in the number of interneurons in the basal ganglia

of brains obtained from Parkinson's diseased patients (Hornykiewicz, 1973; Lloyd et al., 1975; Marsden and Parkes, 1977). Since the majority of interneurons in the basal ganglia appear to be either GABAergic or cholinergic and since the GABA-containing cells appear to be intact in Parkinson brains (Hornykiewicz, 1977), the present findings and those of Lloyd et al. (1975) indicate that a significant amount of the interneuronal cell loss is cholinergic. Accordingly, the finding of a decrease in serotonin receptors in the putamen which correlates with the decrease in ChAC activity strongly suggests that receptors for serotonin are localized to cholinergic cells in this brain area. This hypothesis is reinforced by the previous report that serotonin receptors are significantly reduced in the putamen of brains from individuals who had Huntington's disease, a disorder characterized by a profound loss of both GABA and acetylcholine-containing cells (Enna et al., 1976). On the other hand, the unchanged serotonin receptor binding observed in the globus pallidus in the presence of significantly reduced ChAC activity suggests that serotonin modulates a system other than the cholinergic in the globus pallidus.

The enhanced ^{3}H-QNB binding observed in the putamen coupled with the decrease in ChAC activity implies a possible "denervation supersensitivity" for acetylcholine in this brain area in Parkinson's disease.

Finally, the fact that GABA receptor binding was unchanged in all areas studied suggests that in human brain the primary site of action for this transmitter system is neither the dopamine terminals nor the cholinergic cells, both of which are reduced in these regions in Parkinson's disease. Similarly, in studies in which the rat

nigral-striatal dopamine pathway was destroyed with 6-hydroxydopamine, striatal GABA receptor binding was unchanged (Reisine, Nagy, Fibiger, and Yamamura, 1979). These results confirm the hypothesis that GABA receptors are not located on nigral-striatal dopamine neurons, as was previously proposed by Bartholini and Stadler (1977). The results of this present study also confirm the previous observation of Lloyd, Dreskler, and Bird (1977) that GABA receptor binding is unchanged in these brain areas in Parkinson's disease.

With regard to the therapy of Parkinson's disease, the results of this study suggest that the "on-off" response to L-DOPA observed in Parkinson patients may be related to a loss of postsynaptic dopamine receptors in the caudate nucleus of these individuals. Since the majority of the Parkinson patients used in this study had received long term L-DOPA therapy, it is possible that some of the biochemical alterations observed are the result of this treatment rather than to the disease itself. However, the lack of any correlation between drug treatment and the significant differences observed in this study would suggest that this is not necessarily the case. Nevertheless, the results do indicate that Parkinson patients receiving L-DOPA have a significant reduction in dopamine receptors in the caudate nucleus. This finding provides a biochemical basis for the lack of effectiveness of dopamine receptor agonists in a significant number of Parkinson patients (Lieberman et al., 1976; Kartzinel, Shoulson, and Calne, 1976; Gerlach, 1976).

Huntington's Disease

A number of neurochemical alterations have been detected in postmortem brain tissue obtained from Huntington's diseased patients. Both the activity of ChAC and density of muscarinic cholinergic receptors are decreased in the corpus striatum of Huntington's diseased patients (Bird and Iversen, 1974; Enna et al., 1976; P. McGeer, E. McGeer, and Fibiger, 1973). These findings reveal a major defect in the striatal cholinergic neurotransmitter system in Huntington's disease with both pre- and postsynaptic elements of the cholinergic synapse having degenerated. Brain GABAergic neurons are also affected in Huntington's disease. Glutamic acid decarboxylase activity and GABA levels are reduced in the striatum of Huntington's diseased brains (Bird, 1976; Enna et al., 1976; E. McGeer and P. McGeer, 1976). The density of receptors for GABA is also diminished in the Huntington's diseased striatum (Lloyd et al. 1977; Lloyd and Davidson, 1979; Olsen, van Ness, and Tourtellotte, 1979; Olsen, van Ness, Napias, et al., 1979; Reisine, Beaumont, et al., 1979). However, some controversy exists whether GABA receptors are significantly changed in other Huntington's diseased brain regions such as the cerebellum (Olsen, van Ness, Napias, et al., 1979). Thus, both striatal GABA-containing neurons and the neurons which GABA acts upon in the striatum are partially degenerated in this disease. Unfortunately, little is known regarding the nature of the neurons upon which GABA receptors are located in the striatum.

Several other neurotransmitter and proposed neurotransmitter systems are affected in the brains from Huntington's diseased patients.

