Co-sensitization of Dopamine and Serotonin Receptors Occurs in the Absence of a Change in the Dopamine D1 Receptor Complex After a Neonatal 6-ohda Lesion

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Co-sensitization of dopamine and serotonin receptors occurs in the absence of a change in the dopamine D1 receptor complex after a neonatal 6-OHDA lesion

Gong, Li, Ph.D.

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CO-SENSITIZATION OF DOPAMINE AND SEROTONIN RECEPTORS OCCURS IN THE ABSENCE OF A CHANGE IN THE DOPAMINE D1 RECEPTOR COMPLEX AFTER A NEONATAL 6-OHDA LESION

A Dissertation Presented to
The Faculty of the Department of Pharmacology
James H. Quillen College of Medicine
East Tennessee State University

In Partial Fulfillment of the Requirements for the Degree
Doctor of Philosophy in Biomedical Science

by
Li Gong
December 1993
APPROVAL

This is to certify that the Graduate Committee of

LI GONG

met on the

20th day of August, 1993.

The committee read and examined his dissertation, supervised his defense of it in an oral examination, and decided to recommend that his study be submitted to the Graduate Council and the Associate Vice-President for Research and Dean of the Graduate School, in partial fulfillment of the requirements for the degree Doctor of Philosophy in Biomedical Science.

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Associate Vice-President for Research and Dean of the Graduate School
ABSTRACT

CO-SENSITIZATION OF DOPAMINE AND SEROTONIN RECEPTORS OCCURS IN THE ABSENCE OF A CHANGE IN THE DOPAMINE D_{1} RECEPTOR COMPLEX AFTER A NEONATAL 6-OHDA LESION

by

Li Gong

To test whether SKF 38393 could ontogenetically sensitize dopamine (DA) D_{1} receptors and whether this sensitization would be associated with biochemical changes, intact and neonatal 6-hydroxydopamine (6-OHDA)-lesioned rats (200 μg i.c.v.) were treated daily from birth with SKF 38393 (3.0 mg/kg i.p. x 28 days) or its vehicle. In DA D_{1} neonatally sensitized 6-OHDA rats, enhanced locomotor responses were observed with the first SKF 38393 challenge dose (3.0 mg/kg i.p.) at 6 weeks. This response increased further with weekly SKF 38393 treatments. Enhanced stereotyped behaviors were seen in both lesioned and sensitized rats at 8 weeks. There was no change in the percentage of high affinity D_{1} sites in these groups of rats. Striatal mRNA levels for D_{1} receptors were reduced in the lesioned rats, but restored to control level after treatments with SKF 38393 in adulthood. Basal, DA-, NaF- and forskolin-stimulated adenylate cyclase activities were similar among treatment groups. Striatal DA content was reduced (>99%), whereas serotonin (5-HT) content was elevated (>50%) in the 6-OHDA groups. To study possible interaction between DA and 5-HT systems, the effects of a series of 5-HT agents on the induction of oral activity were determined. The 5-HT_{1C} receptor agonist, m-chlorophenylpiperazine (m-CPP), produced a marked increase in oral activity in 6-OHDA-lesioned rats. The respective 5-HT_{1A} and 5-HT_{1B} agonists, 8-OH-DPAT and CGS-12066B did not increase oral activity. The m-CPP-induced oral response in the lesioned rats was attenuated by mianserin, a 5-HT_{1C} antagonist, but not by ketanserin or MDL-72222, 5-HT_{2} and 5-HT_{3} antagonists, respectively. Although the supersensitized oral response of lesioned rats to m-CPP was not attenuated by SCH 23390, the enhanced response of SKF 38393 was attenuated by mianserin. Additionally, mRNA levels for 5-HT_{1C} receptor were not altered in both intact and lesioned rats. These findings demonstrate that ontogenetic treatments of neonatal 6-OHDA-lesioned rats with a D_{1} agonist produce partial sensitization of DA D_{1} receptors in adulthood without altered biochemical markers, and that this neonatal lesion is associated with both supersensitized DA D_{1} and 5-HT_{1C} receptors. Moreover, induction of oral activity by DA agonists is mediated via a serotonergic system.
DEDICATION

To my parents for their love and encouragement.
ACKNOWLEDGMENTS

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LIST OF PHARMACOLOGICAL AGENTS

SKF 38393 ------ DA D₁ agonist
SCH 23390 ------ DA D₁ antagonist
m-CPP -------- 5-HT₁C₂ agonist
8-OH-DPAT ------ 5-HT₁A agonist
CGS 12066B ------ 5-HT₁B agonist
Mianserin ------ 5-HT₁C₂ antagonist
Ketanserin ------ 5-HT₂ antagonist
MDL 72222 ------ 5-HT₃ antagonist
6-OHDA -------- catecholamine neurotoxin
Desipramine ----- noradrenergic reuptake blocker
Chapter 1
Introduction

**Anatomical Review of Dopamine System**

Dopamine (DA), a veritable newcomer to the field of monoamine transmitters in mammalian central nervous system (CNS), belongs to a class of compounds termed catecholamines (Montagu, 1957). Until the mid-1950s DA was simply considered to represent an intermediate precursor in the biosynthesis of norepinephrine and epinephrine. Because the concentration of DA is almost equal to those of norepinephrine in the brain, and because the regional distribution of these two catecholamines is markedly different, it was proposed that the biological role for DA in CNS is independent of its function as a precursor in norepinephrine synthesis (Carlsson et al., 1958; 1959). With the development of histochemical and immunocytochemical methods for visualizing catecholamines in brain tissue sections (Dahlstrom and Fuxe, 1964; Fuxe et al., 1978), it is now convenient to localize dopaminergic pathways under several major categories as follows:

1. **Nigrostriatal DA system.** A group of cell bodies, designated the A9 group, are located primarily in the substantia nigra pars compacta. These neurons project mainly into neostriatum (principally the caudate and putamen), globus pallidus and
subthalamic nucleus.

2. *Mesolimbic DA system*. One group of DA-containing neurons, known as the A10 group, originates in the ventral tegmental area and terminates in the nucleus accumbens, olfactory tubercle and nucleus interstitialis stria terminalis.

3. *Mesocortical DA system*. Neuronal cell bodies are also located in the ventral tegmental area (A10 group), but their projections end in the septum, prefrontal cortex, cingulate, hippocampus and amygdala.

4. *Mesothalamic DA system*. Another group of cell bodies originates in the ventral tegmental area (A10 group) and terminates in the medial and lateral habenula.

5. *Tuberoinfundibular and tuberohypophyseal DA system*. A system consisting of both short and intermediate-length neurons, designated the A12 group, arises from the arcuate and periarcurate nucleus and the ventral periventricular hypothalamic nucleus, and terminates in the median eminence, infundibular stalk, intermediate and neural lobes of the pituitary.

**Ontogeny of Dopamine System**

Neuron production in the rat is largely prenatal
(Brasel et al., 1970). The features of the postnatal developmental process are mainly related to events involving neuronal growth, axonal outgrowth and the synaptic formation. The development of dopaminergic fiber systems in rats takes place in the first few postnatal weeks. Several striatal components including DA-containing nerve endings, the neurotransmitter DA and DA receptors show transformed patterns during development.

At birth, dopaminergic fibers can be visualized in distinct 'patches' revealed by histofluorescence and immunocytochemistry (Moon Edley and Herkenham, 1984; Voorn et al., 1988). Soon after birth, denser appearance of DA terminals in a 'matrix' pattern in the striatum can be discerned (Gerfen et al., 1987). By 3-4 weeks after birth, the striatal pattern of DA fiber innervation continues to mature and appears to reach an adult-like alignment (Moon Edley and Herkenham, 1984; Voorn et al., 1988).

Similarly, DA content in the developing brain exhibits the same picture (Loizou and Salt, 1970). At the first day after birth, the striatum contains only about 12% of the adult level of DA (Giorgi et al., 1987). The striatal DA level increases slowly thereafter, reaching adult levels 7-8 weeks after birth and remaining constant throughout adulthood (Breese and Traylor, 1972; Giorgi et al., 1987; Broaddus and Bennett, 1990).

Ontogenetic studies with neonatal rats demonstrated
that a first-week postnatal striatum lacks spontaneous neuronal activity (Cheronis et al., 1979; Napier et al., 1985). Striatal neuronal activity can be recorded from 17 day old pups. As the variety of striatal cell firing patterns increased with age, 28 day old rats have been observed to have similar patterns often seen in adult striatum (Napier et al., 1985).

The above mentioned findings indicate that developmental changes occur in nigrostriatal dopaminergic projections and their connections especially during the first 8 weeks after birth.

**Proposed Functions of Dopamine System**

Based on anatomical, biochemical, behavioral and pharmacological characteristics, there is compelling evidence that DA serves as both a neurotransmitter and a modulator in the brain. The physiological role of the central dopaminergic system has been proposed as follows:

1. Behavioral modulation. A variety of behaviors are found to be regulated by the dopaminergic system. In general, the nigrostriatal pathway may largely modulate motor behavior (Ungerstedt and Arbuthnott, 1970; Ungerstedt, 1971a,b), as evidenced by characteristic motor disorder associated with its degeneration in Parkinson’s disease (Hornykiewicz, 1966). Numerous studies have suggested that
mesolimbic and mesocortical pathways regulate not only motor function, but mainly emotional and reward behavior, as well as cortical and subcortical functions, i.e. stereotyped behaviors (Creese and Iversen, 1973), self-stimulation behavior (Phillips and Fibiger, 1973), stimulus control behavior (Ho and Huang, 1975) and locomotion (Pijnenburg et al., 1976). Dysfunction of mesolimbic dopaminergic pathway has been hypothesized to underlie the etiology of schizophrenia and depression (Stevens, 1973).

2. Neuroendocrine function. The tuberinfundibular DA system regulates secretion of prolactin and gonadotropin releasing hormone, and inhibits release of somatostatin and thyrotropin releasing hormone.

Dopamine Receptors

Ample evidence has been provided that there are multiple receptors for DA in the CNS, on the basis of pharmacological, biochemical, anatomical and physiological features (Kebabian and Calne, 1979; Creese and Leff, 1982; Creese et al., 1983; Stoof and Kebabian, 1984). Classically, upon agonist stimulation, DA D₁ receptors are associated with activation of adenylate cyclase (AC) (Kebabian and Calne, 1979; Seeman, 1980). DA D₂ receptors, on the other hand, are either unlinked to stimulation of AC,
or have an inhibitory effect on the enzyme (Memo et al., 1986; Onali et al., 1984; 1985).

Characterization and Localization of Dopamine D₃ Receptors

Pharmacological studies, performed with radioligand binding to discriminate between these two receptor categories, have aided substantially in characterizing DA receptor populations. D₃ receptors have been characterized by using [³H]-cis-(Z)-flupenthixol (Hyttel, 1978; Murrin, 1983) and [³H]-cis-(Z)-piflutixol (Hyttel, 1981). However, both radioligands produce similar affinities for D₁ and D₃ receptors and cause a high degree of nonspecific binding.

SCH 23390 [R-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepine-7-ol] was later introduced as the first highly selective D₃ receptor antagonist and designated as the most suitable ligand for D₃ receptors (Hyttel, 1983; Iorio et al., 1983; Billard et al., 1984; Schulz et al., 1984; Andersen et al., 1985). In vitro, SCH 23390 potently blocks DA-stimulated adenylate cyclase (Iorio et al., 1983) and selectively displaces [³H]-piflutixol binding from striatal slices (Hyttel, 1983). However, in the [³H]-spiperone binding assay (a ligand for D₂ receptor), SCH 23390 displays a relatively low affinity at the micromolar range and is about 460 times weaker than other neuroleptics such as haloperidol. In vivo, this compound is a potent and selective antagonist of the D₃ receptor, with little
affinity toward D₂ receptors in the CNS (Andersen and Groenvald, 1986; Andersen and Nielsen, 1986).

Regional distribution and density of DA D₁ receptors in rat brain have been described by using autoradiographic studies with [³H] SCH23390. The highest density of D₁ receptors was found in the caudate-putamen, nucleus accumbens, olfactory tubercle and the substantia nigra par compacta or pars reticulata (Dawson et al., 1986; Savasta et al., 1986). High densities was also observed in amygdala, nucleus interstitialis stria terminalis, entopeduncular nucleus and island of Calleja. Moderate to low numbers of D₁ sites was found in cerebral cortex, several thalamic, hypothalamic and hippocampal areas, the habenula, the ventral tegmental area, the cingulate and the cerebellum. The topographical distribution of D₁ receptors in the striatum was much greater in the ventrolateral and medial margin subregion than in the ventromedial and dorsolateral sectors, and a rostrocaudal decrease in the densities of D₁ receptors was also noticed (Savasta et al., 1986).

The regional localization of mRNA for D₁ receptors has been determined by northern blot and by in situ hybridization histochemistry. In general, the areas of highest expression of D₁ receptors are the caudate-putamen, nucleus accumbens and olfactory tubercle. D₁ receptor mRNA is also observed in the cerebral cortex, limbic system, hypothalamus and thalamus (Fremeau et al., 1991; Sibley and
Striatal DA D<sub>1</sub> receptors have been shown to mediate the DA-stimulated increase of adenylate cyclase (AC) activity (Kebabian and Caine, 1979). In membrane preparations, the stimulation of AC activity can also be elicited by GTP and forskolin, which specifically act on stimulatory guanine nucleotide regulatory protein (N<sub>s</sub>) or catalytic subunit of the enzyme, respectively, rather than the receptor site (Rodbell, 1980; Seamon et al., 1981). DA, GTP and forskolin thus have been widely used as biochemical markers in adenylate cyclase assays to determine the functional integrity of each subunit in DA D<sub>1</sub> receptor complex system, and to investigate D<sub>1</sub> receptor/effecter mechanisms.

There are also growing lines of evidence that stimulation of DA receptors leads to changes in phosphoinositide metabolism. An early study by Hokin (1970) showed variable effects of DA on incorporation of 32P into phosphatidate and phosphatidylinositol in slices of the guinea pig brain. Later, both stimulatory and inhibitory effects of DA on phosphoinositide labeling were found (Abdel-Latif et al., 1974; Odarjuk et al., 1986). Undie and Friedman (1990) demonstrated that the stimulation of DA D<sub>1</sub> receptors enhances inositol phosphate formation in rat brain. By injecting mRNA from rat striatum into oocytes of
Xenopus laevis, Mahan et al. (1990) also showed the expression of striatal D₁ receptors coupled to inositol phosphate production.

**Dynamics of Dopamine D₁ Receptors**

The binding of antagonists to DA D₁ receptors show mass action characteristics, whereas agonist binding displays two separate states of high and low affinity (Hamblin et al., 1984; Leff et al., 1985). This heterogeneity was demonstrated by shallow two-component curves of agonist competition for radiolabeled antagonist binding. In the presence of guanine nucleotides, the high affinity agonist-receptor complex is converted to low affinity agonist binding states of the receptors (Hamblin et al., 1984; Leff et al., 1985; Hess et al., 1986). These studies have proposed a generalized ternary complex model to explain these phenomena, which has been modified from the original models of Boeynaems and Dumont (1975) and Jacobs and Cuatrecasas (1976), and proposed for several other monoaminergic receptors (Limbird, 1981).

