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Persistent Oral Dyskinesias Induced by Long-term Haloperidol Treatment is Dissociated from Changes in Neostriatal B(max) and Mrna Content for Dopamine D(2) Receptors

Nuoyu Huang
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PERSISTENT ORAL DYSKINESIAS INDUCED BY LONG-TERM HALOPERIDOL TREATMENT IS DISSOCIATED FROM CHANGES IN NEOSTRIATAL $B_{max}$ AND mRNA CONTENT FOR DOPAMINE $D_2$ RECEPTORS

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 Sciences

by
Nuoyu Huang M.D.
May 1995
APPROVAL

This is to certify that the Graduate Committee of

NUOYU HUANG

met on the

Twentieth day of March, 1995

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 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.

Richard M. Kratzer
Chair, Graduate Committee

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Signed on behalf of

the Graduate Council

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

Persistent Oral Dyskinesias Induced by Long-Term Haloperidol Treatment Is Dissociated from Changes in Neostriatal B_max and mRNA Content for Dopamine D_2 Receptors

by

Nuyou Huang

Due to the presumed associations of dopamine (DA) receptor supersensitivity phenomena in both long-term neuroleptic-treated tardive dyskinetic rats and neonatal 6-hydroxydopamine (n6-OHDA)-lesioned rats, we studied the influence of haloperidol on n6-OHDA-lesioned rats. At 3 days after birth rats received 6-OHDA-HBr (200 μg, bilateral intracerebroventricularly; desipramine pretreatment, 20 mg/kg, i.p., 1 h) or vehicle. Two months later haloperidol (1.5/kg/day x 2 days/week for 4 weeks, then 1.5 mg/kg/day, every day for 10 months) was added to the drinking water. Spontaneous oral activity of intact and n6-OHDA-lesioned rats receiving haloperidol was reached and maintained at significantly higher levels after 15 weeks of haloperidol treatment. Haloperidol treatment produced greater oral activity in n6-OHDA-lesioned rats as compared to intact rats. At 11 months there were 35.8±4.9 vs 18.4±2.1 oral movements in lesioned vs. intact rats receiving haloperidol. This high level of spontaneous oral activity was not attenuated by scopolamine and persisted in the lesioned rats for at least 8 months after haloperidol withdrawal. Reverse transcription polymerase chain reaction (RT-PCR) analysis of alternatively-spliced isoforms of DA D_2 (D_2s and D_2l) receptors showed that D_2s receptor mRNA levels of intact and n6-OHDA-lesioned rats receiving haloperidol were significantly elevated after 11 months of treatment and returned to normal level 8 months after haloperidol withdrawal. Similarly, the B_max for [3H]raclopride binding to striatal homogenates was significantly increased in intact and n6-OHDA lesioned rats receiving chronic haloperidol. The B_max was at the control level after 8 months of haloperidol cessation. D_2s and 5-HT_2c receptor mRNA levels were not altered by chronic haloperidol treatment. The effects of assorted receptor-specific drugs on oral activity were tested in our rats to study possible mechanisms underlying the regulation of oral activity. The findings of this study demonstrate that alterations at mRNA and receptor levels of DA D_2 receptors are not critical for maintaining persisting enhanced oral dyskinesias after long-term haloperidol treatment. The long-lasting stable high frequency of oral dyskinesias after haloperidol withdrawal in these rats provides a means for testing agents that have the potential to attenuate dyskinetic oral activity.
DEDICATION

To my parents for their love, encouragement and support
ACKNOWLEDGEMENTS

I would like to express my deepest appreciation to Dr. Richard Kostrzewa, my major advisor, who guided me step by step throughout this project. I thank him for his invaluable advice, guidance and friendship.

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<td>(1R,3S)-3-(1’-adamantyl)-1-aminomethyl-3-4-dihydro-5,6-dihydroxy-1H-2-benzopyran</td>
</tr>
<tr>
<td>cAMP</td>
<td>cyclic adenosine monophosphate</td>
</tr>
<tr>
<td>CGS 12066B</td>
<td>7-trifluoromethyl-4(4-ethyl-1-piperazinyl)pyrrolo[1,2-alquinoxaline</td>
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<tr>
<td>CNF</td>
<td>cerebrospinal fluid</td>
</tr>
<tr>
<td>CNS</td>
<td>central nervous system</td>
</tr>
<tr>
<td>CPu</td>
<td>caudate-putamen</td>
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<td>DA</td>
<td>dopamine</td>
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<tr>
<td>DEPC</td>
<td>diethyl pyrocarbonate</td>
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<td>DTG</td>
<td>1,3-di-o-tolylguanidine</td>
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<td>Epi</td>
<td>epinephrine</td>
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<td>GABA</td>
<td>r-aminobutyric acid</td>
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<tr>
<td>Hal</td>
<td>haloperidol</td>
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<tr>
<td>HVA</td>
<td>homovanillic acid</td>
</tr>
<tr>
<td>icv</td>
<td>intracerebroventricular</td>
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<tr>
<td>IR</td>
<td>immunoreactive</td>
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<tr>
<td>L-DOPA</td>
<td>L-dihydroxyphenylalanine</td>
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<tr>
<td>m-CPP</td>
<td>m-chlorophenylpiperazine</td>
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<td>MDL-72222</td>
<td>3-tropanyl-3,5-dichlorobenzoate</td>
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<td>NAS</td>
<td>Accumbens septi</td>
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<td>NE</td>
<td>norepinephrine</td>
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<td>n6-OHDA</td>
<td>neonatal 6-hydroxydopamine</td>
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<td>PCR</td>
<td>polymerase chain reaction</td>
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<td>RT-PCR</td>
<td>reverse transcription polymerase chain</td>
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reaction

SCH 23390  R-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride

QAR  quantitative autoradiography

SKF 38393  [(±)-1-phenyl-2,3,4,5-tetrahydro-(1H)-3-benzazepine-7,8-diol hydrochloride

TD  Tardive Dyskinesia

THIR  T-OH immunoreactive

UV  ultraviolet

TM  transmembrane

T-OH  tyrosine hydroxylase

5-HT  Serotonin

5,7-DHT  5,7-dihydroxytryptamine

5-HTP  L-5-hydroxytryptophan

5-HIAA  5-hydroxyindoleacetic acid

8-OH-DPAT  (±)-8-hydroxydipropylaminotetralin
Introduction

A. Central Dopamine Systems

Soon after the discovery of dopa decarboxylase enzymes dopamine (DA) was identified as an intermediate in the catecholamine synthesis pathway (Holtz et al., 1938). In 1958 Carlsson and colleagues correctly proposed that DA serves not only as a precursor of norepinephrine (NE) and epinephrine (Epi) synthesis, but also as a neurotransmitter in the brain (Carlsson et al., 1958). There are far more DA neurons than other catecholamine neurons in brain. In terms of the functional and anatomical organization, brain DA systems can be classified as follows (Ganong, 1991; Cooper et al., 1991):

1. The nigrostriatal dopaminergic system:

   DA cell bodies in the pars compacta of the substantia nigra have axonal projections to basal ganglia in the forebrain. This extrapyramidal system has a major role in regulating fine motor control. The destructive loss of nigral DA cells is correlated with a Parkinsonian syndrome.

2. The mesocortical dopaminergic system:

   DA cell bodies in the ventral tegmental nucleus and portions of the substantia nigra have axonal projections to the limbic cortex. This system is considered to have a major role in modulating thinking patterns and motivational state. An imbalance of this system might be expressed as neurosis,
psychosis or schizophrenic state.

3. The tuberoinfundibular, incertohypothalamic, and periventricular dopaminergic system:

Cell bodies of these three DA systems arise in or near the hypothalamus and have short axonal projects to adjacent brain regions, like the median eminence. DA, released by the tuberoinfundibular system, is the primary inhibitory regulator of prolactin hormone release. Other pituitary hormones, including growth hormone, are influenced in part by this DA system.

B. Dopamine Receptors and Behavior

DA receptors can be broadly classified into presynaptic receptors (autoreceptors) and postsynaptic receptors. Autoreceptors exist in most portions of DA cells such as the soma, dendrites and nerve terminals. Somatodendritic autoreceptors regulate the firing rate of dopamine neurons while terminal autoreceptors regulate the synthesis and release of DA. Therefore, autoreceptors can be defined as synthesis-modulating, release-modulating and impulse-modulating autoreceptors in terms of their functional roles (Cooper et al., 1991). Postsynaptic DA receptors are located postsynaptically to DA-releasing cell types.

The cDNAs of five DA receptor subtypes have been cloned (Sibley et al., 1993; Gingrich and Caron, 1993; Zhang et al., 1994). The most popular classification of DA receptors divides these five receptor subtypes into D<sub>1</sub>-like receptors
and D₂-like receptors, on the basis of their biochemical characteristics (Kebabian and Calne, 1979; Breese and Creese, 1986; Seeman and Van Tol, 1994).