Thus, the density of serotonin (Enna et al., 1976). Kainic acid (Beaumont et al., 1979) and benzodiazepine (Reisine, Wastek, et al., 1979) receptors are decreased in density in the Huntington's disease patients. These findings reveal a multiplicity of neurochemical abnormalities in the striata of Huntington's disease patients. This is not surprising, since the brain region exhibiting the most pronounced neuropathology in Huntington's disease is the corpus striatum.

At present, the most widely used drugs for the treatment of chorea are dopamine receptor antagonists. The rationale for the use of these agents in Huntington's disease follows from the results of a number of human clinical and biochemical studies. Of all the drugs tested, neuroleptics were found the most efficacious in reducing chorea. In addition, both the levels of dopamine and the activity of tyrosine hydroxylase appear normal in the basal ganglia of Huntington's disease patients (Bird, 1976). This suggests that nigral-striatal dopamine containing neurons are intact in Huntington's disease. The loss of the major inhibitory influence on nigral dopamine neurons (the striatal-nigral GABA containing neurons) suggests that nigral-striatal dopamine neurons might be hyperactive in Huntington's disease. Dopamine receptor antagonists, in turn, might be effective in the treatment of chorea by counteracting the effects of the overactive nigral-striatal dopamine neurons. In fact, Klawans (1970) has shown that increasing the levels of brain dopamine by treating choreic patients with L-DOPA severely exacerbates their symptoms, thus suggesting that elevated levels of dopamine at its receptor might be responsible in part for the appearance of choreatic symptoms. However, despite all of these

data, neuroleptic therapy is limited in Huntington's disease by both the appearance of debilitating side-effects and the gradual occurrence of either partial or total refractoriness to the beneficial effects of the treatment.

One puzzling aspect regarding the mechanism by which neuroleptics reduce chorea is that if these drugs are acting in the striatum to cause their pharmacological effects, and if most of the neurons in the striatum of Huntington's disease patients are destroyed, where then is the site of action of these compounds? In the present study, there was approximately a 45% reduction in the density of dopamine receptors in the striata of Huntington's disease patients. No change in the affinity of these receptors for ^{3}H-spiroperidol was detected. Similar findings have been reported in the corpus striatum, 22 days after intrastriatal kainic lesion (Fields et al., 1977). These data support the hypothesis that intrastriatal kainic acid lesions might serve as an animal model for Huntington's disease. These data, together with the large decrease reported for the ChAC and glutamic acid decarboxylase activity in the striata of Huntington's disease patients suggest that a substantial percentage of striatal dopamine receptors associated with striatal perikarya or dendrites are lost in Huntington's disease.

It is possible that the remaining dopamine receptors in the striata of Huntington's disease patients are functional. Their location may be on cortical-striatal afferents, remaining cholinergic or GABAergic cell bodies, or striatal neuronal elements of some undetermined nature. Also, their continued activity could very well be

important in the control or disappearance of control of movement in the choreatic patients. The finding by Schwarcz et al. (1979) that striatal dopamine receptors are still functional after intrastriatal kainic acid lesions supports the contention that significant dopaminergic activity can occur in the striatum despite the loss of a large proportion of dopamine receptors associated with striatal cholinergic and GABAergic neurons. Little is known regarding what neuronal populations these remaining dopamine receptors are associated with or what behavioral activity they may in part initiate. However, if in fact nigral-striatal dopamine neurons are hyperactive in Huntington's disease, it is reasonable to assume that neuroleptics might counteract this neurochemical imbalance by diminishing the access of dopamine to the remaining functional dopamine receptors in the striata of Huntington's disease patients.

In addition to the loss of neuroleptic receptors in the striata of Huntington's disease patients, there is also a decrease in the density of neuroleptic receptors in the frontal cortex of these patients. The frontal cortex of brains obtained from Huntington's disease patients exhibits profound atrophy and widespread neuronal death (Alzheimer, 1911; Bruyn, 1968). Despite these findings, no neurochemical markers had previously been observed to be altered in this brain area (Enna et al., 1976). The nature of the ^{3}H-spiroperidol binding site in the human frontal cortex is somewhat unclear. Leysen, Niemegeers, Tollenaere, and Laduron (1978) have proposed that rat cortical ^{3}H-spiroperidol binding sites are mainly serotonergic. However, Enna et al. (1976) found no alteration in ^{3}H-serotonin binding in the frontal

cortices of Huntington's disease patients. This finding contrasts with the over 70% reduction in ^{3}H-spiroperidol binding found in the present study. These results suggest that ^{3}H-spiroperidol does not significantly associate with serotonin receptors in the human frontal cortex. Thus, it is still unclear as to what is the endogenous substrate for these receptors and what if any importance the reduction of the density of these receptors might have regarding the pathophysiology of Huntington's disease. However, the neuropathology in the cerebral cortex has been suggested to have a role in the appearance of dementia in Huntington's disease patients (Klawans, 1970). Whether the loss of frontal cortical neuroleptic receptors as reported in the present study is associated in some manner with the occurrence of thought disorders is unknown.