**Ontogeny of Dopamine D₁ Receptors**

Dopaminergic innervation of the striatum plays an important role in regulating the development of neurons intrinsic to the striatum (Rosengarten and Friedhoff, 1979). There is increasing evidence that the ontogeny of DA
receptors and their mediated responses are dependent upon the maturation of their corresponding presynaptic nerve terminals. DA D_{1} receptors are at low levels at birth (9% of the adult value) and increase in number thereafter (Giorgi et al., 1987; Zeng et al., 1988), attaining a peak value at postnatal day 35 (Giorgi et al., 1987). There were no alterations in affinity (K_{d}) of [3H]piflutixol and [3H]SCH 23390 for D_{1} receptors during development (Zeng et al., 1988; Gelbard et al., 1989; Broaddus and Bennett, 1990). Giorgi et al. (1987) showed that maximum DA-stimulated adenylate cyclase (AC) activity in striatum has a developmental profile similar to DA D_{1} receptors, suggesting that the postnatal increase in AC activity is attributable to the increase in stimulatory receptor density. The ontogeny of D_{1} receptors parallels those seen with several other markers associated with presynaptic dopaminergic terminals in striatum, including DA content, DA uptake (Coyle and Campochiaro, 1976), and tyrosine hydroxylase activity (Coyle and Axelrod, 1972).

**Molecular Biology of Dopamine Receptors**

With the molecular characterization of DA receptors being facilitated by the cloning of cDNAs and/or genes, two subtypes of DA D_{1} receptors and four subtypes of D_{2} receptors have already been identified. The D_{1} receptor of human and rats in CNS has been cloned, expressed and characterized
(Zhou et al., 1990; Dearry et al., 1990), while its subtype, termed D₃, has recently been identified and cloned as well. The D₃ receptor bears resemblance to the cloned D₁ receptor. But pharmacologically, the D₃ receptor has a 10-fold higher affinity for the endogenous agonist, DA (Sunahara et al., 1991).

Two forms of the DA D₂ receptor, D₂ short and D₂ long, were identified by gene cloning and found to be derived from alternative splicing of a common gene (Dal Toso et al., 1989; Giros et al., 1989). A third subtype of the D₂ receptor, termed D₃ receptor and characterized by its different anatomical distribution and pharmacological profile, was isolated by the screening of rat brain cDNA and genomic libraries (Sokoloff et al., 1990; Bouthenet et al., 1991). The fourth subtype of the D₂ receptor, the D₄ receptor, has been recently cloned. Its pharmacological profile is similar to that of the D₂ and D₃ receptors. The affinity of the D₄ receptor for the atypical antipsychotic drug, clozapine, however, is an order of magnitude higher than for D₂ and D₃ receptors (Van Tol et al., 1991).

A better understanding of which DA receptor subtype mediates particular physiological effects would greatly facilitate the development of more specific dopaminergic agents and provide insight into the mechanisms of disorders thought to be related to the malfunctioning of the DA system.
Innervation of Serotonin Fibers to the Striatum

The existence of serotonin (5-HT) innervation in mammalian neostriatum was first shown by regional biochemical examination of this monoamine, and by activity of its biosynthetic enzyme, tryptophan hydroxylase in the dog and cat brain (Bogdanski et al., 1957; Peters et al., 1968). Fluorescent histochemical and autoradiographic investigations further provided evidence of this 5-HT innervation in rat striatum (Arluison and Delamanche, 1980; van der Kooy and Hattori, 1980; Parent et al., 1981). The distributional characteristic of the 5-HT innervation in adult rat striatum demonstrated a marked heterogeneous pattern (Ternaux et al., 1977; Steinbusch, 1981). The neostriatal 5-HT fiber network has a higher density in ventral vs. dorsal parts, and a caudo-rostral decreasing density gradient was also found (Steinbusch, 1981; Soghomonian et al., 1987). This topographic distribution was consistent with regional biochemical measurements, demonstrating that higher levels of intrastriatal 5-HT content or $[^3H]5$HT uptake is noted in the ventral vs. dorsal, and the caudal vs. rostral parts of adult striatum (Beal and Martin, 1985; Widmann and Sperk, 1986). Although the heterogeneous distribution of the striatal 5-HT innervation remains of obscure significance, a complementary striatal pattern of the distribution of DA and 5-HT terminals has been found in the rostral to caudal, and
dorsal to ventral axes (Tassin et al., 1976; Doucet et al., 1986). On the other hand, the striatal 5-HT innervation density is much less than that of DA fiber innervation (Doucet et al., 1986). A critical balance between dopaminergic and serotonergic neurons has been postulated to underlie normal motor activity, based on biochemical and lesion studies (Sourkes and Poirier, 1968).

Lidov and Molliver (1982) reported that 5-HT innervation in neonatal rats does not show a mosaic pattern of patches and matrix organization, and the major development of this innervation takes place 12-15 days after birth. This is in contrast with the DA innervation in striatum, where at the earlier postnatal stage, the prominence of DA islands is found (Olson et al., 1972; Voorn et al., 1988).

**Serotonin Receptors in Striatum**

Based on biochemical, pharmacological and behavioral studies, at least seven distinct subtypes of 5-HT receptors are currently identified in mammalian central nervous system (Frazer et al., 1990; Peroutka et al., 1990; Glennon and Dukat, 1991). Many subtypes of this receptor family have now been cloned (Julius et al., 1988; Pritchett et al., 1988; Albert et al., 1990; Hamblin and Metcalf, 1991; Maricq et al., 1991; Voight et al., 1991; Weinshank et al., 1992). 5-HT₁ group can be divided into four classes (labeled 5-HT₁A
through 5-HT$_{1D}$), while the three other groups are designated 5-HT$_2$, 5-HT$_3$, and 5-HT$_4$.

There is a relative lack of 5-HT$_{1A}$ receptor in rat striatum (Marcinkiewicz et al., 1984; Verge et al., 1986), recently confirmed by immunocytochemistry and in situ hybridization of the mRNA (El Mestikawy et al., 1991; Riad et al., 1991; Chalmers and Watson, 1991; Miquel et al., 1991). Autoradiographic studies show that the 5-HT$_{1B}$ receptor subtype occurs in rat striatum (Pazos and Palacios, 1985). Destruction of 5-HT innervation to striatum with the 5-HT neurotoxin, 5,7-dihydroxytryptamine (5,7-DHT), results in increased striatal $[^{125}\text{I}]$cyanopindolol binding, suggesting postsynaptic 5-HT$_{1B}$ sites in the striatum (Offord et al., 1988; Palacios and Dietl, 1988). 5-HT$_{1D}$ receptors have been identified in the brain of rat, guinea pig and other species (Herrick-Davis and Titeler, 1988; Herrick-Davis et al., 1989; Waeber et al., 1990). Quinalinic acid lesion of striatal neuronal bodies induces a decrease of 5-HT$_{1D}$ receptors in substantia nigra, globus pallidus and striatum, suggesting that their possible location either on cell bodies, dendrites or terminals of interneurons (Waeber et al., 1990). In the striatum, 5-HT$_2$ receptors have an intermediate density, as determined by autoradiographic studies (Pazos et al., 1985a; Palacios and Dietl, 1988). Lesion of 5-HT system by 5,7-DHT did not reduce the number of 5-HT$_2$ sites in rats (Fischette et al., 1987). Therefore,
it is likely that the 5-HT₁ receptors are located on non-5-HT neurons.

The 5-HT₁c receptors were initially found to be present at high density in the choroid plexus (Pazos et al., 1985b). Since the pharmacological characteristics of this receptor subtype are similar to those of 5-HT₂ receptors, it was suggested that they might be in the same receptor family (Hoyer et al., 1988). Autoradiographic studies demonstrated that 5-HT₁c receptors are present at low levels in many areas of the brain. But studies of the distribution of the mRNA encoding for 5-HT₁c receptors confirmed and also extended these results (Hoffman and Mezey 1989; Molineaux et al., 1989; Mengod et al., 1990), indicating that mRNA and binding sites for this particular 5-HT receptor subtype are heterogeneously distributed in the rodent brain including striatum. It was proposed that 5-HT₁c receptors could be involved in the regulation of many different brain functions (Julius et al., 1988; Hoffman and Mezey, 1989; Mengod et al., 1990). The 5-HT₁c receptors have been studied more extensively in choroid plexus than in any other brain area. They appear to be located on epithelial cells where they regulate the production and composition of cerebrospinal fluid (Pazos and Palacios, 1985). Since lesion of the dopaminergic system did not alter 5-HT₁c binding in this area, it was suggested that this receptor subtype is not located on dopaminergic neurons (Palacios and Dietl, 1988).
Supersensitization of 5-HT$_1$c receptors produced by 5,7-DHT lesion, suggested that these receptors are present on non-serotonergic neurons (Conn et al., 1987). The postnatal development of 5-HT$_1$c receptors has been studied in rat brain choroid plexus, although little is known about their ontogeny in other brain regions. The number of binding sites increases markedly between day 3 and 20 after birth (Zilles et al., 1986), and these receptors appear to develop earlier than other 5-HT$_1$ receptor subtypes. Accompanying a 2-fold increase in 5-HT$_1$c binding sites in rat brain between day 17 and 27 after birth, there is a 5-fold increase in corresponding mRNA levels (Roth et al., 1991). The mechanisms responsible for regulating 5-HT$_1$c receptors by exogenous or endogenous compounds are not currently clear (Roth et al., 1990).

5-HT$_4$ receptors have been found mainly in brain postrema and other brain areas including cortex, amygdala and hippocampus, rather than striatum (Kilpatrick et al., 1987; Barnes et al., 1990). When compared to other serotonergic receptors, 5-HT$_4$ receptors have a particularly low density in the brain. 5-HT$_4$ receptors might belong to another functionally important 5-HT receptor family in the CNS. So far, the recognition sites for these receptors have not been identified because they have been hampered by the absence of a high affinity radioligand (Bockaert et al., 1992).
6-Hydroxydopamine and Dopamine Receptor Supersensitivity

6-hydroxydopamine (6-OHDA), a neurotoxin, selectively destroys brain catecholamine-containing neurons and their terminals (Breese and Traylor, 1970; Uretsky and Iversen, 1970; Kostrzewa and Jacobowitz, 1974). 6-OHDA provides a pharmacological means to explore central dopaminergic function in adult (Ungerstedt and Arbuthnott, 1970; Kelly et al., 1975; Creese et al., 1977) and developing rats (Breese et al., 1984; 1985a; 1985b). In many brain regions, the reduction or depletion of a dopaminergic afferent input has been shown to result in a functional postsynaptic receptor supersensitivity. Lesions of nigrostriatal pathway in adult rats produces a motor dysfunction (Breese and Traylor, 1970; Ungerstedt, 1971c; Zigmond and Stricker, 1973) and behavioral supersensitivity (Ungerstedt, 1971a,b), which is accompanied by an increase in the density of DA D1 receptors in the denervated striatum (Creese et al., 1977; Staunton et al., 1981).

Although neonatal lesioned rats possess the capacity to develop apparently normal motor and ingestive behaviors in the virtual absence of the emerging DA system (Erinoff et al., 1979; Pearson et al., 1980; Bruno et al., 1986), the enhanced behavioral responsiveness towards DA D1 and D2 agonists and antagonists has also been well documented after lesion with the toxin (Breese et al., 1984; 1985; 1987; Duncan et al., 1987). In a series of studies, Breese and
co-workers (1985) demonstrated that the sensitivity of neonatal 6-OHDA lesioned rats is often enhanced, with the animals becoming particularly sensitive to DA D$_1$ agonists. This enhanced behavioral responsiveness was not accompanied by a change in density ($B_{max}$) and affinity for either D$_1$ or D$_2$ receptors in the striatum (Breese et al., 1987; Luthman et al., 1990), suggesting that the enhanced response to DA agonists in neonatal 6-OHDA-lesioned rats could be reflecting a change in the sensitivity of secondary and tertiary messenger systems linked to the DA recognition sites; or an alteration in non-dopaminergic neural mechanisms which influence the response to DA receptor stimulation.

Furthermore, results from the same laboratory showed that repeated treatment of neonatal 6-OHDA-lesioned animals with SKF 38393, a specific D$_1$ receptor agonist (Setler et al., 1978), led to a progressive increase in locomotor activity and several other behaviors in adulthood, a phenomenon referred to as 'priming' (Breese et al., 1985b; Criswell et al., 1989). These actions of SKF 38393 were blocked by SCH 23390, a specific D$_1$ antagonist (Breese et al., 1985b; Criswell et al., 1989, 1990). Therefore, priming of D$_1$ receptor responsiveness in neonatal 6-OHDA-lesioned rats represents a model of a long-term alteration in neural function related to repeated activation of a receptor system.
Recently, Simson et al. (1992) found that behavioral supersensitivity in neonatally lesioned rats was not related to the alteration in the activation of adenylate cyclase, because no enhancement of cAMP production was observed with activation of DA D₁ receptors in lesioned animals. These investigators, however, suggested that differences of regionally enhanced electrophysiological sensitivity of striatal neurons to D₁ agonists and regional distribution pattern of tyrosine hydroxylase-like immunoreactivity and c-fos-like immunohistochemical expression within the striatum of neonatal lesioned rats, might contribute to the unique behavioral pattern seen in lesioned rats when they are challenged with DA D₁ agonists in adulthood (Johnson et al., 1992; Simson et al., 1992).

6-Hydroxydopamine and Serotonin System

Another feature of the 6-OHDA lesion in neonatal rats is a substantial increase in the adult striatal levels of 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) (Breese et al., 1984; Stachowiak et al., 1984). This lesion-induced elevation in 5-HT content is associated with a corresponding increase in [³H]5-HT uptake (Stachowiak et al., 1984) and with a proliferation of 5-HT containing fibers in the rostral portion of striatum (Berger et al., 1985; Snyder et al., 1986). The same 6-OHDA denervation of striatum associated with 5-HT hyperinnervation has also been
observed in adult lesioned rats and thus it is proposed as heterotypic terminal outgrowth, in the sense that it involves excessive growth of a neuronal system different from that which was lesioned (Zhou et al., 1991).

Autoradiographic study by Radja et al. (1993) demonstrated that 5-HT$_{1B}$, 5-HT$_{1D}$, 5-HT$_{1AAB}$ (including 5-HT$_{1C}$ and 5-HT$_{1D}$), 5-HT$_2$ receptor subtypes were upregulated in adult rat brain after a neonatal 6-OHDA lesion to striatum that is associated with 5-HT hyperinnervation in that brain region. This upregulation of several 5-HT receptor subtypes was likely to represent increases in receptor number ($B_{max}$) rather than the affinity of the receptors ($K_a$) (Radja et al., 1993).

Little is known about the functional implications of such striatal 5-HT hyperinnervation and its corresponding 5-HT receptor subtype upregulation in lesioned animals. In other studies on neonatal 6-OHDA-lesioned rats it was reported that the sprouted 5-HT fibers did not seem to have an inhibitory control on endogenous 5-HT- and acetylcholine-containing neurons (Jackson et al., 1988). Towle et al. (1989) found that 5-HT hyperinnervation was not responsible for supersensitized locomotor and stereotyped responses to either a DA or 5-HT agonist in neonatally lesioned rats, although their emphasis was focused on 5-HT$_2$ receptors.