1. D₁-like receptors:

D₁-like receptors include D₁ and D₅ subtypes. When DA binds to these receptors on postsynaptic neurons, there is generally an increased intracellular synthesis of cyclic adenosine monophosphate (cAMP) (Kebabian and Calne, 1979).

2. D₂-like receptors:

D₂, D₃, and D₄ DA receptor subtypes fall into the D₂-like receptor category. DA stimulation of these receptors generally inhibits the activity of adenylate cyclase and thereby reduces synthesis of intracellular levels of cAMP (Kebabian and Calne, 1979). D₂ receptors trigger signal transduction pathways in many ways that are different from that of D₁ receptors. In addition to their ability to inhibit the activity of adenylate cyclase, cloned D₂ receptors are able to stimulate phosphatidylinositol turnover (Vallar et al., 1990), regulate K⁺ and Ca²⁺ channel activity (Vallar et al., 1990; Castellano et al., 1993) and increase arachidonic acid release (Kanterman et al., 1991).

Autoreceptors can be classified into D₂-like receptors in terms of their biochemical and pharmacological characteristics (Cooper, 1991).

Behavioral studies of dopaminergic systems was initiated by Randrup and co-workers about 30 years ago.
The availability of relative specific DA receptor ligands enabled functional studies of the behavioral roles of DA receptor subtypes. The overall body of evidence suggests that DA D₁ and D₂ receptors are associated directly or indirectly with the mediation of behavior responses (Andersen and Nielsen, 1986). Central DA systems play important roles in the regulation of motor activities and mental functions (Ganong, 1991). Motor behaviors are usually considered to be under the control of nigrostriatal DA pathway (Ungerstedt and Arbuthnott, 1970; Ungerstedt, 1971) while mesolimbic and mesocortical DA pathways regulate emotional and reward behaviors (Creese and Iversen, 1973). The dysfunctions of nigrostriatal and mesocortical dopaminergic systems are linked to Parkinson's disease and schizophrenia, respectively (Ganong, 1991; Stevens, 1973). DA agonists and antagonists are effective agents in the treatment of these two types of neuronal disorders.

C. Neonatal 6-hydroxydopamine (n6-OHDA) Treatment and Supersensitivity of Central DA Systems

Removal of neuronal input is one of the important means to study the functions of a particular neuronal system (Kostrzewa and Jacobowicz, 1974). The remarkable discovery of Tranzer and Thoenen revealed that 6-OHDA, an isomer of NE, selectively destroyed peripheral catecholamine-containing neurons (Tranzer and Thoenen, 1967; Tranzer and
Thoenen, 1968). When adrenergic neurons are protected by desipramine pretreatment, 6-OHDA produces a selective lesion of the DA system (Breese and Traylor, 1972). Upon stimulated by DA agonists, behavioral responses, such as stereotyped behavior, locomotor and oral activities, are greatly enhanced in n6-OHDA-lesioned rats. This evidence indicates that central dopaminergic systems are supersensitized by n6-OHDA treatment (Breese et al., 1984; 1985a, 1985b; 1987; Kostrzewa and Hamdi, 1991; Kostrzewa and Gong, 1991; Hamdi and Kostrzewa, 1991, Gong et al., 1992, Kostrzewa and Neely, 1993).

D. Neonatal 6-OHDA Treatment and Number of DA Neurons in Rat Brain

Using an antibody to tyrosine hydroxylase (T-OH) Berger et al. (1985) found that the number of T-OH immunoreactive (THIR) cell bodies in pars compacta of the substantia nigra were too numerous to count in intact rats. They estimated >4000 THIR cells in this nucleus. In contrast, in n6-OHDA-lesioned rats (150 µg 6-OHDA, base) there were only 12 to 110 THIR remaining cells in this nucleus (mean of 63 cells). Similarly, unilateral intrastratal injection of 6-OHDA (8 µg) on postnatal day 2 (P2) caused a significant retrograde degeneration of THIR neurons in the substantia nigra of the lesioned side at P9 and P22 (Caboche et al., 1991).

Bilateral intracerebroventricular (icv) injection of 6-OHDA (100 µg each side) resulted in a marked loss of THIR
neurons in mesencephalon. The loss of THIR neurons was more severe in the substantia nigra pars compacta and in the ventral A9 cell group of the A9 complex while the A10 region and the lateral cell group of the A9 complex were less severe by affected (Luthman et al., 1990a). Yokoyama et al. (1993) reported that intracisternal injection of 6-OHDA (67 µg) on P1 induced degeneration of THIR neurons. The decrease of immunoreactive intensity and the appearance of dot-like THIR processes, was first found in the substantia nigra 12 h after the injection of 6-OHDA. The immunoreactive neurons at the substantia nigra were marginally detected 96 h after 6-OHDA. In contrast to the DA neurons at the substantia nigra, there was no detectable degeneration of THIR neurons or terminals in the hypothalamic arcuate nucleus and in the median eminence at least 96 h after the injection of 6-OHDA. In addition, when n6-OHDA-lesioned rat brain tissues were analyzed at 2-5 months after birth, it was found that intracisternal injection of 6-OHDA (67 µg) on P1 or unilateral intraparenchymal injection of 6-OHDA (8 µg on P3) produced almost complete depletion of THIR neurons in the substantia nigra and striatum. Less extensive changes of THIR neurons were found in the ventral tegmental nucleus, arcuate nucleus (A12), zona incerta (A13), periventricular area (A14) and posterior hypothalamic area (A11). In order to exclude the possibility that the difference in regional 6-OHDA
concentrations by intracisternal injection may contribute to the difference in the sensitivity of substantia nigra and hypothalamic THIR neurons to 6-OHDA toxicity, the same dose of 6-OHDA was injected at P1 by different routes (intralateral or third ventricles). Similarly, the degeneration of THIR neurons and fibers was found only in the substantia nigra and caudate putamen, not in the hypothalamus. Therefore, it is unlikely that the difference in sensitivity to 6-OHDA is due to a difference in the regional concentration of 6-OHDA (Yokoyama et al., 1993).

Differences in uptake-accumulation and/or intraneuronal factors that interfere with the auto-oxidation reaction of 6-OHDA may be responsible for differences in sensitivity to 6-OHDA (Jonsson, 1983). There are 2 to 3 times more high affinity dopamine uptake sites in nigrostriatal DA neurons than in tuberoinfundibular DA neurons (Annunziato et al., 1980).

E. Neonatal 6-OHDA Treatment and DA Uptake Sites

Using quantitative autoradiography (QAR) with [3H]mazindol as the ligand for high-affinity DA uptake sites (a DA terminal density marker), it was found that the developmental pattern for DA uptake sites was parallel to that of DA receptors. That is, patch compartments developed at an early stage in life and this was followed by maturation of the matrix compartment (Rao et al., 1991). Bilateral intrastriatal injection of 6-OHDA (4, 8, or 20 µg
each side) at P0 or P1 resulted in an extensive alteration in the number of DA uptake sites (Neal and Joyce, 1991, 1992; Neal-Beliveau and Joyce, 1993). n6-OHDA-lesions caused a dose-dependent patchy loss of high affinity DA binding sites (Neal-Beliveau and Joyce, 1993). There was an overall 22-75% reduction of [3H]mazinol binding sites in the striatum of the n6-OHDA-lesioned rats at low dose (4 μg each side) and intermediate dose (8 μg each side), whereas the high dose (20 μg each side) caused almost complete loss of [3H]mazindol binding sites (Neal-Beliveau and Joyce, 1993). The most extensive loss of [3H]mazindol binding sites was in the dorsomedial quadrant of the left striatum which showed a 43% loss of binding sites. Accumbens septi (NAS) was less affected, there being a loss of only 15% of binding sites (Neal and Joyce, 1992).

F. Neonatal 6-OHDA treatment and DA receptor mRNA

*In situ* hybridization and autoradiographic studies showed that DA D1 receptors are detectable at P1 in the caudate-putamen. D1 receptors density reaches adult levels at P16. The development and maturation of D1 receptors occurs later. DA D2 receptor binding can not be observed until P3 in the striatum and not until P7 in the globus pallidus and substantia nigra. The density of D2 receptors approaches adult level between P16 and P30 (Rao et al., 1991). Using slot bolt and *in situ* hybridization as tools, it was reported that D2 mRNA was detectable in striatum on
the day of birth, then increased gradually and reach peak levels at P16. The different peak time for slot blot and in situ hybridization may be due to the difference of D₄ mRNA expression at the subpopulations of the neurons. Similar to the DA receptor binding characteristics, the distribution of D₄ mRNA also showed a lateral to medial gradient in the striatum. Ontogenetic development of D₄ receptor mRNA was reported to be correlated to the expression of D₄ receptor (Chen and Weiss, 1991). In another study using polymerase chain reaction (PCR) techniques, it was reported that whole rat brain D₄ receptor (D₄M₄ and D₄M₈ receptors) mRNA was measurable at P14 and day P17 respectively and then increased to the adult level (Mack et al., 1991). In this study mRNA was not detected prior to P14. The possible of the expression of D₄ receptor mRNA prior to P14 can not be excluded. Therefore, there are dramatic ontogenetic changes in striatal D₁ and D₄ receptor densities and their mRNA levels. D₁ and D₄ receptor densities and mRNA levels increase after birth until P15 to P30, then decline slightly (Chen and Weiss, 1991; Mack et al., 1991; Xu et al., 1992).