Finally, the stability of dopamine receptors in the substantia nigra of Huntington's disease patients suggests that these receptors are not located on striatal-nigral neurons, since these neurons are mainly lost in Huntington's disease. Similarly, nigral dopamine receptors are not affected by intrastriatal kainic acid lesions (Reisine, Nagy, Fibiger, and Yamamura, 1979). Thus, the lack of any significant change in ^{3}H-spiroperidol binding in the substantia nigra of Huntington's disease patients as well as in a number of other brain regions of the Huntington's disease patients (Reisine, Fields, Stern, et al., 1977) suggests that those alterations detected in the striatum and frontal cortex of Huntington's disease patients were not the result of post-mortem artifacts, but instead results from the disease processes of this affliction.

Schizophrenia

The observation of abnormalities in the concentrations of various neurochemicals in the central nervous system of schizophrenics has led to the development of a variety of hypotheses concerning the biochemical basis of the symptoms inherent in this disease. The "dopamine hypothesis" of schizophrenia evolved from the finding that dopamimetics such as amphetamine induce schizophrenic-like symptoms whereas drugs such as reserpine which deplete brain dopamine or dopamine antagonists such as phenothiazines or butyrophenones alleviate the schizophrenic-like symptoms.(Snyder, 1975; Angrist et al., 1975; Janowsky and Davis, 1976; van Rossum, 1966; Carlsson and Lindqvist, 1963). These results have led some researchers to suggest that an overactive brain dopamine system is responsible in part for some of the clinical abnormalities associated with schizophrenia (Randrup and Munkvad, 1972; Matthysse, 1977; Hornykiewicz, 1977). In contrast, Lee, Seeman, Tourtellotte, Farley, and Hornykiewicz (1978); Owen et al. (1978); Reisine, Pedigo, et al. (1979), Reisine, Rossor, et al. (1979); and Crow et al. (1978) have demonstrated an increased density of striatal and limbic dopamine receptors in schizophrenic postmortem brain samples. The appearance of supersensitive dopamine receptors would suggest that underactivity rather than overactivity of dopaminergic neurons occurs in these brain regions, since both chemical lesions of the nigral-striatal dopamine pathway which depletes striatal dopamine levels or blockade of dopamine's access to its receptor sites by chronic neuroleptic therapy, elevate striatal dopamine receptor densities (Nagy et al., 1978; Reisine, Nagy, Fibiger, and Yamamura,

1979; Burt et al., 1976). These data, although conflicting, add support to the theory of the occurrence of some type of imbalance in brain dopaminergic mechanisms in schizophrenia.

A number of reports have also suggested that an elevation of endorphin levels or an imbalance in endorphin metabolism in the human central nervous system might be associated with the emergence of schizophrenic symptoms (De Wied, 1979; Terenius et al., 1976; Watson et al., 1978). Bloom et al. (1976) found that intracerebral administration of β-endorphin into rats produced a naloxone reversible catatonic state that appeared to have behavioral characteristics similar to those found in schizophrenia. Human clinical investigations have revealed an above normal level of endorphins in the cerebrospinal fluid of schizophrenic patients (Terenius et al., 1976). Since the concentration of a substance in the cerebrospinal fluid is a crude measure of its levels in the brain, these data would suggest that endorphin levels are elevated in schizophrenic brains. Treatment of some schizophrenic patients with the opiate antagonist naloxone alleviated their auditory hallucinations (Gunne, Lindstrom, and Terenius, 1977; Watson et al., 1978). Presumably, naloxone treatment caused these results by counteracting and thus normalizing an overactive endorphin system in the brains of schizophrenics. However, some controversy exists concerning the effectiveness of naloxone therapy in the treatment of schizophrenia, since Kurland et al. (1977) and Janowsky et al. (1977) could not observe any beneficial effects of naloxone therapy in the treatment of schizophrenia. Yet Watson et al. (1978) have suggested that the lack of effectiveness seen by these authors in the treatment of schizophrenia was due to the

relatively low doses of naloxone administered to their patients as well as the short period of time between drug administration and the time at which the authors looked for changes in schizophrenic symptoms in their patients. Recently, De Wied (1979), De Wied et al. (1977), and De Wied et al. (1978) have put forth the hypothesis that metabolites of β-endorphin, such as des-tyrosine-gamma-endorphin (DTγE) alleviate psychotic symptoms in much the same manner as the neuroleptic haloperidol. In support of this theory, they demonstrated that intravenous administration of DTγE diminished some schizophrenic symptoms in a number of psychotic patients (Verhoeven et al., 1979). These findings are highly suggestive of an alteration in the normal activity of endorphins in the nervous systems of schizophrenics.