**Dopamine System and Animal Behavioral Models**

Denervation supersensitivity models have been widely
used to study central dopaminergic function, to elucidate mechanisms of action of DA related agents, and to develop models of neurological and psychiatric diseases. As measured by a variety of enhanced behavioral changes, the mechanism responsible for this phenomenon of denervation is supposedly associated with either receptor up-regulation or functional changes in second messenger systems with regard to these receptors. The best studied animal models are as follows.

1. Rotational model. This was first described by Ungerstedt (1971a), and was produced by injection of 6-OHDA into one substantia nigra of the rat. The resultant unilateral lesion of the nigrostriatal pathway leads to a permanent depletion of DA in the ipsilateral striatum. Behavioral supersensitivity (i.e. contralateral circling) is seen when the rat is challenged with a DA agonist. This increased sensitivity to agonist administration has been correlated with an increase in striatal DA D2 receptor density ipsilateral to the lesion (Creese et al., 1977; Mishra et al., 1980; Heikkila et al., 1981).

2. Stereotypy model. Tarsy and Baldessarini (1974) demonstrated that apomorphine-induced stereotyped behavior was increased in rats after prolonged pretreatment with the DA synthesis inhibitor, α-methyl-p-tyrosine. This was comparable to the effect after chronic administration of antipsychotic drugs, suggesting that this behavioral
stereotypy is probably specifically related to compensatory supersensitivity of DA receptors. This stereotypy includes sniffing, rearing, licking, gnawing, chewing and locomotion behaviors.

3. Oral movement model. Rats chronically treated with neuroleptics show an increased incidence of purposeless or vacuous chewing movements, when challenged by DA receptor agonists (Clow et al., 1979; Klawans et al., 1972; Waddington et al., 1980). This oral activity is similar to the oral-bucial-masticatory movements classically described in patients with tardive dyskinesia. The phenomenology of this oral activity in rats is defined as 'vacuous (or abortive or spontaneous) chewing, whereby what appear to be robust chewing sequences are manifested, but are not directed onto any evident physical material' (Waddington, 1990). In neonatal 6-OHDA-lesioned rats, the effects of a D₁ agonist or D₂ antagonist on oral responses are greatly potentiated, even though the Bₘₐₓ and Kₐ for striatal D₁ and D₂ receptor binding do not accompany the marked reduction in DA content (Kostrzewa and Hamdi, 1991). This indicates that the enhanced oral movements in these neonatally lesioned rats are due to supersensitized DA receptors.

4. Catalepsy model. It is one of the classic rodent models to induce a state of transient immobility in testing DA antagonist activity, which is characterized by placing the forepaw of the rat on a testing bar or on an inclined wire
mesh screen and measuring the cataleptic time on the bar or the screen (Costall and Olley, 1971, Kostrzewa and Kastin, 1993). This effect is mediated by blockade of striatal DA receptors (Costall and Olley, 1971; Koffer et al., 1978; Meller et al., 1985, Kostrzewa and Kastin, 1993).

**Brain Dopamine System and Clinical Diseases**

Many clinical diseases are known to be specifically related to malfunctioning of DA systems.

Parkinson's disease is a progressive neurodegenerative disorder of the basal ganglia. Although characterized pathologically by loss of pigmented neurons in the substantia nigra, the basic biochemistry of the disease was not known until the 1960s. It was found that there is a substantial loss of DA in the striatum (Hornykiewicz, 1966). It is clear now that the most striking degenerative loss of DA neurons occurs in the nigrostiatal system. Studies have also been carried out on the relationship between the sensitivity of postsynaptic DA receptors and the supply of available DA in this disease. Hornykiewicz (1975) reported that increased activity of dopaminergic neurons may occur in Parkinsonism as a compensation for the decrease of available DA at the receptor.

Altered function of mesolimbocortical DA system has been implicated in the pathophysiology of schizophrenia. The disease was once considered to be related to a relative
excess of central dopaminergic neuronal activity, in view of the conviction that antipsychotic agents act therapeutically through blockade of central DA transmission (Matthysse, 1973; Snyder, 1982). Increased brain dopaminergic activity in schizophrenia could occur through several mechanisms such as increased synthesis and release of DA or altered sensitivity of DA receptors (Lozonczy et al., 1987).

Tardive dyskinesia, characterized by involuntary mouth movements, tongue fibrillation and thrusting, occurs in some patients who have been taking neuroleptics for long periods. This motor disturbance is believed to result from compensatory supersensitivity of striatal DA receptors (Klawans, 1973). Gilles de la Tourette's syndrome is characterized by childhood onset of chronic motor tics and involuntary vocalization. The often dramatic response to treatment with haloperidol, a DA D₂ antagonist, the similarity of the movements to those seen in patient with L-DOPA intoxication and the disturbed decrease in DA metabolism (Snyder et al., 1970; Butler et al., 1979), have suggested that excessive DA transmission may have occurred in the syndrome. Likewise, changes in DA receptor binding and coupling have been reported in Huntington's disease (Reisine et al., 1977; Cross and Rossor, 1983; De Keyser et al., 1989), which is a genetically determined neurological disorder, and associated with a progression of involuntary choreiform movements, psychological change and dementia.
Lesch-Nyhan disease, which is associated with an inborn deficiency of the enzyme hypoxanthine-guanine phosphoribosyl transferase (Seegmiller et al., 1967) and characterized by choreoathetoid movement and hypertonicity (Lesch and Nyhan, 1964; Wilson et al., 1983), has been found to be related to reduced brain DA in children (Lloyd et al., 1981). Because treatment of neonatal rats with 6-OHDA resulted in increased susceptibility for self-mutilation behavior due to the destruction of dopaminergic neurons, this useful neurochemical model has been proposed for screening of a variety of pharmacological agents that could minimize the self-mutilation behavior observed in Lesch-Nyhan patients (Breese et al., 1984; Breese et al., 1985a,b).

5-HT<sub>c</sub> Receptor-Related Animal Behaviors and Clinical Implications

Recent evidence suggests that although 5-HT<sub>c</sub> receptors are at high density in the choroid plexus, they are widely distributed in the brain and that their activation may have numerous behavioral and physiological effects. They may thus be implicated in a number of CNS disorders (Julius et al., 1988; Molineaux et al., 1989; Mengod et al., 1990).

1. Locomotor effects: A clear role of 5-HT<sub>c</sub> in behavior has not yet been established due to the lack of a specific agonist for the receptor. However, motor effects were reported to be associated with the activation of 5-HT<sub>c</sub>
receptors. The piperazine agonists TFMPP \[1-(m-
trifluoromethylphenyl)piperazine\], m-CPP \[1-(m-
chlorophenyl)piperazine\] and non-selective ligands, such as
MK 212 \[6-chloro-2-(1-piperazinyl)pyrazine\], dose-
dependently suppress spontaneous locomotor activity in rats
(Kennett and Curzon, 1988a; Lucki et al., 1989; Klodzinska
et al., 1989). This effect was found to be prevented only
by 5-HT antagonists with high affinity for 5-HT\textsubscript{1C} receptors,
such as metergoline and mianserin, suggesting specific
involvement of these receptors.

2. Feeding: Systemic administration of m-CPP and TFMPP
reduces food intake in freely feeding rats (Kennett et al.,
1991). Studies with 5-HT antagonists suggested that this
hypophagic effect is mediated by activation of 5-HT\textsubscript{1C}
receptors, though activation of 5-HT\textsubscript{1B} sites was also needed
for the response to occur (Kennett and Curzon, 1988b).
Dourish et al. (1989) proposed that postsynaptic 5-HT\textsubscript{1C}
receptors located in the ventromedial hypothalamus play an
important role in regulating feeding.

3. Psychiatric disorders: Administration of m-CPP in normal
human volunteers has been reported to increase feelings of
anxiety, dysphoria and panic (Charney et al., 1987; Murphy
et al., 1989). Anxiogenesis was indicated by both decreased
social interaction and decreased activity in rats (Kennett
et al., 1989). These effects might be related to 5-HT\textsubscript{1C}
receptors, because they are blocked by mianserin,
metylgoline and cyproheptadine (i.e., compounds with high affinity for the 5-HT$_1$c receptor).

4. Hypertonic saline consumption: Administration of fenfluramine, fluoxetine, m-CPP, or MK 212 decreases hypertonic saline intake at doses that have negligible effects on water or physiological saline consumption (Neil and Cooper, 1989). Since the more selective 5-HT$_1$b agonist CGS 12066B [7-trifluoromethyl-4(4-ethyl-1-piperazinyl)-pyrrolo(1,2-alquinoxaline] has no such an effect, 5-HT$_1$c receptors have been suggested to be involved in mediating salt-drinking behavior. Further pharmacological characterization of this effect is necessary.

5. Migraine effect: Recent evidence indicates that ergotamine and dihydroergotamine, two effective antimigraine agents, are potent 5-HT$_1$c receptor antagonists in piglet choroid plexus (Brown et al., 1991). Therefore, it is suggested that 5-HT$_1$c receptors may play a role in initiating migraine attacks (Fozard and Gray, 1989).

The above findings of so many potential roles for 5-HT$_1$c receptors suggest that the effects of many drugs acting on brain 5-HT systems might have been targeting on these particular receptor sites. The availability of more selective tools will clarify the roles of 5-HT$_1$c receptors in behavior and in CNS diseases.
Rationale

The availability of 6-OHDA to destroy brain DA-containing neurons in both adult and neonatal rat provides a pharmacological means to study motor functions and lesion-related biochemical changes that may depend on the age at which the dopaminergic pathway is destroyed (Breese and Traylor, 1970, 1972; Smith et al., 1973; Breese et al., 1984, 1985b). Treatment of adult rats with 6-OHDA produces a profound motor dysfunction such as aphagia, adipsia and akinesia (Breese and Traylor, 1970; Ungerstedt, 1971c; Zigmond and Strieker, 1973). In contrast, neonatal treatment of rats with the toxin does not result in such an acute motor syndrome despite the fact that near-total destruction of this emerging DA system is produced (Erinoff et al., 1979; Bruno et al., 1986; Duncan et al., 1987). However, these rats exhibit an increased susceptibility for self-mutilation behavior (Breese et al., 1984, 1985a,b).

Clinically, Parkinson’s disease is associated with reduced brain DA and motor dysfunction (Hornykiewicz, 1973), and Parkinsonian patients display tremor, bradykinesia and rigidity. Another disorder related to the same brain biochemical deficiency is Lesch-Nyhan syndrome. The latter syndrome is characterized by choreoathetoid movement, hypertonicity and unique compulsive self-mutilation of digits and tissue about the mouth (Lesch and Nyhan, 1964). It is believed that the adult 6-OHDA-treated rat serves as a
neurochemical model for Parkinson’s disease (Ungerstedt, 1971), whereas the neonatal lesioned rat provides a model for central dysfunctions observed in childhood diseases, one of which is Lesch-Nyhan syndrome (Breese et al., 1984, 1985a,b).

Neonatal 6-OHDA-lesioned rats are also known to be supersensitized to DA D_1 receptor agonists in the absence of an altered density and affinity of the receptor. SKF 38393, a selective D_1 agonist, produces marked changes in stereotyped and locomotor behaviors in the neonatal lesioned rats (Breese et al., 1984, 1985a,b, 1987). This change in sensitivity is a latent one, which must be unmasked by repeated doses of SKF 38393. This is referred to as a ‘priming’ phenomenon (Criswell et al., 1989). Priming of neonatal 6-OHDA-lesioned rats has been implicated in learning, development of craving following repeated intake of some drugs, or the appearance of motor disturbances following prolonged administration of drugs that influence dopamine receptor functions (Criswell et al., 1989, 1990).

Hamdi and Kostrzewa (1991) demonstrated that the D_1 receptor could be ontogenetically sensitized by repeated treatments with a D_1 agonist during development. As in adult-primed rats, there were enhanced D_1 agonist-induced stereotyped behaviors in adulthood. In order to further investigate ontogenetic sensitization of neonatal 6-OHDA-treated rats and examine its underlying biochemical association, the
selective D₁ receptor agonist, SKF 38393, was used alone, or in combination with neonatal 6-OHDA lesion during postnatal ontogeny in this study.

This project was thus undertaken with the objective of (1) assessing the degree or extent of ontogenetic sensitization of neonatal 6-OHDA-lesioned rats by repeated treatments of the animals with a DA D₁ agonist in development (2) investigating the status of dynamics of the DA D₁ receptors, such as high and low affinity binding sites in the neonatal 6-OHDA-lesioned rat model, or in neonatal 6-OHDA-lesioned rats repeatedly treated in postnatal ontogeny with SKF 38393 (3) evaluating the association of D₁ receptors with second messenger systems in the above animal models, and also (4) determining mRNA levels for D₁ receptors in these rat models.

The above investigations can lead to a better understanding of whether ontogenetic DA D₁ agonist treatments modulate the behavioral responses in adult rats that were lesioned neonatally with 6-OHDA. These studies would also resolve the problem of whether several biochemical markers would be associated with the enhanced behavioral responses generated by a neonatal chemical lesion or combined neonatal lesion plus ontogenetic DA D₁ agonist treatments.

Moreover, in neonatal 6-OHDA-lesioned rats, acute treatment with a DA D₁ receptor agonist results in a great
potentiation of the incidence of oral activity, even though the $B_{max}$ and $K_d$ for striatal $D_1$ receptor binding do not accompany the marked reduction in DA content (Kostrzewa and Hamdi, 1991). It is likely that the enhanced oral response in neonatal 6-OHDA-lesioned rats is due to supersensitized $D_1$ receptors. Since the striatal DA depletion in neonatal 6-OHDA-treated rats is accompanied by elevation in 5-HT levels (Breese et al., 1984; Stachowiak et al., 1984; Luthman et al., 1987), hyperinnervation of rostral striatum by 5-HT-containing fibers (Berger et al., 1985; Snyder et al., 1986), and upregulation of several 5-HT receptor subtypes in striatum (Radja et al., 1993), it is reasonable to study possible interactions between DA and 5-HT neurochemical systems in the $D_1$ supersensitized induction of oral activity by using this animal model.

Another aim in the present project, therefore, was to evaluate whether 5-HT receptors were involved in the induction of supersensitized oral responses in neonatal 6-OHDA-lesioned rats. The piperazine compound, m-CPP, was used in this study, because this 5-HT receptor agonist was found to induce oral activity in intact rats (Stewart et al., 1989). A series of other 5-HT agonists and antagonists was also used to determine which probable 5-HT receptor subtype would be related to such involvement. This study should provide evidence of whether there is a functional correlation between DA $D_1$ receptor and 5-HT fiber
innervation of the striatum.
Chapter 2
Materials and Methods

Animals and Treatment

Timed-pregnant Sprague-Dawley derived albino rats were obtained from Charles River Laboratories (Research Triangle, NC) approximately 1 week before delivery. Animals were housed singly in clear Perspex cages in a room with controlled temperature (22 ± 1 °C) and lighting (12 h-12 h light-dark cycle, lights on at 07:00), and had free access to food and water. Date of litter delivery was noticed within 12 hours and the date of birth was considered as day 0. At birth, litters were reassigned, so that each dam had rats from several litters, with each reconstituted litter consisting of a maximum of ten pups.