The daily treatments of intact rats with SKF 38393 [(±)-1-phenyl-2,3,4,5-tetrahydro-(1H)-3-benzazepine-7,8-diol hydrochloride, 3.0 mg/kg/day, i.p.], for the first 28 days from birth did not alter the expression of D₁ mRNA in rat striatum when analyzed at 11 weeks of age. Similar ontogenetic SKF 38393 treatment of neonatal 6-OHDA-lesioned
rats (67 µg in each lateral ventricle at P3) significantly reduced rat striatal DA D₁ mRNA level. However, the level of DA D₁ mRNA was restored by 4 weekly SKF 38393 treatments (3.0 mg/kg/day, i.p) during the 6th through the 9th weeks after birth (Gong et al., 1994). It has been reported that the presence of dopaminergic neurons is required for the normal expression of D₁ receptors (Saleh and Kostrzewa, 1989). Also the loss of DA D₁ and D₂ receptor sites has been found after a neonatal 6-OHDA-lesion (Deskin et al., 1981; Gelbard et al., 1990). These series of studies suggest that endogenous DA might be important for the development of DA receptors (Gelbard et al., 1990; Saleh and Kostrzewa, 1989).

In other investigations the ontogenetic development of central DA systems was impaired either by icv injection of 6-OHDA (134 µg) on P3, daily injection of the D₁ receptor antagonist SCH 23390 [R-(+)
-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride, 0.25 mg/kg/day, i.p.] from P4 to P20, or the combined treatments with 6-OHDA and SCH 23390. When analyzed at P21, it was found that none of these three treatments was able to alter the level of D₁A receptor mRNA in both caudate-putamen and the nucleus accumbens. There was also no alteration in D₁ receptor binding ([³H]SCH 23390) (Duncan et al., 1993). Similarly, striatal D₂ mRNA levels in the brain of 16 and 32 day old rats was not altered by n6-OHDA (bilateral icv, 67
μg each side) treatment at P3 and P6 (Chen and Weiss, 1991). Striatal D₃ mRNA was found significantly increased (15%) after high dose of bilateral icv 6-OHDA treatment (150 μg each side) at P3 as assessed at 60 days after birth. The increase of D₃ mRNA was more obvious in the lateral than in the medial striatum. Lower dose of 6-OHDA (67 μg) did not cause change in D₃ mRNA level (Soghomonian, 1993). These findings do not support the view that dopaminergic input is a critical factor for postnatal ontogenetic expression of DA mRNA, although it is possible that central DA systems may be involved in striatal DA receptor development prior to n6-OHDA treatment. More work is needed to clarify the relationship between ontogenetic dopaminergic input and the expressions of DA receptor mRNAs and their proteins.

G. Neonatal 6-OHDA treatment and DA receptors

Autoradiography studies with specific ligands have revealed the ontogenetic developmental characteristics of DA receptors (Rao et al., 1991; Cabeche et al., 1991; Murrin and Zeng, 1989). Using [³H]SCH 23390 as a ligand, clear heterogeneous patchy binding was found in rat striatum at P1, most obviously in the rostrocaudal extension of the lateral two-thirds of the striatum. At P3 and P4, the patchy distribution of [³H]SCH 23390 binding was observed in the whole latero-medial and rostro-caudal extension of the striatum. The distribution of D₃ patches was parallel to that of the THIR staining. Beginning from P9, there was
dissociation between the relative distribution of [³H]SCH 23390 binding and THIR staining. Patchy binding was observed only in the ventral part, not the dorsal part of the striatum at this age. However, THIR fibers were present to a greater extent in the dorsal vs. ventral striatum. By 22 days after birth [³H]SCH 23390 binding and THIR staining were found homogeneously distributed in the whole striatum. (Caboche et al., 1991). This suggests that there is a separate organization of patchy and matrix compartments during development. The density of D₁ receptor was 16% of the adult level at P1 and reached 38% at P10. Afterwards, there was a dramatic elevation of D₁ density between P10 to P16. In contrast, the development characteristics of D₂ receptors is different from that of D₁ receptors. D₂ receptors were not detectable until P3 and reached the adult level between P16 to P30 (Rao et al., 1991). These differences in the ontogenetic development of these DA receptor subtypes may give rise to their difference of vulnerability to 6-OHDA toxicity. Therefore, the time at which neonatal lesion is performed may be critical for changes in DA receptor subtypes.

The effects of 6-OHDA depletion on central DA systems and DA receptors are highly controversial. It was found that DA receptors were unaltered (Breese et al., 1987; Dewar et al., 1990; Hamdi and Kostrzewa, 1991; Kostrzewa and Hamdi, 1991; Caboche et al., 1991), decreased (Duncan et
al., 1987; Dewar and reader, 1989; Neal and Joyce, 1992; Neal-Beliveau and Joyce, 1993; Radja et al., 1993) or increased (Broaddus and Nennett, 1990) in adult rats after the depletion of central DA systems with n6-OHDA treatment.

In a study involving n6-OHDA treatment (67 μg, intracisternally) of rats at P3, autoradiography with the DA D₁ ligand [³H]SCH 23390 revealed no change from control, of [³H]SCH 23390 binding in dorsolateral striatum, dorsomedial striatum, ventrolateral striatum, ventromedial striatum and nucleus accumbens (Simson et al., 1992). When rats were observed at P9 and P22, Caboche et al. (1991) demonstrated that unilateral intrastriatal injection of 6-OHDA (8 μg) at P2 did not cause any alteration in the [³H]SCH binding pattern and density at lesioned and unlesioned sides of ventral, dorsal and whole striatum. This treatment resulted in a significant decrease of THIR fibers in the striatum and a retrograde degeneration of THIR neurons in the substantia nigra. Because of the dissociations between THIR fiber distributions and [³H]SCH 23390 binding patterns, the authors suggested that ontogenic development of D₁ receptors may not be depend upon the presynaptic DA elements (Caboche et al., 1991).

In contrast to the above results, bilateral intrastriatal injection of 6-OHDA (4, 8, or 20 μg each side) at P0, P1 or P2 produced significant loss of [³H]SCH 23390 binding of D₁ receptors in the striatum. This patchy
reduction of D₁ receptor binding was not dose-dependant. The loss of D₁ binding was preferential to the dorsomedial caudate-putamen (CPu) and the patchy compartment. In contrast to the changes in D₁ receptor subtypes, this treatment did not cause a significant alteration in striatal D₂ receptor number. Although the reduction of D₃ receptor binding density was greater in the medial CPu than that in the central and lateral CPu regions, the normal lateral to medial gradient D₃ binding characteristic in the striatum was not altered by intrastriatal n6-OHDA treatment. The absence of change in D₃ receptors may due to the fact that D₃ receptors are not present in the striatum at the time of 6-OHDA treatment (Neal and Joyce, 1992; Neal-Beliveau and Joyce, 1993). Radja and associates (1993) reported that bilateral icv injection of 6-OHDA (50 μg each side) at P3 caused significant changes in both D₁ and D₃ receptors as determined by autoradiography. D₁ receptor binding ([³H]SCH 23390) in the rostral striatum was significantly decreased whereas there was no marked alteration in the caudal striatum. DA D₂ receptor binding ([³H]raclopride) was significantly increased (23-27%) in all parts of the caudal striatum and most parts (dorsolateral, dorsomedial and ventrolateral) of rostral striatum (except ventromedian quadrants of the rostral striatum). In the substantia nigra, the density of D₂ binding was dramatically (80%) elevated. In situ hybridization study revealed that there
was no alteration in the amount of D<sub>2</sub> receptor mRNA in the striatum of n6-OHDA-lesioned rats vs control rats.