A mechanism by which opiates might induce or potentiate psychotic symptoms is by affecting the activity of dopaminergic neurons in the brain. Recently, evidence has been presented showing a direct interaction of enkephalinergic and dopaminergic systems in the brain (Eidelberg, 1976; Lal, 1978). Thus, opiates inhibit the potassium-evoked release of striatal dopamine by a mechanism that is naloxone reversible (Celsens and Kusehinsky, 1974; Loh et al., 1976). Also, lesion and neurotransmitter receptor binding studies have demonstrated the presence of opiate receptors located on dopamine nerve terminals in both the corpus striatum and mesolimbic regions (Carenzi, Frigeni, and Della Bella, 1978; Pollard, Llorens, and Schwartz, 1977; Reisine, Nagy, Beaumont, et al., 1979). Since the levels of dopamine and the dopamine metabolite homovanillic acid are slightly, but significantly, altered and dopaminergic receptor density is dramatically elevated in the

caudate nucleus of postmortem brain samples obtained from schizophrenic patients, it is conceivable that these neurochemical changes could directly or indirectly be the result of increases in brain endorphin concentrations in schizophrenic nervous tissue (Crow et al., 1978; Lee, Seeman, Tourtellotte, Farley, and Hornykiewicz, 1978; Owen et al., 1978).

The alterations in ^{3}H-spiroperidol and ^{3}H-naloxone binding observed in the present study in the neostriatum of postmortem schizophrenic tissue could be the result of the neuroleptic therapy or inherent biochemical abnormalities in the neostriatum of schizophrenic brains. Several studies have demonstrated that chronic administration of neuroleptics to rats increases the density of striatal dopamine receptors (Burt et al., 1976). However, neuroleptics are also known to interact in <u>in vitro</u> experiments with alpha-adrenergic and serotonin (Leysen, Niemegeers, Tollenaere, and Laduron, 1978) receptors. Yet ^{3}H-WB-4101 (an alpha-adrenergic receptor antagonist) binding was unaltered in the schizophrenic brain tissue in this study. Also, ^{3}H-spiroperidol binding and ^{3}H-serotonin binding (Bennett et al., 1979) in the frontal cortex of brain tissues obtained from schizophrenic patients that had received neuroleptic therapy was similar to control values. In addition, Owen et al. (1978) demonstrated an increase in striatal ^{3}H-spiroperidol binding in brains from schizophrenic patients who had not been on neuroleptic therapy for over one year prior to death. In the present study, ^{3}H-spiroperidol binding was also significantly elevated (150% of control values) in the caudate nuclei obtained from the brains of the three schizophrenic patients that were not receiving neuroleptic

drugs. These data suggest that the neuroleptic therapy was not the primary cause for the increase in striatal ^{3}H-spiroperidol binding in the brain tissue obtained from schizophrenic patients.

Long-term morphine administration to rats enhances dopaminergic receptor activity. Thus, chronic morphine treatment increases apomorphine's (a dopamine agonist) potency in inducing agression (Puri and Lal, 1973), jumping, and stereotype behaviors (Cox, Ary, and Lomax, 1976; Herz, Blasig, and Papeschi, 1974). Also, both morphine and β-endorphin inhibit the potassium-evoked release of rat striatal dopamine (Celsens and Kusehinsky, 1974; Loh et al., 1976). Decreased release presumably lowers the concentration of dopamine at the synapse thereby inducing receptor supersensitivity. The abnormally high levels of endorphins in cerebrospinal fluid samples obtained from schizophrenic patients (Terenius et al., 1976) and ability of naloxone to alleviate auditory hallucination in schizophrenics (Gunne et al., 1977; Watson et al., 1978) suggests that brain endorphin concentrations may be elevated in schizophrenic brains. High levels of endorphins in the striatum of schizophrenic patients could conceivably lower striatal dopamine release and increase dopamine receptor density. At the same time, the increased levels of striatal endorphins could possibly induce opiate receptor desensitization in the caudate nucleus of schizophrenic brains. Interestingly, only in the caudate nucleus did we find a loss of opiate receptors in the schizophrenic brains. Owen et al. (1978) also could only detect an increase in dopamine levels and a decrease in homovanillic acid levels in the caudate nucleus and not in the putamen or nucleus accumbens. These data support our hypothesis that

the possible presence of abnormally high levels of endorphins in the caudate nucleus of schizophrenic brains could lower dopamine release, elevate dopamine receptor density, and desensitize opiate receptors in this brain region.