Starting at birth all pups were treated once a day for 28 consecutive days by intraperitoneal (i.p.) injection of SKF 38393 HCl (1-phenyl-2,3,4,5-tetrahydro-[1H]-3-benzazepine-7,8-diol hydrochloride; 3.0 mg/kg, salt form; Research Biochemicals, Inc., Natick, MA) or saline vehicle. At 3 days from birth all rats also received a bilateral intracerebroventricular (i.c.v.) injection of 6-OHDA hydrobromide (100 µg, salt form, on each side; Regis Chemical Co., Chicago, IL) or the vehicle, saline-ascorbic acid (0.1%). Neonates were pretreated with desipramine (20 mg/kg i.p.) 1 hr before 6-OHDA injection to prevent
destruction of noradrenergic neurons. Starting 3 days after birth, all animals were divided into four treatment groups: (1) saline (2) SKF 38393 (3) 6-OHDA (4) SKF 38393 plus 6-OHDA. At the age of 6 weeks, the first portion of the rats from each group was challenged with SKF 38393, to study (1) locomotor activity and (2) stereotyped behaviors (see behavioral observations). These rats were continuously tested with a weekly challenge dose of SKF 38393 for another three weeks. At 9 weeks of age, a second portion of the rats from all treatment groups without weekly challenge dose of SKF 38393 in adulthood, was sacrificed for biochemical analyses only.

At 3 and 9 months of age, another two separate groups of rats consisting of neonatal saline and 6-OHDA treatment, respectively, were used for determination of oral activity response to DA D₄ agonist and antagonist as well as a series of 5-HT receptor agents.

All animals were sacrificed by decapitation. Brains were immediately removed and the striata were dissected free, quickly frozen on dry ice, and stored at -70 °C for later in vitro neurochemical assessments (see biochemical assays below).

**Behavioral Observations**

1. Locomotor activity. Between 6 and 9 weeks of age, rats were placed singly in donut-shaped test chambers (46 cm in
diameter) with six equidistant markings along the circumference. After a 60 min habituation period in a quiet and well-lighted room, a challenge dose of SKF 38393 HCl (3.0 mg/kg i.p., salt form) or vehicle was administered. Locomotor activities were then videotaped by a camcorder for 60 min starting 10 min after the challenge agent. Tapes were replayed and the distance traveled by each rat was thus determined.

2. Stereotyped behaviors. At 8 weeks of age, rats were placed singly in clear Perspex cages (48 x 26 x 18) with wood chip bedding, in a quite and well-illuminated room, and were acclimated to the testing surroundings for 30 min. An individual rat was challenged first with saline vehicle 10 min before behavioral testing. The rat was then observed for stereotyped behaviors for 10 min. A challenge dose of SKF 38393 HCl (3.0 mg/kg i.p., salt form) was then administered and rats were again observed for 10 min for stereotyped behaviors, starting 10 min after injection. Behaviors were quantified as seconds in which each particular behavior was performed by a rat. Therefore, the total time an individual rat spent in performing or not performing an activity was 600 seconds (10 min observation period). The behaviors observed were locomotion, sniffing, grooming, eating, taffy pulling (coordinated movement of front paws toward the mouth and then away from the body), digging, gnawing, licking, jumping, paw treading and
locomotion. Yawning and rearing were counted as numbers of incidents, not seconds. Immobility was defined as the time period in which a rat was not observed in any stereotypic activity.

3. Oral activity: Rats were observed for oral responses to test agents between 3 and 9 months of age. For each test session, rats were placed in individual clear plastic cages (48 x 26 x 18 cm) with steel grid floors in a quiet, well-ventilated and well-lighted room. Rats were allowed to acclimate to the new environment for at least 1 hr.

Between 9:00 A.M. and 4:00 P.M. each rat was given a single i.p. or s.c. treatment with vehicle, SKF 38393 HCl (0.03-3.0 mg/kg i.p.); m-CPP 2HCl [1-(3-chlorophenyl)piperazine dihydrochloride, 0.5-8.0 mg/kg i.p.]; CGS 12066B maleate [7-trifluoromethyl-4(4-ethyl-1-piperazinyl)pyrrolo[1,2-alquinoxaline], 1:2 maleate salt, 3.0 mg/kg i.p.]; or 8-OH-DPAT HBr [(±)-8-hydroxydipropylaminotetralin hydrobromide, 0.1 mg/kg s.c.]. Each rat was then observed one at a time, for 1 min every 10 min, over a 60-min period, beginning 10 min after any of these treatments. Numbers of rapid jaw movements were counted. Some rats were pretreated with mianserin HCl (1.0 mg/kg s.c., 30 min); ketanserin tartrate (5.0 mg/kg i.p., 30 min); MDL 72222 (3-tropanyl-3,5-dichlorobenzoate, 10.0 mg/kg s.c., 30 min); or SCH 23390 HCl [R-(+)7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetra-hydro-1H-3-benzazepine
hydrochloride, 0.30 mg/kg i.p., 1 hr]. At least 1 week intervened between these observation sessions.

Oral activity in these studies is of the type described by Waddington (1990) as "vacuous (or abortive or spontaneous) chewing, whereby what appear to be robust chewing sequences are manifested, but are not directed onto any evident physical material." No differentiation between lateral and vertical jaw movements was made. Also, there was occasional tongue thrusting noted in the these rats. Oral activity that occurred in eating, grooming or taffy pulling was not counted.

Determination of DA, 5-HT and Their Metabolites in the Striatum

Striatal concentrations of DA, 5-HT and their metabolites were measured by liquid chromatography with electrochemical detection as follows. The striata were sonicated in 1 ml of 0.1 M trichloroacetic acid containing 0.2 mg/ml cysteine as a stabilizing agent and an internal standard which was 0.2 nmol/ml 5-hydroxyindole carboxylic acid (5HICA). The resulting homogenate was centrifuged at 12,000 x g for 5 min and DA, homovanillic acid (HVA), 3,4-dihydroxyphenylacetic acid (DOPAC), 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) were assayed by injecting 30 µl of supernatant directly onto the C18 analytical column. The column was an Econosphere C18 (5 micron, 4.6 x
150 mm) from Alltech Associates. The mobile phase was 0.1 M monochloroacetic acid, 1 mM EDTA, 220 mg/l sodium octanesulfonic acid, 8% acetonitrile, pH 2.6 at a flow rate of 1.3 ml/min and temperature of 40 °C.

The instrumentation consisted of a Bioanalytical System LCEC Analyzer and a Waters WISP automatic sample injector with a refrigerated sample compartment (samples kept at 5°C). The glassy carbon electrode was used at a potential of +0.75 volts. Peak heights and sample concentration were calculated with a Hewlett-Packard HP1000 chromatography data system.

**Determination of DA Competition for [³H]SCH 23390 Binding to Striatal Homogenates**

To assess DA competition for [³H]SCH 23390 binding, a modified method of Hess et al. (1986) was used. Briefly, striata were homogenized with a Teflon on glass mortar and pestle, in 100 volumes of ice cold 50 mM Tris buffer (pH 7.4). This gentle homogenizing procedure was used to avoid destruction of D₁ receptors during this tissue preparation step (Norman et al., 1989). Homogenates were then centrifuged at 35,100 x g for 10 min at 4 °C using a Beckman J2-21M centrifuge and JA-20.1 rotor. After one wash, the homogenates were preincubated at 37 °C for 15 min, chilled in ice and centrifuged as before. Tissue pellets were finally resuspended in 200 volumes of fresh buffer.
Aliquots (300 µl) of homogenates were added to 450 pM of [³H]SCH 23390 (specific activity 294 mCi/mg; Amersham, Arlington Heights, IL) in Tris buffer containing 120 mM NaCl, 5 mM KCl, 2 mM CaCl₂, 0.1% ascorbic acid, 10 µM pargyline (final concentrations) and varying concentrations of DA as competitor (1 nM - 1 mM), to yield a 2.5 ml final assay volume. Samples were incubated for 40 min at 37 °C in a shaking water bath, and then were rapidly filtered under partial vacuum on Whatman GF/F glass fiber filters using a Millipore filtration unit. Filters were washed 3 times with 5 ml of ice-cold Tris-salt solution each time. After drying overnight, filters were placed in 9 ml of fluor, and tritium activity was determined in a Beckman LS 9800 liquid scintillation spectrometer. A GraphPad program (GraphPad Software Inc., San Diego, CA), using a nonlinear regression model for complex ligand-receptor binding systems, was used to fit the agonist-antagonist competition curves matching two binding sites. The IC⁵₀ values for DA at the high and low affinity site and the percentage of each receptor binding component were estimated by the computer program. The Kᵢ for each IC⁵₀ was determined according to following equation:

\[ Kᵢ = \frac{IC⁵₀}{1 + \frac{L}{K₄}} \]

where L is the concentration of [³H]SCH 23390 and K₄ is its affinity for the receptors.
Determination of mRNA Levels for D₁ and 5-HT₁C Receptors in the Striatum

Two mixtures of 48-base oligonucleotide probes for the respective rat DA D₁ and 5-HT₁C receptor mRNA were used (NEN Research Products, Boston, MA). The sequences for D₁ receptor mRNA are complementary to bases 13-60 (5'-GAA ATC CCT CTC CGT TGG CAG CCC GGC CTC ATC CAT GGT AGA AGT GTT-3'), 520-567 (5'-GGA CTG CTG CCC TCT CCA AGG CTG AGA TGC CCC GGA TTT GCT TCT GGG-3') and 664-711 (5'-TGT CAC ACT TGT CAT CCT CGG TGT CCT CCA GGG AGG TAA AAT TGC CAT-3'), while the sequences for 5-HT₁C receptor mRNA are complementary to bases 4-51 (5'-GCC GAT TAG GTG CAT CAG GAG CGA GCG CAC CGC GTT GCC AAG GTT CAC-3'), 721-768 (5'-CTC CTC GGT GTG ACC TCG AAG TAA CAT CAG AGT TTG ACG GCG CAG GAC-3') and 856-903 (5'-GGT GCC TCT GGG ACG CTT TTG TTT CTT CTT TCG ACG TGG TTT CTG ATC-3').

Rat striatal tissues from above treatment groups were homogenized in denaturing solution containing 4.0 M guanidine thiocyanate, 42 mM sodium citrate, 0.83% N-lauroyl sarcosine and 0.2 mM β-mercaptoethanol, using a Tekmar Tissumizer (setting 5, 20 sec). After adding 2.0 M sodium acetate (pH 4.0), the homogenates were extracted with equal volume of phenol:chloroform:isoamyl alcohol mixture and then centrifuged at 10,000 x g (20 min at 4 °C). The upper aqueous phase was collected and the total RNA was precipitated with isopropanol by centrifugation. RNA was
quantified by UV spectrophotometry at 260 nm wavelength.

A sample representing 5-10 μg of striatal RNA denatured at 95 °C for 2 min, was fractionated by electrophoresis on 1% agarose gel containing 2% formaldehyde, 20 mM 3-(N-morpholino)propanesulfonic acid, 5 mM sodium acetate and 1 mM EDTA. The gel was stained with ethidium bromide and visualized under UV light to check the integrity and loading of the RNA in each lane. The RNA was then transferred to nylon membranes (Schleicher & Schuell, Keene, NH), which were subsequently crosslinked under UV light for 5 min and baked at 80 °C for half an hour. Some of the striatal RNA samples from each treatment group were denatured in 7.4% formaldehyde at 65 °C and then transferred directly to nylon membrane on an apparatus for dot blot analysis. The membranes were probed with respective D1 and 5-HT1c receptor mRNA probe according to following procedure.

Membranes were incubated for 1 hr at 58 °C in 20 ml of prehybridization buffer containing 5 x SSC, 20 mM Na2HPO4, 7% SDS, 1 x Denhardt's solution and 100 μg/ml denatured salmon sperm DNA. Following prehybridization, the buffer was replaced with the same new fresh buffer and the membranes were hybridized with oligonucleotide probes at 58 °C for 24 hr, which were end-labelled with [γ-32P]ATP using oligonucleotide 5'-end labelling system (Promega Corporation, Madison, WI). The membranes were washed twice in 2 x SSC, 1% SDS and 20 mM Na2HPO4 at room temperature for
15 min. They were finally washed twice in 0.5 x SSC and 1% SDS at 58 °C for 15 min and then were exposed to Kodak X-OMAT AR film (Sigma Chemical Co., St. Louis, MO) at -70 °C with two intensifying screens for 48-72 hr to ensure that the exposure time was within the linear range of the film. The optical densities of the bands or the dots were measured by a Bio Image Analysis System (Millipore Corporation Imaging Systems, Ann Arbor, MI). The probes were removed from the membranes by washing in solution containing 50% formamide and 2 x SSPE buffer at 65 °C for 1 hr before rehybridization with cDNA probe for β-actin mRNA. The β-actin mRNA probe was labelled with [α-32P]dCTP by a Prime-a-Gene labelling system (Promega Corporation, Madison, WI) to give a specific activity of 1-2 x 10⁹ cpm per μg of DNA. The β-actin mRNA measurements were used mainly for correcting errors that occurred in preparation or loading of the RNA samples.

**Determination of DA-Stimulated Adenylate Cyclase Activity in the Striatum**

The striatal tissues from all above treatment groups were homogenized with a Teflon on glass mortar and pestle, in 50 volumes of ice-cold 10 mM Tris maleate/2 mM EGTA buffer (pH 7.4). Homogenates were then centrifuged at 35,100 x g for 10 min in Beckman model J2-21M superspeed centrifuge, using a JA-20.1 rotor. After one wash with the
same buffer and centrifugation as before, the resultant pellet was resuspended in 70 volumes of fresh buffer and kept on ice prior to use for adenylate cyclase activity assays.

Adenylate cyclase activity was assayed in a 200-μl incubation mixture containing 80 mM Tris maleate, 0.4 mM EGTA, 4.0 mM MgSO₄, 1.0 mM isobutylmethylxanthine, 5.0 mM phosphocreatine, 50 unit/ml creatine phosphokinase, 0.02% ascorbic acid, 10 μM pargyline, 0.1 mM GTP, 50 nM spiperone, and varying concentrations of DA (0-10⁻³ M). In some instances, 12.2 μM forskolin or 10 mM NaF in combination with 10 μM AlCl₃ ([AlF₄]⁻), was added to the mixture instead of DA, in order to stimulate the catalytic subunit of the enzyme or the N₅ protein, respectively. Spiperone was included in all incubation solutions to block DA D₂ receptor-mediated inhibition of adenylate cyclase activity. Membrane suspensions (30-40 μg protein) were preincubated at 30 °C for 5 min in the reaction mixture. The reaction was then started by adding ATP (final concentration: 1.0 mM) and the mixtures were incubated at 30 °C for 15 min. After termination of the reaction in boiling water for 4 min, the mixtures were centrifuged at 13,000 x g for 5 min. Aliquots of the supernatant were assayed for cAMP content using an Amersham cAMP enzymeimmunoassay kit (RPN 225, Amersham, UK; sensitivity: 38.4 pg/ml). Protein content of striatal homogenates was determined by the method of Lowry et al.
(1951).

**Data Analysis**

Locomotor activity: Data are expressed as the mean distance (meters) ± S.E.M. in different groups of rats traveling within activity testing chambers. The statistical significance of the data was determined by a two-way analysis of variance (ANOVA), followed by a post-ANOVA Newman-Keuls test. A p value of < 0.05 was considered to be statistically significant.