Using [\(^{3}H\)]raclopride as a ligand, Dewar et al (1990) found that n6-OHDA treatment was not associated with an alteration in 

\[ B_{\text{max}} \] or \[ K_{d} \] for DA D<sub>2</sub> receptors in the rostral striatum at 15 days and 1 month of age. However, at 3 months the \[ B_{\text{max}} \] was increased by 25%. In caudal striatum the \[ B_{\text{max}} \] and \[ K_{d} \] of [\(^{3}H\)]raclopride binding did not change at these intervals after n6-OHDA treatment. Broaddus and Bennett (1990) applied 3 different protocols for the n6-OHDA lesion to obtain selective damage of DA systems: (1). Bilateral intrastriatal (i.s.) injection of 6-OHDA (20 \( \mu \)g) at P2 to cause selective destruction of the 'patches'; (2). Four weekly (1 to 4 weeks) injections (i.s.) of 6-OHDA (20 \( \mu \)g) to destroy DA terminals in both patchy and matrix systems; (3). Intracisternal injection of 6-OHDA (120 \( \mu \)g) at P3 to cause extensive damage of midbrain DA neurons. It was found that all these treatment protocols resulted in an increase (15-40%) in the \[ B_{\text{max}} \] of SCH 23390 binding in the striatal homogenates. The first and second protocols also led to an increase of \[ K_{d} \] for SCH 23390 binding to D<sub>1</sub> receptors. This may implicate a depletion supersensitivity of D<sub>1</sub> receptors. However, these lesion protocols decreased D<sub>2</sub> receptor density (\[ B_{\text{max}} \]) in the striatal homogenates, using [\(^{3}H\)](-) sulpiride as ligand. These results indicate that the development of D<sub>2</sub> receptors is probably regulated by DA
innervations (Broaddus and Bennett, 1990).

Receptor binding studies indicate that n6-OHDA treatment supersensitizes DA D_1 receptors without altering D_1 receptor density ($B_{mm}$) and affinity ($K_a$) (Hamdi and Kostrzewa, 1991; Luthman et al., 1990b; Duncan et al., 1987). It is known that DA D_1 receptors have high and low affinity states of agonist binding (Leff et al., 1985; Seeman et al., 1985). No change in the distribution of D_1 receptor high and low agonist binding sites was found in n6-OHDA-lesioned rats (Gong et al., 1994).

The mechanisms underlying the changes in DA receptors after n6-OHDA are still unclear. It was proposed that the depletion of endogenous DA may contribute to the DA receptor changes after n6-OHDA treatment (Gelbard et al., 1990). It was found that in intact rats, there was a negative correlation between the densities of DA receptor binding and the levels of DA in forebrain tissue. This inverse correlation was eliminated by daily administration of exogenous DA (SKF 38393). Similarly, the changes of D_1 receptor binding induced by n6-OHDA treatment was restored by SKF 38393. These findings indicate that there is an important role of endogenous DA in regulating D_1 receptor development (Gelbard et al., 1990). In adult animals, transplantation of fetal mesencephalic DA neurons did not reverse the loss of DA receptors (Blunt et al., 1992). The developmental stage at which the central DA system is
depleted is an important determinant of the changes in DA receptors by n6-OHDA treatment (Neal and Joyce., 1992; Neal-Beliveau and Joyce., 1993). Furthermore, DA receptor supersensitization in n6-OHDA-lesioned rats can not be simply explained by changes in either DA receptor binding parameters or the expressions of DA receptor mRNAs. It has been suggested that an imbalance among DA, 5-HT and other neurotransmitter systems underlies the phenomenon of DA receptor supersensitivity (Gong et al., 1994; Kostrzewa and Neely, 1993, Kostrzewa, 1995).

H. Anatomy of Central Serotonin Pathways

Serotonin (5-HT) was isolated and synthesized and its chemical structure was defined in late 40’s and early 50’s (Hamlin and Fisher, 1951; Rapport, 1949; Rapport et al., 1948). The significant abundance of 5-HT in various regions of the central nervous system (CNS) suggests that it is a major CNS transmitter (Amin et al., 1954). The discovery of the involvement of 5-HT in the major psychoses (Jacobs, 1984) led to a great deal of interest in this neurotransmitter. Although the numbers of 5-HT cells in the CNS constitute only about 1/1,000,000 of all CNS neurons, the influence and regulation of 5-HT systems on their target sites seem to be much greater than their numbers (Jacobs and Azmitia, 1992).

The 5-HT neurons can be defined into superior and inferior groups that are located in the brain stem around
the midline (Jacobs and Azmitia, 1992). Projections of 5-HT axons from cell bodies in the dorsal and medial raphe located in the midbrain innervate various regions of the brain such as substantia nigra, striatum, mesolimbic system, hippocampus, hypothalamus and forebrain region (Meltzer and Nash, 1991).

5-HT projections to substantia nigra and striatum are originate from the rostral potion of the dorsal raphe (Imai et al., 1986; Molliver, 1987). Afferent 5-HT fibers from both dorsal and medial raphe innervate mesolimbic regions such as the ventral tegmentum (Simon et al., 1979; Parent et al., 1981). Medial raphe 5-HT neurons project to the hippocampus (Moore and Halaris, 1975). 5-HT fibers arising from both the dorsal and medial raphe pass through the hypothalamus and innervate forebrain regions (Meltzer and Nash, 1991).

I. Serotonin Receptors and their Functional Roles

The latest classification of Serotonin Club Receptor Nomenclature Committee defined 5-HT receptors into seven classes base on three fundamental properties: operational (drug-related), transductional (receptor-coupling) and structural (primary amino acid sequence) characteristics (Humphrey et al., 1993; Martin and Humphrey, 1994). The characteristics of each 5-HT receptor class are outlined as follows:

1. The 5-HT₁ receptor
Five 5-HT receptor subtypes are recognized as 5-HT₁ receptors. These include 5-HT₁A, 5-HT₁B, 5-HT₁D, 5-HT₁F and 5-HT₁G. They all have seven transmembrane (TM) domains and are linked to the inhibition of adenylate cyclase (Martin and Humphrey, 1994).

a. 5-HT₁A receptors

This subtype of 5-HT receptors is located primarily in the limbic system, with a lesser extent in dorsal and median raphe nuclei. These regions regulate mood, anxiety, temperature, feeding and locomotion. 5-HT₁A receptors are important for the action of antipsychotic drugs (Meltzer and Nash, 1991; Martin and Humphrey, 1994).

b. 5-HT₁B receptors and 5-HT₁D receptors

5-HT₁B receptors are unique in rats and some other rodents. These receptors are not present in humans. The exact physiological functions of 5-HT₁B and 5-HT₁D receptors are not clear, because of the lack of specific ligands. They may function either as autoreceptors or regulate the release of acetylcholine, glutamate or other neurotransmitters. Substantia nigra and basal ganglia are two areas with the highest densities of 5-HT₁B and 5-HT₁D receptors (Engel et al., 1986, Maura and Raiteri, 1986).

c. 5-HT₁D and 5-HT₁F receptors

The distribution of 5-HT₁D receptors is similar to that of 5-HT₁F receptors in the CNS (Leonhardt et al., 1989). However, unique distributions of 5-HT₁F receptors, which
include the pyramidal cells of the cortex and, the dorsal raphe and the hippocampus, have been revealed (Amlaikey et al., 1992; Martin and Humphrey, 1994). The physiological roles of these two receptor subtypes are yet to be determined. There are still no selective ligands for these receptor subtypes.

2. 5-HT$_2$ receptor

5-HT$_2$ receptors consist of three subtypes, namely 5-HT$_{2A}$, 5-HT$_{2B}$ and 5-HT$_{2C}$ receptors. They all have seven TM and are coupled to phospholipase C and increased production of IP$_3$ (Hoyer et al., 1994; Martin and Humphrey, 1994).

a. 5-HT$_{2A}$ and 5-HT$_{2B}$ receptors

5-HT$_{2A}$ receptors exist primarily in cortex, and to a lesser extent in hippocampus and caudate nuclei (Hoyer et al., 1986). These receptor subtypes are known to have regulating roles for motor activity and sleep (Sharpley et al., 1990). 5-HT$_{2B}$ receptors are found only in peripheral tissues, such as the stomach fundus (Kursar et al., 1992).

b. 5-HT$_{2C}$ receptors

5-HT$_{2C}$ receptors distribute with highest density in choroid plexus and to a lesser extent in the limbic system, basal ganglia, hypothalamus and hippocampus (Pazos et al., 1987; Julius et al., 1988; Molineaus et al., 1989). 5-HT$_{2C}$ receptors have been proposed as having a regulating role for oral activity (Gong et al., 1992; Kostrzewa and Gong, 1991), locomotion, eating and anxiety (Kennett and Curzon, 1988;
Kennett et al., 1989; Lucki, 1992).

3. 5-HT₁ receptors

5-HT₁ receptors are structurally and functionally distinct from all other 5-HT receptors. In fact, these 5-HT receptors are ligand-gated cation channels (Martin and Humphrey, 1994). In the CNS 5-HT₁ receptors are found in the area postrema, the nucleus tractus solitarius, the dorso-vagal complex and the trigeminal nucleus caudalis (Waeber et al., 1988; Kilpatrick et al., 1990). 5-HT₁ receptors modulate the release of other neurotransmitters such as DA (Blandina et al., 1988) and γ-aminobutyric acid (GABA) (Alhaider et al., 1991).

4. 5-HT₄ receptors

The structure of 5-HT₄ receptors is currently unknown. In the CNS, activation of 5-HT₄ receptors in the hippocampus elevates the formation of cAMP (Dumuis et al., 1988) and decrease K⁺ conductance (Andrade and Chaput, 1991). It was shown that 5-HT₄ receptors are also localized in the striatum, substantia nigra and olfactory tubercle of rats and guinea-pigs. However, the functional role of 5-HT₄ receptors remains to be determined (Grossman et al., 1993).