The opiate receptor loss in the caudate nucleus of schizophrenic brains could have been promoted by the neuroleptic therapy. However, the lack of opiate receptor alterations in either the putamen or frontal cortex (areas rich in ^{3}H-naloxone binding) of schizophrenic brains argues against this notion and suggests that a specific abnormality in brain opiate mechanisms occurs in the caudate nucleus of schizophrenic nervous tissue.

The primary sites of opiate effects on behavior are not necesarily limited to the striatum as demonstrated by morphine's ability to induce catalepsy despite the destruction of the striatum by electrolytic lesions (Koffer, Berney, and Hornykiewicz, 1978). Thus, alterations in brain opiate mechanisms could also be localized to non-striatal brain regions, such as the limbic system. Therefore, more extensive studies in a greater number of regions of schizophrenic brains may further elucidate the biochemical abnormalities associated with the schizophrenic central nervous system.

Recently, it has been proposed that an analogue of gamma-endorphin, DTγE might be an endogenous neuroleptic (De Wied et al., 1978; De Wied, 1979). An interesting aspect of this compound is that despite its similarity in structure to various endogenous opiates (except for the loss of the N-terminal tyrosine moiety), it is devoid of opiate activity (De Wied et al., 1978). However, from behavioral

studies, the administration of DTYE appears to result in similar behavioral responses as elicited by haloperidol (De Wied et al., 1978). In contrast, DTYE does not compete with ^{3}H-haloperidol or ^{3}H-spiroperidol for the same receptor in in vitro binding experiments (Pedigo et al., 1979; van Ree, Witter, and Leysen, 1978). It has been suggested, however, that DTYE must be metabolized in the body to substances which in turn would be active at neuroleptic receptors in the brain (Pedigo et al., 1979). Therefore, it is possible that in regions of the brain such as the caudate nucleus, an inhibition of the normal metabolism of endorphins occurs which would adversely affect behavioral activities dependent on normal dopaminergic or opiate neural transmission. Such a neurochemical alteration may be responsible for initiating psychotic symptoms.

Conclusion

The studies reported here suggest that spiroperidol acts specifically at dopamine receptors in the corpus striatum and substantia nigra and is a useful ligand both for the in vitro and in vivo measurement of brain neuroleptic receptors.

Lesion studies investigating the location of dopamine receptors in the basal ganglia indicate that dopamine receptors are predominately postsynaptic to dopamine terminals in the corpus striatum. In the substantia nigra, dopamine receptors are localized to dopamine perikarya and dendrites, but not to the terminals of striatal-nigral afferents.

In Parkinson's disease, the caudate nucleus has a lower density of dopamine receptors than similar tissue obtained from controls. This dopamine receptor desensitization could be associated with the excessive amounts of dopamine being formed in the striatum of Parkinson's disease patients receiving L-DOPA therapy. The loss of dopamine receptors in the caudate nucleus could be a causative factor for the gradual loss of effectiveness of L-DOPA therapy in these patients. The presence of other neurochemical abnormalities in the brains of Parkinson patients suggests that alterations in non-dopaminergic neurotransmitter systems may also contribute to the appearance of the symptoms of this disorder.

In Huntington's disease, there is a loss of neuroleptic receptors in both the corpus striatum and frontal cortex. The alterations found in the corpus striatum may in part be related to the appearance of choreiform movements in this disease. The neuroleptic receptor changes found in the Huntington's disease frontal cortex might be associated in some manner with the dementia present in all Huntington's disease patients.

The observation of supersensitive neostriatal dopamine receptors in brain tissue from schizophrenic patients suggests that either dopaminergic transmission in this brain region is reduced compared to normal tissue or that the neurons on which these receptors are located are defective in some manner. Since brain opiates can diminish dopamine release in the striatum, it is conceivable that overactivity of striatal opiate containing neurons may release above normal levels of opiates into the corpus striatum which in turn could cause the decreased release of dopamine in the striatum. Lowered release of dopamine might reduce

the availability of dopamine at its receptor sites thereby inducing various cellular processes that might increase the density of dopamine receptors in this region of schizophrenic brains. The presence of lowered levels of opiate receptors in the caudate nucleus of schizophrenic brains supports this hypothesis since elevated concentrations of brain opiates in the vicinity of opiate receptors in the striatum might be expected to promote the desensitizatin of these receptors.

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