Stereotyped behaviors: Results are expressed as the mean time (seconds) ± S.E.M. in which rats from different treatment groups perform a particular behavior. The statistical significance of the results was evaluated by a one-way ANOVA, followed by a post-ANOVA Newman-Keuls test. A p value of < 0.05 or smaller was considered to be statistically significant.

Oral activity: Results are expressed as the mean number of oral movements ± S.E.M. of rats receiving neonatal 6-OHDA treatment or vehicle. The statistical significance was determined by a one-way ANOVA, followed by a post-ANOVA Newman-Keuls test. A p value of < 0.05 or smaller was considered to be statistically significant.

Monoamine determinations: The mean nanomoles of monoamine or metabolite per gram of tissue ± S.E.M. in different treatment groups were analyzed by the ANOVA, followed by a post-ANOVA Newman-Keuls test, in order to
determine the statistical significance among treatment groups. A p value of < 0.05 was considered to be the level for statistical significance.

Binding studies: Competition curves were analyzed according to models for the competition of radioligand and competitor, DA, to one or two binding sites. The statistical difference between models was tested by comparing the sum of squares which resulted from different fits of the data. A sequential F-test was used according to the following equation:

\[
F = \frac{SS_1 - SS_2}{df_1 - df_2} \cdot \frac{SS_2}{df_2}
\]

where SS refers to the sum of squares, and df refers to the number of degree of freedom. The subscripts 1 and 2 refer to the model for one, and two binding sites, respectively. A model for two binding sites was retained only when it fitted the data statistically significantly better than a model for one binding site (p < 0.05). Also, the mean (± S.E.M.) percent of high affinity binding sites (%RH), and the respective mean (± S.E.M.) K_H and K_L in each treatment group were analyzed by one-way ANOVA. A p value of < 0.05 was considered to be the level for statistical significance.

mRNA analyses and adenylate cyclase activity assays: The mean (± S.E.M.) ratio of D_1 or 5-HT_{1C} receptor mRNA to β-
actin mRNA, and mean (± S.E.M.) picomoles of cAMP per milligram of protein per min, respectively, were analyzed by ANOVA, followed by a post-ANOVA Newman-Keuls test, in order to evaluate the statistical significance among different treatment groups. A p value of < 0.05 was considered to be the level for statistical significance.
Chapter 3
Results

DA D1 Receptor Supersensitivity

Locomotor activity of rats

Starting at 6 weeks adult rats from the different treatment groups were tested at weekly intervals for spontaneous and SKF 38393-induced locomotor activity. At 6, 7 and 9 weeks there was little locomotor activity in each group after administration of saline, and no statistically significant difference between the groups (p > 0.05, left 4 bars in figs. 1, 2 and 3).

At 6, 7 and 9 weeks, SKF 38393 HCl treatment (3.0 mg/kg i.p.) did not increase locomotor activity in the vehicle control rats, nor in the rats treated repeatedly during development with SKF 38393 HCl (3.0 mg/kg per day x 28 days from birth, i.p.; Fig. 1, 2 and 3, 5th and 6th bars). At 6 weeks acute SKF 38393 HCl treatment (3.0 mg/kg i.p.) produced a slight increase in locomotor activity in the group of rats that was lesioned at 3 days after birth with 6-OHDA HBr (200 μg, i.c.v.), although this effect was not different from that after saline treatment (fig. 1, 7th bar). However, in the group of rats that was both lesioned at 3 days after birth with 6-OHDA and treated repeatedly in development with SKF 38393 HCl (3.0 mg/kg per day x 28
days), acute SKF 38393 treatment at 6 weeks produced a large increase in locomotor activity (p < 0.05, fig. 1, 8th bar). Rats in this group traveled about 210 meters in 1 hr, vs. about 40 meters for the 6-OHDA-lesioned group (p < 0.05, 8th vs. 7th bar of fig. 1). The greater SKF 38393-induced activity in this group suggests that ontogenic SKF 38393 treatments produced homologous sensitization of the DA receptors.

One week later when rats were challenged again with SKF 38393, there was an increase in locomotor activity in the 2 groups of rats lesioned at 3 days after birth with 6-OHDA. In the group treated at 3 days with 6-OHDA, locomotor activity increased substantially from one week before, now 155 meters in the one hour following the challenge dose of SKF 38393. In the group that was lesioned with 6-OHDA at 3 days and also treated repeatedly in development with SKF 38393, the challenge dose of SKF 38393 at 7 weeks increased the distance traveled to more than 590 meters, nearly triple that from the week before (8th bar of fig. 2 vs. 8th bar of fig. 1).

In the 8th week rats were tested for stereotyped activities following the SKF 38393 injection, so there is no locomotor activity data. In the 9th week the challenge dose of SKF 38393 produced more than a 2-fold increase in locomotor activity in the group of rats lesioned at 3 days after birth with 6-OHDA (p < 0.05, 7th bar of fig. 3 vs 7th
bar of fig. 2). However, in rats receiving both 6-OHDA and SKF 38393 treatments in development, the challenge dose of SKF 38393 at 9 weeks increased the distance traveled only slightly and not significantly from that at 7 weeks (8th bar of fig. 3 vs. 8th bar of fig. 2). Nevertheless, SKF 38393-induced locomotor activity in this group was higher at 9 weeks vs. 6 weeks (p < 0.05, 8th bar of fig. 3 vs. 8th bar of fig. 1).

**Stereotyped behaviors of rats**

At 8 weeks, following a saline injection, there was little stereotyped activity in the 4 groups of rats, with immobility time being 340 to 490 seconds (i.e., 600 seconds of observation, table 1). Following the challenge dose of SKF 38393 HCl (3.0 mg/kg i.p.) activity increased in all groups (table 2). However, for rats treated repeatedly in development with SKF 38393, there was no difference from control for time spent in sniffing, locomotion, grooming, eating, taffy pulling, digging, gnawing and paw treading, following the challenge dose of SKF 38393 at 8 weeks. Also, numbers of yawns and rearings was not different between these two groups.

In the group of rats lesioned at 3 days with 6-OHDA, the challenge dose of SKF 38393 at 8 weeks substantially increased time in locomotion (351.7 ± 59.6 vs. 1.7 ± 0.9 sec) and paw treading (89.0 ± 50.2 vs. 0.0 sec), compared to
the vehicle control group (all p < 0.05, table 2).
Conversely, in the 6-OHDA vs. control group, the challenge dose of SKF 38393 decreased time in grooming (4.3 ± 4.3 vs. 135.4 ± 21.8 sec), gnawing (1.1 ± 0.7 vs. 12.1 ± 2.3 sec) and immobility (84.1 ± 56.3 vs. 396.1 ± 34.4 sec) (all p < 0.05, table 2).

In the group of rats lesioned at 3 days with 6-OHDA and also treated daily for the first 28 days from birth with SKF 38393 HCl (3.0 mg/kg per day, i.p.), the challenge dose of SKF 38393 at 8 weeks produced several changes in stereotyped behaviors. Time in locomotion (228.9 ± 74.8 sec), taffy pulling (172.5 ± 87.8 sec) and paw treading (140.3 ± 75.8 sec) was increased vs. the control group (p < 0.05); taffy pulling was different vs. the 6-OHDA group (p < 0.05, table 2). Time in grooming (3.5 ± 3.5 sec) and immobility (0.0 sec) were different vs. control (p < 0.05); while immobility time was different vs. the 6-OHDA group (p < 0.05).

**Striatal levels of DA, DOPAC, HVA, 5-HT and 5-HIAA**

As shown in table 3, neonatal 6-OHDA treatment produced a profound depletion of DA (>99% reduction), DOPAC (>99% reduction) and HVA (>99% reduction) in the striatum of rats. Conversely, neonatal 6-OHDA treatment was associated with a 70% increase in striatal 5-HT and 35% increase in 5-HIAA content (all p < 0.001). SKF 38393 treatments for the first 28 days after birth did not produce an effect on striatal
levels of DA, 5-HT and their metabolites, nor did this
treatment modify the effect of 6-OHDA.

**Agonist competition for DA D1 receptor binding in striatal homogenates**

Computer-modeled DA agonist-[³H]SCH23390 antagonist competition binding curves are shown in figs. 4-7 for the respective groups receiving saline, ontogenetic SKF 38393 and/or neonatal 6-OHDA treatments. Each curve fits a two-site model. Dissociation constants to high affinity ($R_\text{H}$) and low affinity ($R_\text{L}$) have been designated $K_\text{H}$ and $K_\text{L}$, respectively. Table 4 summarizes computer-assisted binding parameters for DA competition curves, demonstrating that these curves exhibit high and low affinity binding components. No apparent difference was observed in the distribution of high and low affinity agonist binding sites between 6-OHDA vs. saline group. Also, there was no significant effect of ontogenetic SKF 38393 treatments on the affinity of DA at either high or low affinity states of the receptor. Postnatal treatment of 6-OHDA lesioned rats with SKF 38393 for 28 consecutive days likewise did not affect high or low affinity agonist binding sites.

**Striatal levels of DA D1 receptor mRNAs**

Dot blot analysis revealed that in the first portion of rats that did not receive weekly challenge doses of SKF
38393 in adulthood, striatal mRNA levels for DA D₁ receptor were significantly reduced (p < 0.05) in neonatal 6-OHDA-lesioned rats, regardless of whether ontogenetic SKF 38393 treatments were given (fig. 8A). SKF 38393 treatments for the first 28 days after birth per se did not produce an effect on striatal mRNA content of the D₁ receptor (fig. 8A). In the second portion of rats receiving weekly adult challenge doses of SKF 38393, the striatal mRNA levels for D₁ receptors in neonatal 6-OHDA-treated rats were restored to the control level, so that there was no statistically significant difference in D₁ receptor mRNA content among the treatment groups (fig. 8B).

**Striatal DA-stimulated adenylate cyclase activity**

No significant difference in basal adenylate cyclase activity was observed between treatment groups (fig. 10). Stimulation of D₁ receptors by DA significantly increased adenylate cyclase activity in the striatum at higher doses of DA (10⁻⁶-10⁻³ M) in all treatment groups. The relative increase among saline, SKF 38393, 6-OHDA, and 'SKF 38393 + 6-OHDA' treatment groups was similar (fig. 9). To evaluate the effects of neonatal 6-OHDA and ontogenetic SKF 38393 treatments on the receptor coupling with G-protein, NaF was used to stimulate N₅. The adenylate cyclase activity was enhanced by NaF, but there were no statistical differences among the treatment groups (fig. 10, p=0.051). To examine
the effects of neonatal 6-OHDA and ontogenetic SKF 38393 treatments on the activity of adenylate cyclase, the enzyme was stimulated directly with forskolin. The activity of adenylate cyclase was markedly increased by forskolin. However, no significant difference was found in the treatment groups of saline, SKF 38393, 6-OHDA, and SKF 38393 plus 6-OHDA (fig. 10, p=0.759).

**Mediation of DA Agonist-Induced Oral Behavior by the Serotonin System**

**Dose-response study of SKF 38393-induced oral activity**

To determine the optimal doses of SKF 38393 for inducing oral activity, a dose-response curve was constructed (fig. 11). In the saline control group, SKF 38393 at a dose of 0.1 mg/kg and higher resulted in only a slight increase in oral activity. In the 6-OHDA group, the threshold dose of SKF 38393 was 0.03 mg/kg, with oral activity increasing to 15 ± 2.9 movements (p < 0.01). At higher doses of SKF 38393 there were about 40 oral movements during the session (p < 0.005).

**Effect of combined D1 agonist and D1 antagonist treatments**

To determine whether a DA D1 receptor antagonist would attenuate the oral responses of rats to a D1 agonist, rats were treated with SCH 23390 (0.3 mg/kg, i.p.) 1 hr before
SKF 38393 (0.3 mg/kg, i.p.). As shown in fig. 12, SCH 23390 attenuated the action of SKF 38393 (p < 0.005), indicating that SKF 38393-induced oral activity is a D1-receptor-mediated event.

**Dose-response study of m-CPP-induced oral activity**

To determine the optimal dose of m-CPP for induction of the oral response, a series of doses of m-CPP was administered to rats. As shown in fig. 13, the oral response was increased in both the control and 6-OHDA-lesioned groups of rats after m-CPP doses in the range of 0.5 to 8.0 mg/kg i.p. The overall response was greater in the 6-OHDA vs. the control group of rats (p < 0.001), with the apparent maximal response in the 6-OHDA-lesioned rats occurring at the 4.0 mg/kg dose of m-CPP.

**Effects of 5-HT\textsubscript{1A} and 5-HT\textsubscript{1B} receptor agonists on oral activity**

To determine the possible involvement of 5-HT\textsubscript{1} receptors on induction of oral activity, rats were treated with either the 5-HT\textsubscript{1A} receptor agonist, 8-OH-DAPTHBr (0.1 mg/kg s.c., 10 min before observation) or the 5-HT\textsubscript{1B} receptor agonist, CGS-12066B maleate (7-trifluoromethyl-4(4-methyl-1-piperazinyl)pyrrolo[1,2-alquinoxaline], 1:2 maleate salt, 3.0 mg/kg i.p., 10 min before observation). As shown in fig. 14, 8-OH-DPAT did not induce an oral response in
rats. Also, CGS-12066B did not produce a statistically significant increase in oral activity in control and 6-OHDA-lesioned rats (fig. 14).

Effects of 5-HT receptor antagonists on m-CPP-induced oral activity

To assess whether m-CPP might produce enhanced oral activity through an action on several different types of 5-HT receptors, a variety of 5-HT receptor antagonists were administered in conjunction with m-CPP (1.0 mg/kg i.p.). After pretreatment with mianserin (1.0 mg/kg s.c.), a mixed antagonist for 5-HT₁c and 5-HT₂ receptors, there was complete attenuation of the oral response to m-CPP (p < 0.001, fig. 15). Ketanserin (5.0 mg/kg i.p.), a relatively selective antagonist for the 5-HT₂ receptor, failed to modify the oral response to m-CPP (fig. 15). Similarly, MDL 72222 (10.0 mg/kg s.c.), a relatively selective antagonist for the 5-HT₃ receptor, failed to alter the response to m-CPP (fig. 15).

Effect of a DA D₁ receptor antagonist on m-CPP-induced oral activity

To determine whether a DA D₁ receptor antagonist would attenuate the oral response of rats to the 5-HT agonist, m-CPP, rats were treated with SCH 23390 (0.3 mg/kg i.p.). As shown in fig. 16, SCH 23390 failed to attenuate the enhanced oral response to m-CPP, indicating that 5-HT agonist-induced
oral activity is not mediated through the DA D_1 receptor.

**Effect of a 5-HT_{1C} receptor antagonist on DA D_1 agonist-induced oral activity**

To determine whether a 5-HT_{1C} receptor antagonist would attenuate the oral response to a DA D_1 receptor agonist, rats were treated with mianserin (1.0 mg/kg s.c.) 30 min before SKF 38393 (1.0 mg/kg i.p.). As shown in fig. 17, pretreatment with mianserin attenuated the enhanced oral response to SKF 38393, in both control (p < 0.05) and 6-OHDA-lesioned rats (p < 0.001). This finding indicates that the oral response of rats to a D_1 agonist is mediated via a 5-HT system involving 5-HT_{1C} receptors.