5. Other 5-HT receptors

5-HT₅, 5-HT₆, and 5-HT₇ receptors are the newest numbers of the 5-HT receptor family. Their genes were cloned recently (Plassat et al., 1993; Monsma et al., 1993; Ruat et al., 1993). It has been proposed that 5-HT₅ receptors
feature seven TM and are coupled to G-protein. Their second messenger system is unknown. Very little is known about the functional roles of 5-HT₁, 5-HT₄, and 5-HT₇ receptors (Martin and Humphrey, 1994).

J. Neonatal 6-OHDA Treatment and 5-HT Fiber Sprouting in Rat Striatum

The 5-HT content of the striatum of rats treated neonatally with 6-OHDA (100-200 μg; desipramine pretreatment) has been found to be elevated by 50 to >100% (Breese et al., 1984; Stachowiak et al., 1984). A 20% reduction in size of the striatum could not account for the elevation in striatal 5-HT content in 6-OHDA-treated rats. Further study revealed that 5-HT content of caudal striatum remained unchanged whereas 5-HT content of rostral striatum was elevated as much as 4 to 5-fold (Stachowiak et al., 1984; Dewar et al., 1990). The elevation in 5-HT content of rostral striatum was accompanied by a proportionate increase in the [³H]5-HT accumulation by striatal homogenates (Stachowiak et al., 1984). These findings indicated that the loss of DA innervation of the striatum did not influence the 5-HT fiber innervation of caudal striatum, which has a 5-HT content and 5-HT fiber input that is 3 to 6 times that of rostral striatum. However, the loss of DA input to the striatum seemed to induce heterotypic sprouting and hyperinnervation of rostral striatum by 5-HT fibers.
Using an antibody to 5-HT, it was shown that n6-OHDA treatment was associated with a several-fold increase in the number of 5-HT-immunoreactive (IR) fibers in rostral striatum. The fine and highly varicose fibers increased in number by only a slight amount in caudal striatum (Snyder et al., 1986; Luthman et al., 1987).

The change in 5-HT content in rostral striatum of n6-OHDA rats was not accompanied by a change in 5-HT content of the olfactory tubercle or septum (Stachowiak et al., 1984), nor frontal cortex, occipital cortex, mesencephalon, raphe nucleus or cerebellum (Luthman et al., 1987). A 30% increase in 5-HT was measured in the cingulate cortex, 3 months after n6-OHDA (Dewar et al., 1990). Numbers of 5-HTIR fibers in the basal forebrain, septum, cortex and substantia nigra were also not altered (Snyder et al., 1986).

K. Neonatal 6-OHDA treatment and 5-HT Receptors

In the striatum of intact and n6-OHDA-lesioned rats 5-HT$_{1A}$ receptors are not present. The low numbers of 5-HT$_{1B}$ and 5-HT$_{1A}$ receptors were increased in the rostral striatum of n6-OHDA-lesioned rats by 30% and 60%, respectively. 5-HT$_{1nonAB,2C}$ receptors, which were most abundant in the striatum of intact rats, were increased by 35% in rostral striatum of n6-OHDA-lesioned rats (Radja et al. 1993).

In the caudal striatum, as in rostral striatum, 5-HT$_{1B}$ and 5-HT$_{1nonAB,2C}$ receptors were increased by 30 to 35%, while
5-HT\textsubscript{1A} receptors were not altered in numbers in n6-OHDA-lesioned rats. Because numbers of 5-HT\textsubscript{1A} receptors and numbers of 5-HT fibers are increased in rostral striatum of n6-OHDA rats, it was suggested that 5-HT fiber proliferation accounted for the change in 5-HT\textsubscript{1A} receptor number (Radja et al., 1993).

There was no change in the number of 5-HT\textsubscript{1A} receptors in lateral septum, CA1 area, dentate gyrus and dorsal raphe of n6-OHDA-lesioned rats. Similarly, there was no change in the number of 5-HT\textsubscript{1A} receptors in claustrum and nucleus accumbens of n-6-OHDA-lesioned rats. It was suggested that the cumulative upregulation of assorted 5-HT receptor subtypes would effectively enhance actions of synaptic 5-HT, even if the synaptic content of 5-HT was the same as in intact rats (Radja et al., 1993).

L. Tardive Dyskinesia (TD)

TD is a syndrome of involuntary, repetitive and purposeless hyperkinetic abnormal movements that occur during or after chronic neuroleptic treatment. Orofacial regions and sometimes the limbs and trunk are most commonly involved (Waddington, 1990; Jeste and Caligiuri, 1993).

TD was first described by Schonecker (1957), five years after the introduction of the neuroleptic chlorpromazine as a treatment of psychosis. The term "tardive dyskinesia" was first used by Faurbye et al. in 1964 to describe this late-developing movement disorder (Faurbye et al., 1964). Long-
term use of neuroleptics is the major risk factor for TD (Jeste and Caligiuri, 1993).

The reported prevalence of TD varies from 0.5 to 100%. This wide range may be due to the differences in risk factors, treatment characteristics and diagnostic criteria. Conservatively, it is usually agreed that the prevalence of TD is between 15 to 20%. In high risk patients, such as the elderly, the prevalence is as much as 70% (Casey, 1987). M. Risk Factors for TD

1. Age and Gender

Age is the primary risk factor for TD, with there being a positive correlation between the prevalence and severity of TD with age (Smith and Baldessarini, 1980; Jeste and Wyatt, 1982b). Age-related degeneration of the nigrostriatal DA system may be one underlying basis of this phenomenon (Jeste and Caligiuri, 1993). A higher incidence with greater severity of TD occurs in females (Casey, 1987).

2. Psychiatric diagnosis

Neuroleptic-treated schizophrenic patients are less likely to develop TD than neuroleptic-treated mood disorder patients (Casey and Keepers, 1988; Mukherjee et al., 1986).

3. Organic brain damage

Since TD is the consequence of treatment of patients with mental disorders, it is possible that CNS damage may be a risk factor for TD (Yassa et al., 1984). There is some disagreement with this view (Gold et al., 1991). In future
studies it would be valuable to assess the relationship between TD and brain damage.

4. Other factors

There is reportedly a higher incidence of TD in people that smoke or have diabetes. Asians have the lowest incidence of TD, indicating that ethnicity or genetic differences may represent risk factors for TD (Jeste and Caligiuri, 1993; Yassa et al., 1987).

N. Tardive Dyskinesia and Central DA Neurons

Up until recently the most widely-accepted hypothesis on the origin of TD was that TD was related to development of DA receptor supersensitivity. This was based mainly on the knowledge that movements disorders are a consequence of DA receptor block in the nigrostriatal system. It is extremely difficult to conduct postmortem studies on TD patients, since this is a neuroleptic-induced disorder and the therapy is often sufficiently variable so that it is difficult to reasonably group patients into subsets. Also, residual neuroleptics in these patients would still exert a block of brain DA receptors. Therefore, most evidence on the pathophysiology of TD are derived from animal models (Casey, 1987).

In studies on intact and/or n6-OHDA-lesioned rats it has been found that oral activity is induced by DA D₁ agonists (SKF 38393 and A77636) and DA D₂ antagonists (sulpiride, spiperone and haloperidol). Therefore, it
appears that an imbalance of D1/D2 receptor activity plays a prominent role in the induction of oral activity (Rosengarten et al., 1983; Gong et al., 1992; Huang and Kostrzewa, 1994b). In studies on rats treated long-term with neuroleptics, an increase in the Bmax and Kd for DA receptors has been reported (Chiu et al., 1981; Rupniak et al., 1984). These associated behavioral and biochemical indices are supportive of the hypothesis that an overactive DA system may contribute to the pathophysiology of TD (Klawans, 1973; Chiu et al., 1981; Rupniak et al., 1984; Jeste and Caligiuri, 1993).

In other subsequent studies, there have been findings that conflict with those just described. Waddington et al. (1983), using direct measurements on postmortem brain from humans with TD, found that there was a dissociation between behavioral and DA receptor changes. Blin et al. (1989), using positron emission tomography on brains of humans with TD, also found a discrepancy between oral dyskinesias and changes in DA receptors. Recently, attention has been focused on a multi-neurotransmitter theory of TD, in which there is a seeming imbalance between regulation by different kinds of neurons (Casey, 1987; Waddington, 1990; Jeste and Caligiuri, 1993).

O. Regulation of Oral Activity in Rats by Multiple Neurochemical Systems

It is well-established that DA neuronal systems have an
integral role in regulating oral activity in rats (Rosengarten et al., 1983; Gong et al., 1992; Kostrzewa and Gong, 1991; Kostrzewa and Hamdi, 1991; Huang and Kostrzewa, 1994a). Serotonergic (Stewart et al., 1989; Gong et al., 1992; Kostrzewa et al., 1992), cholinergic (Rupniak et al., 1983, 1985a; Salamone et al., 1990), GABAergic (Gunne et al., 1984; Morelli et al., 1985; Lloyd et al., 1985) and peptidergic (Liminga, 1993) neurons are also involved in regulating oral activity.

Acute treatment with the cholinergic agonist pilocarpine significantly increases oral activity in rats. This effect is produced when pilocarpine is administered directly into the ventrolateral striatum via a cannula implant. Also, when pilocarpine or the cholinesterase inhibitor physostigmine is administered in drinking water for three weeks, there is an increased incidence of oral activity (Rupniak et al., 1985b). The effect of pilocarpine and physostigmine in this last study was attenuated by the muscarinic receptor antagonist, scopolamine (Salamone et al., 1990). The acute treatment of chronic haloperidol-treated rats with pilocarpine or physostigmine results in an increased number of oral movements in rats (Rupniak, 1983). It is yet to be determined, however, whether cholinergic-induced oral activity represents an oral dystonia or an oral dyskinesia.

It is possible that GABA agonists have a therapeutic
effect on dyskinesia, since GABA agonists interact with DA systems to alter behavioral responses (stereotyped and dyskinetic movements) induced by lesions or DA agonists (Gunne et al., 1984; Morelli et al., 1985). It was reported that the GABA agonists muscimol and progabide have beneficial effects on TD patients (Lloyd et al., 1985; Morelli et al., 1985; Tamminga et al., 1979). In animal studies, a microinfusion of the GABA antagonist bicuculline and GABA-depleting agent isoniazid bilaterally in the substantia nigra greatly increased oral activity of rats (Gunne et al., 1988). GABA agonists attenuated oral activities of cats, monkeys and rats (Lloyd et al., 1985; Mithani et al., 1987). These findings suggest that an impaired GABA system may be involved in abnormal oral activities (Mithani et al., 1987).

Central (intranigral) or peripheral administration of receptor agonists [1,3-di-o-tolylguanidine (DTG) and (+)-pentazocine] or the receptor antagonist naloxone resulted in increased oral activity in rats. This may be related to a release of DA in the striatum (Patrick et al., 1993; Cancela et al., 1988). It is also known that the intranigral infusion of the neurokinin NK₁ agonist substance P and NK₂ agonist neurokinin A produce dose-related increases in purposeless oral activity in rats. These findings are compatible with the view that central neuropeptides are involved in the regulation of oral
activity (Liminga, 1993).

Extensive studies have been conducted to explore the effects of DA 5-HT and cholinergic systems in regulating oral activity in neonatal 6-OHDA-lesioned rats. In n6-OHDA-lesioned rats, there were enhanced oral activity responses to the DA D₁ agonist SKF 38393, the 5-HT₂ agonist m-chlorophenylpiperazine (m-CPP) and cholinergic agonist pilocarpine (Kostrzewa and Gong, 1991; Gong and Kostrzewa, 1992; Gong et al., 1992; Kostrzewa and Neely, 1993). These findings indicate that there is co-sensitization of DA, 5-HT and cholinergic systems in n6-OHDA-lesioned rats (Kostrzewa, 1995). Of the several 5-HT agonists that were tested in these rats, only the 5-HT₂₃ agonist m-CPP induced oral activity. The 5-HT₁₆ agonist (±)-8-hydroxydipropylaminotetralin (8-OH-DPAT) and 5-HT₁₆ agonist 7-trifluoromethyl-4(4-ethyl-1-piperazinyl)-pyrrolo[1,2-alquinoxaline] (CGS 12066B) did not increase the incidence of oral activity. Furthermore, the enhanced oral response to m-CPP was attenuated by the 5-HT₂₃ receptor antagonist mianserin, but not by the 5-HT₁₆,₁₅ antagonist pindolol, 5-HT₂₆ antagonist ketanserin or 5-HT₁ antagonist MDL-72222 (3-tropanyl-3,5-dichlorobenzoate). These findings indicate that m-CPP-induced oral activity is mediated by 5-HT₂₆ receptors and not the other 5-HT receptor subtypes (Gong et al., 1992).

In other studies on n6-OHDA-lesioned rats it was
reported that the DA D₁ receptor antagonist SCH 23390 did not attenuate m-CPP-induced oral activity, but that the 5-HT receptor antagonist mianserin attenuated oral activity induced by the DA D₁ agonist SKF 38393. This indicates that DA agonist-induced oral activity is mediated by 5-HT₂c receptors (Gong et al., 1992). In a later study it was found that the muscarinic receptor antagonist scopolamine effectively blocked the oral activity response to both SKF 38393 and m-CPP, but that SCH 23390 and mianserin did not block the response to pilocarpine. This finding indicates that oral activity induced by DA and 5-HT systems are mediated by cholinergic systems. Therefore, these series of studies indicate that DA neurons are acting via 5-HT neurons which are acting via cholinergic neurons (Kostrzewa and Neely, 1993).

P. Rationale

The introduction of neuroleptics into clinical practice in 1952 was a milestone in the treatment of psychiatric disorders. However, the beneficial effects of these drugs were soon found to be counterbalanced by the appearance of the serious syndrome known as tardive dyskinesia (TD). The underlying basis of this adverse effect is still not known. An earlier theory on the origin of TD was assumed to be related to the development of DA receptor supersensitivity (Klawans and Hitri, 1978; Chiu et al., 1981). Today the multi-neurotransmitter theory, based on the involvement of
many neurochemical systems (Waddington, 1990; Caligiuri, 1993), seems to be more in vogue. Waddington (1990) has critically reviewed the literature. Nonetheless, there has been a steady increase in the incidence of TD by about 1% per year during the past 20 years (Casey, 1987; Jeste and Caligiuri, 1993).

Animal models that mimic the pathophysiology of human TD are useful for studying putative mechanisms associated with spontaneous oral activity and in studying potentially useful drugs for TD. Non-human primates (Gunne et al., 1984; Kovacic and Domino, 1984) and rodent models of TD (Ellison and See, 1989; Waddington, 1990) have been used. The most common animal model is chronic neuroleptic (usually haloperidol)-treated rats.

There is still no single animal model which reproduces the exact phenomenology of TD, particularly the characteristic oral movement found in TD patients. This may be related to the fact that neuroleptics are usually administered to psychotics, who are known to have an altered disturbance in the CNS. In contrast, the animals receiving the neuroleptic are normal healthy rodents (Waddington, 1990).

In rats in which the neostriatal DA innervation has been destroyed early in postnatal ontogeny by 6-OHDA, the incidence of oral activity is greatly increased by DA agonists (Kostrzewa and Hamdi, 1991; Kostrzewa and Gong,
1991) and 5-HT agonists (Gong et al., 1992). Rats lesioned neonatally with 6-OHDA have been proposed as a better model for TD (Gong et al., 1992; Huang and Kostrzewa, 1994b).

In order to test this hypothesis the following study was performed. Haloperidol was added to the drinking water of intact and neonatal 6-OHDA-lesioned rats for nearly 1 year, while spontaneous and agonist-induced oral activity was observed at one or two week intervals. The objective was to determine whether:

a. persistent oral dyskinesias might be induced in haloperidol-treated 6-OHDA-lesioned rats,
b. mRNA levels of DA D₄ and 5-HT₁C receptors might be altered to different extents in intact and 6-OHDA-lesioned haloperidol-treated rats,
c. DA D₄ receptor number might be altered by haloperidol treatment of intact and 6-OHDA-lesioned rats and
d. receptor-specific compounds might attenuate spontaneous oral activity in treated rats.

This study was expected to lead to a better understanding of the pathophysiology of TD, particularly of the roles of DA and other neurotransmitter systems in regulating oral activity. More significantly, there was the potential for inducing long-lived spontaneous oral dyskinetic activity in rats, so that putative drugs for TD could be reasonably tested for their ability to attenuate
TD.
Chapter 2
Materials and Methods

A. Animal Treatment

1. Animal

Time-pregnant Sprague-Dawley albino rats were purchased from Charles River Laboratories (Research Triangle, NC) approximately 1 week before delivery and housed singly in clear Perspex cages in a room with constant temperature (21±1°C) and 12 h light-dark cycle (lights on at 07:00). Animals had free access to food and water. The date of birth was considered as postnatal day 0. The time of birth was recorded within 12 hr. Litters were reassigned at birth, so that each reconstituted litter consisted of about equal number of pups from 10 different litters, for a maximum of 10 pups per litter.