**Effect of neonatal 6-OHDA treatment on concentrations of DA, 5-HT and their metabolites in the striatum**

As expected, neonatal 6-OHDA treatment profoundly reduced striatal DA, HVA and DOPAC contents (>98% reduction, p < 0.001; table 5). Accompanying this change was an elevation in striatal 5-HT content by 77% (p < 0.001), and a slight but not statistically significant increase in 5-HIAA content (table 5).
Effect of neonatal 6-OHDA and ontogenetic SKF 38393 treatments on mRNA levels for 5-HT₁C receptors of the striatum

To evaluate the effect of neonatal 6-OHDA and ontogenetic SKF 38393 treatments on 5-HT₁C receptor gene transcription, dot blot analysis was also employed. There was no significant difference in the mRNA levels for 5-HT₁C receptors in the striatum between neonatal 6-OHDA-lesioned and intact rats (fig. 18A). Ontogenetic treatments of either intact or lesioned rats with SKF 38393 did not alter the mRNA level for the 5-HT₁C receptor (fig. 18A). After repeated administration of SKF 38393 in adulthood, there was still no change in 5-HT₁C receptor mRNA in saline, 6-OHDA, SKF 38393, and 'SKF 38393 + 6-OHDA' treatment groups (fig. 18B).
TABLE 1

Behavioral effects of acute saline challenge in rats treated developmentally with SKF 38393 or saline, in combination with single i.c.v. injection of 6-OHDA or its vehicle.

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Saline</th>
<th>SKF 38393</th>
<th>6-OHDA</th>
<th>6-OHDA + SKF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sniffing</td>
<td>49.7 ± 10.7</td>
<td>174.0 ± 50.6</td>
<td>66.8 ± 34.3</td>
<td>93.6 ± 25.0</td>
</tr>
<tr>
<td>Locomotion</td>
<td>1.5 ± 1.0</td>
<td>5.2 ± 2.6</td>
<td>1.7 ± 0.9</td>
<td>3.0 ± 1.2</td>
</tr>
<tr>
<td>Grooming</td>
<td>38.2 ± 12.1</td>
<td>37.4 ± 24.7</td>
<td>21.6 ± 7.8</td>
<td>57.6 ± 31.4</td>
</tr>
<tr>
<td>Eating</td>
<td>4.2 ± 4.2</td>
<td>26.4 ± 16.1</td>
<td>7.5 ± 4.7</td>
<td>10.1 ± 4.8</td>
</tr>
<tr>
<td>Taffy pulling</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Digging</td>
<td>1.0 ± 0.4</td>
<td>1.8 ± 1.2</td>
<td>0.8 ± 0.4</td>
<td>1.1 ± 0.6</td>
</tr>
<tr>
<td>Gnawing</td>
<td>6.5 ± 0.9</td>
<td>7.8 ± 3.1</td>
<td>13.4 ± 2.8</td>
<td>15.2 ± 2.6</td>
</tr>
<tr>
<td>Paw treading</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Yawning^</td>
<td>0.3 ± 0.3</td>
<td>0.0 ± 0.0</td>
<td>0.6 ± 0.3</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>Rearing^</td>
<td>5.6 ± 2.3</td>
<td>10.2 ± 3.8</td>
<td>5.3 ± 2.9</td>
<td>3.8 ± 1.4</td>
</tr>
<tr>
<td>Immobility</td>
<td>493.0 ± 20.7</td>
<td>337.2 ± 64.2</td>
<td>482.3 ± 38.6</td>
<td>414.9 ± 36.2</td>
</tr>
</tbody>
</table>

Behavioral activity was determined in rats that were treated during postnatal development with a DA D₂ agonist, SKF 38393 HCl (3.0 mg/kg per day x 28 days, i.p., from day of birth and/or the catecholamine neurotoxin 6-OHDA HBr (200 μg i.c.v., salt form, at 3 days from birth; desipramine pretreatment, 1 hr). Rats were challenged with saline at 8 weeks from birth and were immediately observed for stereotyped behaviors for 10 min. Behaviors were thus quantified as seconds in which each individual behavior was performed by a rat. The total observation time was 600 seconds. *Yawning and rearing behaviors were counted as numbers of incidents, not seconds. Each group is the mean of 10 rats.
TABLE 2

Behavioral effects of acute SKF 38393 in rats treated developmentally with SKF 38393 or saline, in combination with single i.c.v. injection of 6-OHDA or its vehicle.

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Saline</th>
<th>SKF 38393</th>
<th>6-OHDA</th>
<th>6-OHDA + SKF</th>
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<tbody>
<tr>
<td>Sniffing</td>
<td>49.5 ± 14.2</td>
<td>95.2 ± 24.0</td>
<td>22.8 ± 7.6</td>
<td>54.8 ± 34.6</td>
</tr>
<tr>
<td>Locomotion</td>
<td>1.8 ± 0.9</td>
<td>0.8 ± 0.5</td>
<td>351.7 ± 59.6*</td>
<td>228.9 ± 74.8*</td>
</tr>
<tr>
<td>Grooming</td>
<td>135.4 ± 21.8</td>
<td>95.6 ± 22.0</td>
<td>4.3 ± 4.3*</td>
<td>3.5 ± 3.5*</td>
</tr>
<tr>
<td>Eating</td>
<td>0.5 ± 0.5</td>
<td>1.8 ± 1.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Taffy pulling</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>172.5 ± 87.8*+</td>
</tr>
<tr>
<td>Digging</td>
<td>0.4 ± 0.3</td>
<td>2.1 ± 1.2</td>
<td>2.3 ± 1.9</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Gnawing</td>
<td>12.1 ± 2.3</td>
<td>7.7 ± 1.8</td>
<td>1.1 ± 0.7*</td>
<td>0.0 ± 0.0*</td>
</tr>
<tr>
<td>Paw treading</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>89.0 ± 50.2*</td>
<td>140.3 ± 75.8*</td>
</tr>
<tr>
<td>Yawning</td>
<td>0.1 ± 0.1</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Raring</td>
<td>4.1 ± 1.8</td>
<td>4.3 ± 1.7</td>
<td>7.2 ± 2.9</td>
<td>13.3 ± 5.5</td>
</tr>
<tr>
<td>Immobility</td>
<td>396.1 ± 34.4</td>
<td>392.5 ± 38.0</td>
<td>84.1 ± 56.3*</td>
<td>0.0 ± 0.0*</td>
</tr>
</tbody>
</table>

Behavioral activity was determined in rats that were treated during postnatal development with a DA D₂ agonist, SKF 38393 HCl (3.0 mg/kg per day x 28 days, i.p., from day of birth) and/or the catecholamine neurotoxin 6-OHDA HBr (200 μg i.c.v., salt form, at 3 days from birth; desipramine pretreatment, 1 hr). Rats were challenged with SKF 38393 HCl (3.0 mg/kg i.p.) at 8 weeks from birth and were immediately observed for stereotyped behaviors for 10 min. Behaviors were thus quantified as described in Table 1 legend. Each group is the mean of 10 rats.

*p < 0.000 vs. saline control group; +p < 0.01 vs. 6-OHDA group.
### TABLE 3

Effect of neonatal 6-OHDA and ontogenetic SKF 38393 treatments on concentrations of DA, 5-HT and their metabolites in the striatum of adult rats.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>n</th>
<th>DA</th>
<th>HVA</th>
<th>DOPAC</th>
<th>5-HT</th>
<th>5-HIAA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>6</td>
<td>79.2±2.3</td>
<td>8.6±0.5</td>
<td>16.1±0.4</td>
<td>4.0±0.1</td>
<td>5.2±0.2</td>
</tr>
<tr>
<td>SKF 38393</td>
<td>5</td>
<td>74.1±3.0</td>
<td>8.2±0.3</td>
<td>13.8±0.6</td>
<td>3.9±0.2</td>
<td>5.2±0.3</td>
</tr>
<tr>
<td>6-OHDA</td>
<td>10</td>
<td>0.6±0.07*</td>
<td>0.1±0.03*</td>
<td>0.1±0.02*</td>
<td>6.8±0.2*</td>
<td>7.0±0.3*</td>
</tr>
<tr>
<td>SKF 38393 + 6-OHDA</td>
<td>9</td>
<td>0.6±0.08*</td>
<td>0.2±0.05*</td>
<td>0.2±0.08*</td>
<td>6.2±0.2*</td>
<td>6.8±0.2*</td>
</tr>
</tbody>
</table>

Rats were treated daily with vehicle or SKF 38393 HCl (3.0 mg/kg i.p.) for 28 consecutive days from birth in combination with i.c.v. injection of 6-OHDA (200 µg, salt form, 3 days after birth; desipramine pretreatment, 1 hr) or its vehicle. Striata were removed for assay at 11 weeks. Values are mean nanomoles per gram of tissue ± S.E.M.

*p < 0.001 when compared to control group.
Computer-modeled parameters for dopamine inhibition of \(^{3}\text{H}\)SCH 23390 binding in the striatal homogenates of saline, 6-OHDA, SKF 38393 and 'SKF 38393 + 6-OHDA' treatment groups of rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>(%R_H)</th>
<th>(K_H) (nM)</th>
<th>(K_L) ((\mu)M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>75.1 ± 2.6</td>
<td>212.2 ± 26.8</td>
<td>30.9 ± 9.9</td>
</tr>
<tr>
<td>SKF38393</td>
<td>75.3 ± 3.2</td>
<td>231.6 ± 26.6</td>
<td>29.2 ± 6.8</td>
</tr>
<tr>
<td>6-OHDA</td>
<td>68.7 ± 2.6</td>
<td>272.5 ± 38.5</td>
<td>24.4 ± 4.0</td>
</tr>
<tr>
<td>SKF38393 + 6-OHDA</td>
<td>73.2 ± 2.0</td>
<td>275.0 ± 59.2</td>
<td>30.5 ± 6.8</td>
</tr>
</tbody>
</table>

Rats were treated daily with vehicle or SKF 38393 HCl (3.0 mg/kg i.p.) for 28 consecutive days from birth in combination with i.c.v. injection of 6-OHDA HBr (200 \(\mu\)g, salt form, 3 days after birth; desipramine pretreatment, 1 hr) or its vehicle. Striata were removed for assay at 11 weeks. Competition curves were constructed by using 14 concentrations of inhibitor, DA, to compete for 0.45 nM \(^{3}\text{H}\)SCH 23390 binding in rat striatum and were fit best by a two-site computer derived model where the affinity of \(^{3}\text{H}\)SCH 23390 was constrained to be equal (\(K_d = 0.61\) nM) at both high (\(R_H\)) and low (\(R_L\)) affinity sites. The dissociation constants for high (\(K_H\)) and low (\(K_L\)) affinity agonist-binding sites were thus determined by using computer program (GraphPad Software Inc., San Diego, CA). Data are expressed as mean values or percent ± S.E.M. of 8 determinations in each treatment group.
### TABLE 5

Effect of neonatal 6-OHDA treatment on concentrations of DA, 5-HT and their metabolites in the striatum of rats

<table>
<thead>
<tr>
<th>Monamine or Metabolite</th>
<th>Treatment</th>
<th>% of Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline (n=6)</td>
<td>6-OHDA (n=9)</td>
</tr>
<tr>
<td>DA</td>
<td>67.83 ± 1.80</td>
<td>0.95 ± 0.16*</td>
</tr>
<tr>
<td>HVA</td>
<td>7.50 ± 0.53</td>
<td>0.14 ± 0.04*</td>
</tr>
<tr>
<td>DOPAC</td>
<td>14.45 ± 0.87</td>
<td>0.27 ± 0.05*</td>
</tr>
<tr>
<td>5-HT</td>
<td>3.52 ± 0.15</td>
<td>6.23 ± 0.55*</td>
</tr>
<tr>
<td>5-HIAA</td>
<td>6.53 ± 0.30</td>
<td>7.50 ± 0.60</td>
</tr>
</tbody>
</table>

Rats were treated at 3 days after birth with 6-OHDA HBr (200 µg, i.c.v. salt form; desipramine pretreatment, 1 hr) or its vehicle. Striata were removed for assay at 9 months. Values are mean nanomoles per gram of tissue ± S.E.M. Numbers in parentheses are numbers of samples per group. *p < 0.001 when compared to vehicle control group.
Figure 1. Effect of first acute weekly SKF 38393 HCl (3.0 mg/kg i.p.) injection on locomotor activity in rats. Rats were treated during postnatal ontogeny with either saline (□), SKF 38393 HCl (3.0 mg/kg i.p. daily x 28 days; □), 6-OHDA HBr (200 µg i.c.v., 3 days after birth; desipramine pretreatment, 1 hr; □) or combined SKF 38393 + 6-OHDA treatments (□). Rats were challenged at 6 weeks from birth with SKF 38393 HCl (3.0 mg/kg i.p.), 10 min before observation, and then were videotaped for their locomotor response for 60 min. Locomotor activity represents the distance (meters) traveled by each rat. Each bar indicates the mean of 7 to 10 rats. *p < 0.05, vs. control group challenged with SKF 38393 HCl; +p < 0.05, vs. same group challenged with saline.
Figure 2. Effect of second acute weekly SKF 38393 HCl (3.0 mg/kg i.p.) injection on locomotor activity in rats. Rats were treated during postnatal ontogeny with either saline ( ), SKF 38393 HCl (3.0 mg/kg i.p.) daily x 28 days; ( ), 6-OHDA HBr (200 µg i.c.v., 3 days after birth; desipramine pretreatment 1 hr; ) or combined SKF 38393 + 6-OHDA treatments ( ). Rats were challenged at 7 weeks from birth with SKF 38393 HCl (3.0 mg/kg i.p.), 10 min before observation, and then were videotaped for their locomotor response for 60 min. Locomotor activity represents the distance (meters) traveled by each rat. Each bar indicates the mean of 7 to 10 rats. * p < 0.05, vs. control group challenged with SKF 38393 HCl; + P < 0.05, vs. same group challenged with saline.
Figure 3. Effect of fourth acute weekly SKF 38393 HCl (3.0 mg/kg i.p.) injection on locomotor activity in rats. Rats were treated during postnatal ontogeny with either saline (□), SKF 38393 HCl (3.0 mg/kg i.p. daily x 28 days; ◼), 6-OHDA HBr (200 μg i.c.v., 3 days after birth; desipramine pretreatment, 1 hr; ▼) or combined SKF 38393 + 6-OHDA treatments (■). Rats were challenged at 9 weeks from birth with SKF 38393 HCl (3.0 mg/kg i.p.), 10 min before observation, and then were videotaped for their locomotor response for 60 min. Locomotor activity represents the distance (meters) traveled by each rat. Each bar indicates the mean of 7 to 10 rats. * p < 0.05, vs. control group challenged with SKF 38393 HCl; + p < 0.05, vs. same group challenged with saline.
Figure 4. Competition of DA for 0.45 nM [3H]SCH 23390 binding in the striatum of the saline group of rats. Rats were treated with vehicle i.c.v. injection (bilateral; desipramine pretreatment; 3 days after birth) and the striata were removed 11 weeks from birth. The competition curve in striatal homogenates was fit best by a two-site computer derived model where the affinity of [3H]SCH 23390 was constrained to be 0.61 nM. Each data point represents the mean of 8 determinations ± S.E.M.. The competition curve was analyzed as in table 4.
DOPAMINE INHIBITION OF [\(^3\)H]SCH 23390 BINDING IN RAT STRIATUM