2. 6-OHDA Treatment

At 3 days after birth all rat pups were pretreated with desipramine hydrochloride (20 mg/kg i.p., base form; Sigma Chemical Co., St. Louis, MO, USA), 1 hr before bilateral intracerebroventricular (icv) administration of 6-OHDA. The desipramine was intended to block NE transporters and protect NE-containing neurons from 6-OHDA toxicity, thereby increasing the selectivity of 6-OHDA for DA-containing neurons. 6-OHDA HBr (100 μg, salt form, on each side; Research Biochemicals Inc., Natick, MA, USA) was delivered
via a microliter syringe equipped with a 26-gauge needle having a polyethylene sleeve up to 2 mm from the tip of the needle. The injection was positioned 1.5 mm anterior to lambda and 2 mm lateral to the sagittal plane. The needle was left in place for about 30 seconds after 5 µl of 6-OHDA HBr solutions or 0.1% saline-ascorbic acid vehicle was injected into the lateral ventricle. This procedure has been described in detail (Kostrzewa and Gong, 1991). Rats were weaned at 28 days and group housed in wire cages. Only males were used in this study.

3. Haloperidol Treatment

Starting 2 months after birth about half the rats in the 6-OHDA and vehicle groups received haloperidol (1.5 mg/kg/day) via drinking water, for 2 consecutive days a week for 4 weeks. Starting in the fifth week haloperidol was administered on a continuous schedule (1.5 mg/kg/day) for 10 months. Thus, the study consisted of four groups: (1) non-lesioned rats with tap water as drinking water, (2) non-lesioned rats with haloperidol in drinking water, (3) 6-OHDA-lesioned rats with tap water as drinking water, and (4) 6-OHDA-lesioned rats with haloperidol in drinking water. The dose of haloperidol in this study reputedly produces plasma levels of haloperidol that correspond to that of people treated long-term with haloperidol (Tamminga et al., 1990). Rats were weighed once a week to adjust the dose of haloperidol.
4. Observation of Oral Activity

Spontaneous oral activity was observed one week before instituting haloperidol treatment, then every 1 or 2 weeks until the end of the study. A variety of receptor-specific drugs for different neurotransmitter systems were tested for their effect on oral activity, during and after the phase of haloperidol treatment. These included drugs selective for DA, serotonin, cholinergic, GABAergic and glutamatergic systems. Also, oral activity dose-effect curves for the DA D₁ agonist SKF38393 and 5-HT₁ agonist m-CPP were constructed for all groups of rats at 12, 36 and 38 weeks after haloperidol treatment. Haloperidol was withdrawn after 11 months of treatment. Eight or nine days after withdrawing haloperidol all rats were killed, except for 9 of the 6-OHDA-lesioned group that had received haloperidol for the past 11 months. Oral activity in these rats continued to be observed for the following 8 months.

Oral activity determinations were performed in a well-ventilated and well-lighted room. Rats were placed individually in clear plastic cages (48 X 26 X 36 cm) with steel grid floors and acclimated for at least 30 min. Agonists or saline vehicle were administered ip to each rat (1 mg/kg) 10 min before observation. Antagonists or vehicle were administered ip 1 hour before observation. All observation sessions were conducted with 9 rats per group except when deaths reduced the pool size in 6-OHDA-lesioned
haloperidol-withdrawn rats. Numbers of oral movements were counted in each rat by an experienced observer, for 1 min every 10 min during a 60 min session. Oral activity represents spontaneous chewing (vacuous chewing) which is not directed onto any physical material (Rupniak et al., 1983; Waddington, 1990). No distinction was made between lateral and vertical jaw movements. Directed oral activity and food chewing sequences were not counted (Kostrzewa and Gong, 1991; Huang and Kostrzewa, 1994a,b).

B. Neurochemical Analysis of Striatum

Eight or nine days after the withdrawal of haloperidol rats were killed by decapitation. Each brain was rapidly removed and the striata were dissected free, frozen on dry ice and stored at -70°C until the time of analysis.

Concentrations of DA, 5-HT and their metabolites in rat striatum were determined by liquid chromatography with electrochemical detection (LCEC). Each striatum was sonicated in 1 ml of 0.10 M trichloroacetic acid containing 0.20 mg/ml of cysteine as a stabilizing agent. 5-hydroxyindole carboxylic acid was used as internal standard. Homogenates were centrifuged at 12,000 x g for 5 min and 30 μl of each supernate was injected onto an Econosphere C18 analytical column (5 micron, 4.6 x 150 mm), having a mobile phase of 0.10 M monochloroacetic acid, 1 mM EDTA, 220 mg/l of Na octanesulfonic acid, 8% acetonitrile, pH 2.6, with a flow rate of 1.3 ml/min and temperature of 40 °C (Gong et
al., 1992). A Hewlett-Packard HP1000 chromatography data system was used to calculate peak heights and to determine concentrations of DA, DOPAC, homovanillic acid (HVA), 5-HT, 5-hydroxyindoleacetic acid (5-HIAA) and noreprenophine (NE).

C. Assessment of Striatal D₄ Receptor Binding Parameters

A modification of the procedure of Dewar et al. (1989, 1990) was used to assess DA D₄ receptors in the striatum. Rostral striata were homogenized with a Teflon on glass mortar and pestle in 1:100 ice-cold 50 mM Tris buffer (pH 7.4). This gentle homogenizing procedure was used to avoid damage to 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. The supernatants were discarded and the pellets were washed 4 times in the Tris buffer by repeated pipettings, followed by centrifugation. Final pellets were resuspended in 200 volumes of buffer.

Saturation curves were obtained in the following way. Aliquots of homogenate (2 mg membrane in 400 µl) were added to a series of incubation tubes with 10 different concentrations (93-8245 pM) of the DA D₄ receptor antagonist [³H]raclopride (specific activity: 82.4 Ci/mmol; Dupont). The incubate consisted of Tris buffer (pH 7.4) containing 120 mM NaCl, 5 mM KCl and 40 nM ketanserin (final concs.). Samples were incubated in a final volume of 2.5 ml at 25 ºC for 60 min. The incubation was terminated by rapidly
filtering each sample under partial vacuum on Whatman GF/F glass filters using a Millipore filtration unit. Filters were washed 3 time each with 5 ml ice-cold Tris-salt solution, then placed into scintillation counting vials. After drying overnight, 9 ml of scintillation solution was added to each vial. Tritium activity was determined in a Beckman LS 9800 liquid scintillation spectrometer.

A GraphPad program (GraphPad Software Inc., San Diego, CA) was used to construct the binding curves and calculate receptor binding parameters, B_{max} and K_{d}, by fitting the data to a double rectangular hyperbola equation \[ Y = A*X/(B+X) + C*X/(D+X) + E*X \]. Factor B in this equation was set as a constant of zero since there was only one binding site for D_{1} receptors (Fig. 1, sequential F test, P>0.05) (Dewar et al., 1989, 1990).

D. Determination of mRNA levels for D_{2L}, D_{2S}, and 5-HT_{3C} receptors in the striatum

1. Messenger RNA Extraction and Purification

Total RNA was isolated and purified according to the Ultraspec™-II RNA Isolation System (Biotecx Laboratories, Inc., Houston, TX). The rostral striatum of each rat was homogenized in 1 ml of Ultraspec™ RNA reagent with a Tekmar Tissumizer, setting of 5 for 25 sec. Homogenates were transferred to 1.5 ml polypropylene microcentrifuge tubes and kept at 4°C for 5 min to allow dissociation of nucleoprotein complexes. Then, 0.2 ml of chloroform was
added to each isolation tube, followed by vigorous shaking for 15 seconds. After 5 min on ice, homogenates were centrifuged at 12,000 g for 15 min and the upper aqueous phase which contained RNA was carefully transferred to a fresh tube -- taking care not to disturb the interphase and the lower organic phase. Each tube was vortexed for 30 seconds after the addition of 0.5 volumes of isopropanol and 0.05 volumes of RNA Tack™ Resin. Following a 1 min centrifuge step with a table-top minicentrifuge, supernatants were discarded and the pellets were washed with 2 x 1 ml of 75% ethanol by vortexing for 30 seconds and spinning for 1 min. The supernatants were discarded. All tubes were dried for 5 min in a vacuum centrifuge (VirTis Co., Gardiner, NY) to remove traces of ethanol. Pellets containing RNA were eluted with diethyl pyrocarbonate (DEPC)-treated water by vortexing for 30 seconds and then spinning for 1 min. Supernatants containing RNA were transferred to 0.6 ml polypropylene tubes and stored at -70°C until used. Ten μl of aliquot RNA solution of each sample was transferred into 490 μl of DEPC-treated water and the optical densities of the samples at 260 nM and 280 nM were obtained, to determine the concentration and purity of the final RNA solutions.

2. Polymerase Chain Reaction (PCR)

mRNA determinations were performed with Perkin Elmer GeneAmp RNA PCR Kit (Roche Molecular Systems, Inc.,
One microgram of total RNA from each sample was transcribed into single-stranded cDNA, using MuLV reverse transcriptase and random hexamers and incubating (42°C for 15 min, 99°C and 5°C for 5 min each) in a Perkin Elmer GeneAmp PCR system 9600 (Perkin Elmer, Roche Molecular systems, Inc., Branchburg, NJ).