Figure 5. Competition of DA for 0.45 nM [\(^3\)H]SCH 23390 binding in the striatum of the ontogenetic SKF 38393 group of rats. Rats were treated ontogenetically with SKF 38393 HCl (3.0 mg/kg i.p. daily x 28), the striata were removed at 11 weeks from birth. The competition curve was constructed as in figure 4.
Figure 6. Competition of DA for 0.45 nM [3H]SCH 23390 binding in the striatum of the neonatal 6-OHDA group of rats. Rats were treated neonatally with 6-OHDA HBr (200 µg i.c.v., 3 days after birth; desipramine pretreatment), and the striata were removed at 11 weeks from birth. The competition curve was constructed as in figure 4.
Figure 7. Competition of DA for 0.45 nM [3H]SCH 23390 binding in the striatum of the neonatal 6-OHDA plus ontogenetic SKF 38393 group of rats. Rats were treated ontogenetically with SKF 38393 HCl (3.0 mg/kg i.p. daily x 28) and lesioned neonatally with 6-OHDA HBr (200 µg i.c.v., 3 days after birth; desipramine pretreatment). The striata were removed at 11 weeks from birth. The competition curve was constructed as in figure 4.
Figure 8A. Effects of neonatal 6-OHDA and ontogenetic SKF 38393 treatments on striatal mRNA levels for DA D₁ receptors before treatments with SKF 38393 in adulthood. Each bar represents the mean (± S.E.M.) ratio of optical density for D₁ receptor mRNA vs. that for β-actin mRNA. * p < 0.05 vs. saline group.
Figure 8B. Effects of neonatal 6-OHDA and ontogenetic SKF 38393 treatments on striatal mRNA levels for DA D1 receptors after treatments with SKF 38393 in adulthood. Each bar represents the mean (± S.E.M.) ratio of optical density for D1 receptor mRNA vs. that for β-actin mRNA. No significant differences were observed among saline, SKF 38393, 6-OHDA and 'SKF 38393 + 6-OHDA' treatment groups.
Figure 9. Effects of neonatal 6-OHDA and ontogenetic SKF 38393 treatments on DA-stimulated adenylate cyclase activity. Each point represents the mean (± S.E.M.) cAMP production (pmoles/mg protein/min) of 5 to 6 determinations. The basal enzyme activities for saline, SKF 38393, 6-OHDA and SKF 38393 + 6-OHDA treatment groups are: 19.0 ± 2.7, 19.1 ± 3.6, 14.7 ± 2.3, and 19.6 ± 2.1 pmoles/mg protein/min, respectively. No significant differences in stimulation were observed between the treatment groups. *p < 0.05, vs. basal activity in the same group.
Figure 10. Effects of neonatal 6-OHDA and ontogenetic SKF 38393 treatments on basal adenylate cyclase activity and NaF- and forskolin-stimulated adenylate cyclase activity. No significant differences were observed among saline, SKF 38393, 6-OHDA and 'SKF 38393 + 6-OHDA' treatment groups on basal enzyme activity. NaF and forskolin increased adenylate cyclase activity in all treatment groups, but the relative increase was not statistically significant among these four groups of rats.
SKF 38393-INDUCED ORAL ACTIVITY IN RATS

![Graph showing dose-response relationship for SKF 38393-induced oral activity in rats. Numbers of oral movements were determined for one minute every 10 minutes over a 60-min period, starting 10 minutes after SKF 38393 HCl (0.03 to 3.0 mg/kg i.p.). Each group is the mean of 4 to 6 rats. The number of oral movements in 6-OHDA vs. control rats is different at each respective dose of SKF 38393 (p < 0.01).]
ATTENUATION OF SKF 38393-INDUCED ORAL ACTIVITY IN RATS BY SCH 23390

Figure 12. Attenuation of SKF 38393-induced oral activity in rats by SCH 23390. Numbers of oral movements were determined for one minute every 10 minutes, for 60 minutes, starting 10 minutes after SKF 38393 HCl (0.3 mg/kg i.p.) and 60 minutes after SCH 23390 HCl (0.3 mg/kg i.p.). Each group is the mean of 4 to 6 rats. * p < 0.005 vs. other 6-OHDA groups.
Figure 13. Dose-response relationship for m-CPP-induced oral activity in control and 6-OHDA-lesioned rats. Rats were studied for oral activity as in figure 11. Each bar represents mean number of oral movements ± S.E.M. Each m-CPP dose produced a greater response in the 6-OHDA vs. control group. * p < 0.01; ** p < 0.001 vs. respective control or 6-OHDA group challenged with saline.
Figure 14. Effects of 8-OH-DPAT, a 5-HT₁₅ receptor agonist, and CGS-12066B, a 5-HT₁₅ receptor agonist, on oral activity in control and 6-OHDA-lesioned rats. Rats were observed for oral activity, as described in figure 11. The number of oral movements was determined in the 1-hr interval, starting 10 min after challenge treatment with saline, or 8-OH-DPAT (0.1 mg/kg s.c.), or CGS-12066B (3.0 mg/kg i.p.). Each group is the mean of 8 to 9 (control) or 11 to 14 (6-OHDA lesioned) rats. There was no statistically significant increase in oral activity after 8-OH-DPAT or CGS-12066B treatments.
EFFECTS OF SEROTONIN RECEPTOR ANTAGONISTS
ON m-CPP-INDUCED ORAL ACTIVITY IN RATS

Figure 15. Effects of 5-HT receptor antagonists on m-CPP-induced oral activity in control and 6-OHDA-lesioned rats. Rats were studied for oral activity as in figure 11. Mianserin HCl (1.0 mg/kg s.c.), ketanserin tartrate (5.0 mg/kg i.p.) and MDL 72222 (10.0 mg/kg i.p.) were administered 30 min before m-CPP 2HCl (1.0 mg/kg i.p.) or its vehicle. Each group is the mean (± S.E.M.) of 6 to 11 rats. * p < 0.001 vs. m-CPP effect in the 6-OHDA-lesioned rats.
Figure 16. Lack of effect of SCH 23390 on m-CPP-induced oral activity in control and 6-OHDA-lesioned rats. Rats were studied for oral activity as in figure 11. SCH 23390 (0.3 mg/kg i.p.) was administered 1 hr before m-CPP 2HCl (1.0 mg/kg i.p.) or its vehicle. Each group is the mean (± S.E.M.) of 4 to 11 rats. SCH 23390 did not attenuate the effect of m-CPP (p > 0.05).
ATTENUATION OF SKF 38393-INDUCED ORAL ACTIVITY IN RATS BY MIANSERIN

![Graph showing oral activity in control and 6-OHDA-lesioned rats with and without mianserin and SKF 38393.](image)

**Figure 17.** Attenuation of SKF 38393-induced oral activity in control and 6-OHDA-lesioned rats by mianserin. Rats were studied for oral activity as figure 11. Mianserin HCl (1.0 mg/kg s.c.) was administered 30 min before SKF 38393 HCl (1.0 mg/kg i.p.) or its vehicle. Each group is the mean (± S.E.M.) of 4 to 11 rats. * p < 0.001 vs. other groups.
Figure 18A. Effects of neonatal 6-OHDA and ontogenetic SKF 38393 treatments on striatal mRNA levels for 5-HT1C receptors before treatments with SKF 38393 in adulthood. Each bar represents the mean (± S.E.M.) ratio of optical density for 5-HT1C receptor mRNA vs. that for β-actin mRNA. No significant differences were observed among saline, SKF 38393, 6-OHDA and 'SKF 38393 + 6-OHDA' treatment groups.
Figure 18B. Effects of neonatal 6-OHDA and ontogenetic SKF 38393 treatments on striatal mRNA levels for 5-HT_{1C} receptors after treatments with SKF 38393 in adulthood. Each bar represents the mean (± S.E.M.) ratio of optical density for 5-HT_{1C} receptor mRNA vs. that for β-actin mRNA. No significant differences were observed among saline, SKF 38393, 6-OHDA and ‘SKF 38393 + 6-OHDA’ treatment groups.
Chapter 4
Discussion

DA D₄ Receptor Supersensitization

Neonatal treatment of rats with a catecholamine neurotoxin, 6-OHDA, results in the supersensitization of DA receptors. That is, doses of L-dihydroxyphenylalanine (L-DOPA), apomorphine or SKF 38393 which have little effect in intact rats, produce marked changes in stereotyped and locomotor behaviors in the lesioned rats that were tested as adults (Breese et al., 1984, 1985a,b, 1987; Hamdi and Kostrzewa, 1991). The DA D₁ receptor antagonist, SCH 23390, attenuates the enhanced stereotyped and locomotor behaviors by the D₁ agonist, SKF 38393, in the lesioned rats, indicating that D₁ receptors are specifically involved in the induced receptor sensitization (Breese et al., 1985a,b, 1987). The ability to sensitize D₁ receptors appears to be related to immaturity of the DA system at the time of lesioning, since 6-OHDA treatment of adult rats is not associated with such receptor sensitization (Breese et al., 1984). Actually, a latent supersensitization of D₁ receptors occurs with this lesion, and the sensitization process is only unmasked by repeated DA D₁ agonist treatments in adulthood (Criswell et al., 1989, 1990; Johnson et al., 1992). This process of induction of long-lived D₁ receptor supersensitivity is known as 'priming'
(Breese et al., 1985b; Criswell et al., 1989). Recently, Hamdi and Kostrzewa (1991) reported that the D₁ receptors could be ontogenetically sensitized by repeated D₁ agonist treatments during the first 33 days after birth. As in adult-primed rats, there was an enhancement of the D₁ agonist-induced stereotyped behaviors in adulthood. Also, as in studies by Breese and co-workers, there was no change in the Bₘₐₓ or Kᵢ for D₁ binding in the striatum of these rats (Breese et al., 1987).

The status of the DA D₁ receptor during postnatal ontogeny is not completely resolved. There are some reports of only slight change in the Bₘₐₓ and Kᵢ for [³H]SCH 23390 binding to striatal homogenates in the first 1 to 2 months after birth (Broaddus and Bennett, 1990), and other reports of a dramatic increase in Bₘₐₓ during the first 4 to 5 weeks after birth (Giorgi et al., 1987; Zeng et al., 1988) and even transient increases in the Kᵢ in this interval (Zeng et al., 1988). Using histochemical methods, however, it has been clearly demonstrated that DA D₁ receptors develop in patches in the striatum, being closely associated with DA nerve terminal input to these patches (Zeng et al., 1988). As D₁ receptors increase in number, the striosomal pattern of DA D₁ receptors becomes obscured by the global increase in DA D₁ receptors in the matrix of the striatum. This pattern of DA D₁ receptor development indicates that the early series of SKF 38393 treatments in the present study
would initially interact only with the population of DA \( \text{D}_1 \) receptors that had developed up to that point in time. Interaction of SKF 38393 with striatal DA \( \text{D}_1 \) receptors would change during development, as the population of the receptor continued to increase in number during ontogeny. In theory, each subsequent dose of SKF 38393 was able to act at greater numbers of \( \text{D}_1 \) receptors, which would mean that there was unequal exposure of the DA \( \text{D}_1 \) receptor population to the DA \( \text{D}_1 \) agonist. Perhaps this relates to the inability of SKF 38393 to fully sensitize DA \( \text{D}_1 \) receptors to later SKF 38393-induced locomotor activity effects.

Although many SKF 38393-induced responses of neonatal-sensitized and adult-primed rats are similar, there are some differences in degree of response for many behaviors, which possibly are related to the extent of sensitization. The extent of DA \( \text{D}_1 \) sensitization in those rats treated repeatedly in postnatal ontogeny with the DA \( \text{D}_1 \) agonist, SKF 38393 was investigated in the present study. For that reason the locomotor responses to SKF 38393 were determined after the first 2 doses and after the last dose of the \( \text{D}_1 \) agonist. It is clear from effects observed in rats treated only with neonatal 6-OHDA, that adult priming was essential for unmasking of the latent supersensitivity of \( \text{D}_1 \) receptors. In essence, the first dose of SKF 38393 produced only a slight increase in the locomotor response of rats, while subsequent doses produced increasingly greater effects.
(7th bars of figs. 1-3; p < 0.05, ANOVA). This finding in
the present study is in full agreement with those earlier
reports (Breese et al., 1985b; Criswell et al., 1989, 1990).

In contrast to this effect, sensitization of DA D₁
receptors was evident with the first challenge dose of SKF
38393 at 6 weeks, in neonatal 6-OHDA lesioned rats that were
treated daily with SKF 38393 during the first 4 weeks of
postnatal ontogeny. That is, an enhanced locomotor response
was observed after initial SKF 38393 treatment in adulthood
(8th bar of fig. 1, p < 0.05). Subsequent SKF 38393
challenge doses increased locomotor activity to a level
ultimately about 3-fold greater than that seen after the
first adult challenge dose (8th bars of figs. 2-3).
Therefore, it appears that ontogenetic treatments of
neonatal 6-OHDA-lesioned rats with SKF 38393 produces
partial but not complete DA D₁ receptor supersensitization.
Additional adult SKF 38393 treatments were necessary to
produce the maximal level of D₁ sensitivity.

Because locomotor activity is only one of several kinds
of behaviors induced by DA agonists, we observed rats for
SKF 38393-induced stereotypies at 8 weeks. DA D₁ agonists
produce exaggerated stereotyped behavioral responses in
neonatal 6-OHDA-lesioned rats (Breese et al., 1984, 1985a,b,
1987; Hamdi and Kostrzewa, 1991). It was important to
determine if several SKF 38393 challenge treatments would
substantially modify the stereotypy effect of a DA D₁
agonist in adult rats. We found that ontogenetic SKF 38393 treatments did not markedly influence stereotyped responses in neonatal 6-OHDA-lesioned rats. Virtually all of the behaviors were expressed in both the 6-OHDA group and 'SKF 38393 + 6-OHDA' group, except for taffy pulling, which was expressed in only the latter group. Also, immobility time was less in this group vs. the 6-OHDA group. These findings suggest that ontogenetic SKF 38393 treatments are able to partially modify later stereotyped behavioral effects of a DA D₄ agonist.

Despite the fact that a different method was used in the present study to evaluate stereotyped behavior, virtually all of the SKF 38393-induced responses are similar to those observed previously in rats that were lesioned neonatally with 6-OHDA and ontogenetically sensitized with SKF 38393 (Hamdi and Kostrzewa, 1991). The one exception is that SKF 38393-induced grooming is reduced in the present study, compared to an enhanced grooming response in the previous study (Hamdi and Kostrzewa, 1991). This difference may relate to additional priming in adulthood in the present study. It is likely that, as D₄-related behaviors became more prominent with additional SKF 38393 treatments in adulthood, the competing behaviors resulted in less grooming behavior. In essence, a rat can only exhibit so many behaviors at one time, so an increase in performing of one behavior might be expected to diminish the time spent in
another behavior.

Given the behavioral aberrations observed in both neonatal 6-OHDA-lesioned rats and those receiving combined neonatal 6-OHDA and ontogenetic SKF 38393 treatments in the present study, a DA competition for $[^{3}H]SCH$ 23390 binding analysis was performed from striatal homogenates of the rats. This was done because earlier reports showed a lack of change of $D_1$ receptor number and affinity by either using striatal homogenates (Breese et al., 1987; Duncan et al., 1987; Dewar et al., 1990; Hamdi and Kostrzewa, 1991) or performing autoradiography (Luthman et al., 1990; Simson et al., 1992), after neonatal 6-OHDA treatment. As yet, very little is known about whether numbers of high affinity $D_1$ receptor would be altered in these rats or in rats given both neonatal 6-OHDA and ontogenetic $D_1$ agonist treatments. No change in high affinity binding sites for DA $D_1$ receptors was noted in neonatal 6-OHDA vs. saline rats when using DA competition for radiolabeled $D_1$ antagonist binding (table 4). Also, in the present study postnatal treatment of neonatal 6-OHDA-lesioned rats with SKF 38393 did not affect the distribution of high and low affinity agonist binding sites (table 4). Collectively, these results demonstrate that behavioral supersensitivity to systemic administration of DA $D_1$ agonist in adulthood, or priming of rats lesioned as neonates and those given neonatal lesion plus ontogenetic treatment with the $D_1$ agonist, is neither due to increased $D_1$
receptor number in general, nor is it due to altered high affinity receptor binding in particular.

With the techniques of recombinant DNA technology being used, specific sequences encoding different types of DA receptor including rat (Zhou et al., 1990) and human D₁ receptors (Deary et al., 1990; Sunahara et al., 1991), have been cloned and sequenced, and thereby can be used to analyze the receptor gene expression in the brain. Therefore, availability of the receptor mRNA probe has made it possible to analyze the specific mRNA coding for DA D₁ receptor and thus to have a better understanding of the receptor regulation and supersensitivity after neonatal 6-OHDA lesion. In order to investigate whether the enhanced locomotor and stereotyped responsiveness of the lesioned rats to adult challenge doses of D₁ agonist is attributable to an altered rate of transcription of the gene or the mRNA for DA D₁ receptors, we also evaluated the effect of SKF 38393 treatments on expression of the gene coding D₁ receptors. The finding that the level of D₁ receptor mRNA after neonatal 6-OHDA was reduced, regardless of whether ontogenetic SKF 38393 was co-administered, suggests that 6-OHDA chemical lesion influences the receptor gene expression. Gelbard et al. (1990) demonstrated that neonatal development of DA D₁ receptors in rat forebrain was impaired after 6-OHDA lesion. These investigators suggested that in the absence of endogenous DA, the D₁ receptors may
possibly either fail to develop adequately or fail to be maintained. The present data reveal possible molecular mechanisms underlying this biochemical event. Our finding indicates that neonatal 6-OHDA lesion impairs ontogeny of DA D_1 receptors due to failure of receptor gene expression. After repeated treatments with SKF 38393 in adulthood (adult priming), there was no change in striatal D_1 mRNA levels among the treatment groups, indicating that priming restores D_1 receptor gene expression in the lesioned rats. These results are in accord with previous studies, demonstrating that priming of striatal D_1 receptors in neonatal 6-OHDA lesioned rats occurred in the absence of altered D_1 receptor density and affinity (Breese et al., 1987; Duncan et al., 1987; Dewar et al., 1990; Luthman et al., 1990; Hamdi and Kostrzewa, 1991). Moreover, these findings are fully consistent with the above agonist competition for radiolabeled DA D_1 antagonist binding data, indicating that the DA D_1 receptor recognition site after neonatal 6-OHDA lesion may not be a critical factor in the development of behavioral supersensitivity to DA D_1 agonist.

Based on pharmacological manipulation of adult animals, it has been proposed that alterations in DA D_1 receptor mRNA by repeated reserpine treatments (Butkerait and Friedman, 1993) or by chronic DA D_1 receptor blockade (Schettini et al., 1992) are associated with an increased level of mRNA coding for G protein alpha subunit (N_5α). The present data
showed that after adult priming with a D₁ agonist, the level of D₁ receptor mRNA in neonatally lesioned or combined neonatally lesioned plus ontogenetically sensitized rats did not differ from that of the control at a time when behavioral sensitization of the animals is known to occur. Taken together, these studies suggest that a site distal to the DA D₁ receptor recognition site, or possibly, a site rather than in DA neurochemical system but in a system or systems modulating DA-mediated effects, accounts for the apparent supersensitization of D₁ receptor-related function.

The present study, using DA to stimulate adenylate cyclase in the presence of spiperone, indicates that the link of DA D₁ receptors to this enzyme is not changed in the striatum by lesioning neonates with 6-OHDA, in spite of profound depletion of striatal DA content and supersensitized behavioral responses. Moreover, we found that forskolin- and NaF-stimulated adenylate cyclase activity did not differ in lesioned and intact rats. Additionally, ontogenetic treatments with SKF 38393 failed to affect basal and DA-stimulated adenylate cyclase activity in both neonatal lesioned and intact animals. This finding supports that of Simson et al. (1992), who found that agonist-stimulated adenylate cyclase activity did not change in neonatal 6-OHDA lesioned rats that were primed in adulthood. It appears that DA D₁ receptor linked adenylate cyclase is also not a major determinant of supersensitized
behavioral responses to D₁ agonists in neonatal 6-OHDA-lesioned rats.

It is well established that neonatal 6-OHDA treatment of rats is associated with a marked reduction in the striatal level of DA, due to destruction of nigrostriatal neurons (Breese and Traylor, 1971, 1972; Breese et al., 1984), and an elevation in 5-HT content (Breese et al., 1984; Stachowiak et al., 1984), due to sprouting and ensuing hyperinnervation by 5-HT-containing fibers (Stachowiak et al., 1984; Berger et al., 1985; Luthman et al., 1987; Towle et al., 1989). The findings in this study are in full agreement with those earlier reports. That is, DA content of the striatum in 6-OHDA-lesioned rats is profoundly reduced by nearly 99%, and DOPAC and HVA contents are reduced by a similar amount (table 3). Accompanying these changes is the elevation of striatal 5-HT content by 70%, and 5-HIAA by 35% (table 3). Additionally, ontogenetic treatments of rats with SKF 38393 in the present study, did not produce an effect on striatal DA, 5-HT and their metabolites, nor did this treatment modify the effect of 6-OHDA (table 3). Based on above striatal contents of DA, 5-HT and their metabolites found in this study, we feel that the animal models are similar to those in earlier studies.
Mediation of DA Agonist-Induced Oral Behavior by the 5-HT System

In neonatal 6-OHDA-lesioned rats a supersensitized response to DA D\textsubscript{1} agonists is observed in adulthood (Breese et al., 1985a,b, 1987; Hamdi and Kostrzewa, 1991), despite the lack of change in number or affinity of D\textsubscript{1} receptors (Breese et al., 1987; Hamdi and Kostrzewa, 1991). The oral activity response to D\textsubscript{1} receptor agonists and D\textsubscript{2} antagonists is also enhanced in the neonatal lesioned rats (Kostrzewa and Hamdi, 1991). In the present study, the augmented oral response to SKF 38393 in lesioned rats is attenuated by SCH 23390, a DA D\textsubscript{1} receptor antagonist, indicating that the D\textsubscript{1} receptor subtype is primarily involved in the enhanced response (fig. 12).

Recently, it was found that 5-HT agonists also can induce oral activity in rats (Stewart et al., 1989). Since the striatal DA depletion in neonatal 6-OHDA-treated rats is accompanied by elevated 5-HT levels (Breese et al., 1984; Stachowick et al., 1984; Luthman et al., 1987; table 5 in the present study) and hyperinnervation of striatum by 5-HT fibers (Berger et al., 1985; Snyder et al., 1986), it was of interest to determine whether the induction of oral activity by 5-HT agonists would be potentiated in these rats. The enhanced response to m-CPP over a wide dose range in lesioned rats in this study, demonstrates that 5-HT receptors are also supersensitized in this group of rats.
Production of overt supersensitization of 5-HT receptors in neonatal 6-OHDA-lesioned rats is suggested by the fact that the maximal oral activity response is 2- to 3-fold higher in this group vs. control (fig. 13). It has also been shown that m-CPP acts directly as a 5-HT agonist that lacks 5-HT uptake inhibitor properties (Fuller et al., 1981). Therefore, the most likely explanation for m-CPP action relates to 5-HT receptor supersensitization.

The series of studies with 5-HT receptor agonists and antagonists indicate that 5-HT₁₅ and 5-HT₁₆ receptors are not associated with oral activity. The agonist, 8-OH-DPAT, is relatively selective for 5-HT₁₅ receptors (Arvidsson et al., 1981; Middlemiss and Fozard, 1983), and the dose used in the present study is known to produce a response in vivo (Fuller and Snoddy, 1987; Stewart et al., 1989). CGS 12066B, a selective 5-HT₁₆ receptor agonist with little activity at D₁ sites (Neale et al., 1987), did not induce oral activity. Although we have no direct evidence that CGS 12066B acted at the 5-HT₁₆ site in the brain, the dose used in this study is more than 10-fold greater than that found by others to alter the firing of dorsal raphe neurons (Neale et al., 1987).

Ketanserin and MDL 72222, antagonists with high affinity for 5-HT₂ (Leysen et al., 1981, 1982) and 5-HT₃ (Fozard, 1984) receptors, respectively, were ineffective in attenuating the enhanced oral response to m-CPP. The doses of ketanserin and MDL 72222 are similar or higher than that
known to produce antagonism of 5-HT$_2$ and 5-HT$_3$ receptors in vivo (Fozard, 1984; Awouters et al., 1990; Nash, 1990). These findings suggest that 5-HT$_2$ and 5-HT$_3$ receptors are not involved in the effect of m-CPP. Attenuation of the enhanced oral response to m-CPP by mianserin, an antagonist with affinity for both 5-HT$_1c$ and 5-HT$_2$ receptors, suggests that it is the 5-HT$_1c$ receptor subtype that becomes supersensitized after loss of DA input to the striatum.

It was not practical to use a wide range of doses for the different agonists and antagonists in these studies, and it is conceivable that a different dose and/or different observation time for some of these agents might have resulted in an effect on the oral response. However, based on findings with 5-HT receptor agonists and antagonists in this study, there is an apparent correlation between 5-HT$_1c$ receptors and the oral responses of rats.

In the present study, the other major finding is the lack of effect of SCH 23390 on m-CPP-induced oral activity. This indicates that the DA system is not the final pathway in the behavioral event. This finding is in agreement with our biochemical studies in which there was no alteration in DA D$_1$ receptor high affinity binding sites, no change in basal and DA-stimulated adenylate cyclase activity, and no change in D$_1$ receptor mRNA level after priming in adulthood. SCH 23390 has a high affinity for the D$_1$ receptor, with an IC$_{50}$ for [H]$^3$SCH 23390 binding being about 150 times less
than that for $[3H]$mesulergine binding (Billard et al., 1984; Hoyer et al., 1988). The dose of SCH 23390 in this study is similar or identical to that used to specifically attenuate oral activity responses to a D$_1$ agonist (Levin et al., 1989; Rosengarten et al., 1986); to attenuate D$_1$ agonist-induced behaviors (Breese et al., 1985a,b; Criswell et al., 1989); and to attenuate the development of D$_1$ receptors (Saleh and Kostrzewa, 1988; Kostrzewa and Saleh, 1989).

The effectiveness of mianserin in attenuating the enhanced oral response to SKF 38393, suggests that D$_1$ receptor-mediated oral activity relies on a 5-HT neurochemical system for transduction of the response. The DA and 5-HT pathways associated with oral activity may operate in sequence, with the 5-HT system representing a later stage in this circuit. It is also possible, however, for these two neurochemical systems to operate in parallel but with the 5-HT system exerting greater control. Antagonists of the 5-HT system could conceivably attenuate D$_1$ agonist-induced oral responses in either design. The observed oral response may be a consequence of 5-HT fiber sprouting and hyperinnervation in rostral striatum. Additional studies are needed to resolve these aspects of interaction between the DA and 5-HT neurochemical systems. In other studies on neonatal 6-OHDA-lesioned rats it was reported that the sprouted 5-HT fibers did not seem to possess an inhibitory control on endogenous 5-HT- and
acetylcholine-containing neurons (Jackson et al., 1988), and that 5-HT innervation did not appear to be responsible for supersensitized locomotor and stereotyped responses to a DA agonist (Towle et al., 1989). The present findings demonstrate that 5-HT neurons can be intricately involved in mediation of supersensitized responses to DA agonists.

To study the effects of neonatal 6-OHDA lesion and ontogenetic SKF 38393 treatments on gene expression for 5-HT_{1C} receptors, the mRNA levels for the receptor subtype were also determined in the present study. Unlike DA D_{1} receptors, neither neonatal 6-OHDA nor ontogenetic SKF 38393 treatments affect mRNA coding for the 5-HT_{1C} receptor. Also, repeated treatments of both intact and lesioned rats with SKF 38393 in adulthood did not influence the levels of mRNA for 5-HT_{1C} receptors in the striatum. Therefore, the biochemical change responsible for the enhanced oral response in 6-OHDA-lesioned rats, other than altered gene expression for 5-HT_{1C} receptors, is yet to be determined. It has been known that 5-HT_{1C} receptors are coupled to the breakdown of phosphatidylinositolides (Conn and Sanders-Bush, 1986). An increase in inositol trisphosphates (IP_{3}) was reported to be related to the supersensitization of 5-HT_{1C} receptors generated by chemical denervation (Conn et al., 1987). It may be that coupling of the receptor with this second messenger system could be different in the supersensitized 5-HT_{1C} receptors in our neonatal 6-OHDA rat
model. Alternatively, balance and adjustment among striatal DA, 5-HT and other neuronal systems after neonatal 6-OHDA lesion may be responsible for the supersensitization of multiple neural receptor systems (Kostrzewa and Neely, 1993).

The present series of studies helps to identify the altered 5-HT receptor responses that occur in those rats with greatly increased oral activity after neonatal destruction of DA-containing fibers in the brain. This animal model should be useful in studying functional associations between central dopaminergic and serotonergic neurons. Also, the discovery of a 5-HT neurochemical component in enhanced oral dyskinetic activity implicates another direction that may be taken toward the discovery of agents that modulate dyskinetic oral activity in assorted human clinical disorders.
Chapter 5

Summary

The present series of studies has demonstrated the following:
1. Ontogenetic treatments of neonatal 6-OHDA-lesioned rats with a DA D_{1} agonist, SKF 38393, can partially sensitize D_{1} receptors in adulthood.
2. Neonatal 6-OHDA chemical lesions impair DA D_{1} receptor ontogeny due to failure of gene expression for the receptor, while repeated treatments of the lesioned rats with SKF 38393 in adulthood result in restoration of the mRNA level for D_{1} receptors.
3. Changes in DA D_{1} receptor high affinity binding, D_{1} receptor mRNA levels, and D_{1} receptor-stimulated adenylate cyclase activity, can not account for supersensitized behavioral responses in neonatally lesioned rats.
4. The 5-HT_{1c} receptor subtype becomes functionally supersensitized after neonatal destruction of DA input to the striatum, in the absence of a change in striatal mRNA level for this receptor subtype. Moreover, induction of oral activity by a DA D_{1} agonist is mediated via a serotonergic system.
5. The neonatal 6-OHDA rat model should be useful in exploring functional and neurochemical associations between central dopaminergic and serotonergic systems.
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