Two oligonucleotides (24-mer) flanking the alternative spliced exon 6 of D₂ receptor cDNA were used for amplification of the mRNA levels of two isoforms of D₂ receptor, D₂₅ and D₂₆. The sequences of these two primers (Martres et al., 1992; Della Vedova et al., 1992) are:

- Primer 1: 5'-TTCAGAGCCAAACCTGAAGACACCA-3' (nucleotides 694-717);
- Primer 2: 5'GCTTTCTGCGGCTCATCGTCTTAA-3' (nucleotides 1067-1090).

The amplified fragment of D₂₅ and D₂₆ receptors were 397-bp and 310-bp respectively.

The levels of β-actin mRNA were also determined and this served as the internal standard. Two primers (Martres et al., 1992) for β-actin mRNA detection were 5'-

- GATGTTGGTATGGGTACAGAAGGA-3' (nucleotides 129-152) and 5'-
- GCTCATTGCGATAGTGACCT-3' (nucleotides 737-760). The 5-HT₂c receptor primers were 5'-ACACCGAGGAGGAACTGACTGTAATAT-3' and 5'-CTGGTAACAGAAGACTGCTCGACTAT-3' which define a 601-base-long DNA fragment. Amplification was carried out for 35 cycles using a DNA thermal cycler (Perkin Elmer Cetus) at the following condition: 95°C for 45 seconds, 58°C for 30 seconds and 72°C for 45 seconds. The final products after
amplification were fractionated on 2% Metaphor high-resolution agarose gel and stained with ethidium bromide. Two bands of 397-bp and 310-bp for D_{2a} and D_{1a} receptors, a band of 601-bp for 5-HT_{2c} receptors and a 632-bp band for β-actin were viewed and photographed with Polaroid positive film (type 665) under an ultraviolet (UV) light source. The optical densities and areas of each band were analyzed by a Bio Image Analysis System (Millipore Corporation Imaging Systems, Ann Arbor, MI).

E. Data Analysis

1. Oral Activity:

Oral activity was expressed as the mean number of oral movements in 6 min of a 60 min observation session ± S.E.M. Statistically significant differences in oral activity between groups was determined by one-way ANOVA, followed by the post-ANOVA Newman-Keuls test. In the case where receptor-specific drugs were tested for their ability to attenuate oral activities, the Student paired t-test was used to determine significant differences within a group of animals before and after administration of the drug.

2. Monoamine Determinations:

The mean tissue concentration of each monoamine and metabolite (nmol/gram ± S.E.M.) was determined for all groups of rats. Differences between the groups for each of these substances were determined by one-way ANOVA and a post-ANOVA Newman-Keuls test.
3. \(D_2\) Receptor Binding Parameters:

The double rectangular hyperbola equation of GraphPad, using total membrane binding (DPM/fmol protein) for each incubate concentration of \([\text{H}]\)raclopride (93-8245pM), was used to construct specific- and nonspecific-binding curves and to calculate values for \(B_{\text{max}}\) and \(K_d\). It was determined that there was only one binding site for \([\text{H}]\)raclopride, in agreement with Dewar et al. (1989, 1990). Differences in \(B_{\text{max}}\) and \(K_d\) between the groups were tested by one-way ANOVA, followed by the post-ANOVA Newman-Kuels test.

4. \(D_2\) and \(5-HT_{1c}\) Receptor mRNA levels:

The relative levels of mRNA for each receptor were expressed as a product of optical density and area of the individual gel bands, following adjustment for internal standard variations [i.e., dividing by levels of \(\beta\)-actin mRNA (optical density x area)]. The mean (± S.E.M.) content of the mRNA levels of each group was then determined, and these were compared among the different groups by one-way ANOVA and the post-ANOVA Newman-Keuls test.

A \(P\) value equal to or less than 0.05 was considered to be statistically significant in all of the statistical analyses.
Chapter 3

Results

A. Before Haloperidol Withdrawal

1. Spontaneous Oral Activity

In intact rats receiving tap water, the incidence of spontaneous oral activity was low for the entire duration of the experiment, remaining at less than 5 oral movements per session (Fig.2; Fig.3, 1st, 5th and 9th bars). The spontaneous oral activity of n6-OHDA-lesioned rats that were kept on tap water was slightly but not significantly higher than that in the intact rat and remained stable for the entire experiment duration (Fig. 2; Fig. 3, 2nd, 6th and 10th bars).

Starting in the 15th week of haloperidol treatment, there was a significantly higher incidence of oral activity in all haloperidol-treated rats (Fig.2 and Fig.3, P<0.01). The high level of spontaneous oral activity remained stable for the entire duration of haloperidol treatment (Fig.2). In intact rats oral activity ranged from 14.4 to 20.4 oral movements per session during the 11-month period of haloperidol treatment (observations at 1- or 2-week intervals). In the latest session there were approximately 18 oral movements, a 4-fold increase vs. intact rats on tap water (Fig.2 and Fig.3, 1st, 3rd, 7th and 11th bars, P<0.01). A much greater effect was produced by haloperidol administration to neonatal 6-OHDA-lesioned rats. In these
rats spontaneous oral activity ranged from 27.6 to 41.6 oral movements per sessions during the 11-month period of haloperidol treatment. In the latest session there were 36 oral movements, double that of intact rats which received haloperidol (Fig.3, 7th and 8th, 11th and 12th bars P<0.01).

2. Supersensitization of DA and 5-HT systems

a. DA system

Dose-response curves for SKF 38393-induced oral activity were constructed at 12 weeks and 36 weeks after the administration of haloperidol (Fig.4 and Fig.5). As expected, the 0.3 and 1.0 mg/kg doses of SKF 38393 significantly (P<0.01) increased oral activity in n6-OHDA-lesioned rats during the 12th and 36th weeks of haloperidol treatment. The oral activity of n6-OHDA-lesioned rats receiving haloperidol was also increased at the above 2 time points with the same SKF 38393 doses. This indicates that DA D_1-mediated oral responses were enhanced in both n6-OHDA-lesioned and n6-OHDA-lesioned rats receiving haloperidol treatment.

b. 5-HT system

The sensitivity of 5-HT_2c-mediated oral responses of rats was also tested. It recognized that m-CPP-induced oral activity is mediated by 5-HT_2c receptors (Gong et al, 1992). The dose-response curves for m-CPP-induced oral activity were performed at 12th and 38th week of haloperidol treatment. m-CPP produced bell-shaped dose-response curves
in n6-OHDA-lesioned rats, n6-OHDA-lesioned rats receiving haloperidol and intact rats receiving haloperidol, the optimal dose was 1.0 mg/kg (i.p.). Oral activities of these three groups of rats were significantly increased by m-CPP (Fig. 6 and Fig. 7). These results demonstrate that there is enhanced 5-HT₉-mediated oral activity in n6-OHDA rats and in both groups of haloperidol-treated rats.

3. Effects of assorted receptor agonists and antagonists on oral activity

a. DA D₁ receptor antagonist

At a 1.0 mg/kg dose, the DA D₁ receptor antagonist SCH 23390 had no effect on the oral activity of both intact and n6-OHDA-lesioned rats receiving tap water. However, the same SCH 23390 dose produced a significant reduction of oral activity in both groups of rats that received haloperidol (Fig. 9, P<0.05 for intact rats receiving haloperidol, P<0.01 for n6-OHDA-lesioned rats receiving haloperidol).

b. 5-HT₉ receptor antagonist

m-CPP and SKF 38393 produced significantly increased oral activity in n6-OHDA-lesioned rats and both groups of rats receiving haloperidol (Fig. 9, 6th, 7th, 8th and 10th, 11th, 12th bars vs. 2nd, 3rd and 4th bars respectively, P<0.01). The enhancement of oral activity induced by these two agonists was attenuated by mianserin (1.0 mg/kg). This finding indicates that both SKF 38393 and m-CPP-induced oral activity is mediated by a neural circuit with 5-HT
c. GABA receptor agonist and antagonist

Muscimol, a GABA_{A} receptor agonist, at the dose of 3.0 mg/kg, significantly attenuated the number of oral movements in n6-OHDA-lesioned rats receiving haloperidol (Fig.10, P<0.05). This same dose of muscimol had no effect on oral activity of intact rats receiving tap water or haloperidol; and no effect on oral activity of n6-OHDA-lesioned rats receiving tap water (Fig.10). The GABA receptor antagonist bicuculline (1.0 mg/kg) did not influence oral activity in any of the groups (Fig.11).

d. Cholinergic receptor antagonist

The effect of the muscarinic receptor antagonist scopolamine on oral activity of all rats was also tested (Fig.12). Analogous to SCH 23390, scopolamine (0.1 mg/kg) had no effect on oral activity of intact and n6-OHDA-lesioned rats receiving tap water. However, oral activity of intact and n6-OHDA-lesioned rats receiving haloperidol was significantly attenuated (Fig.12, P<0.05 and <0.01 respectively).

B. After Haloperidol Withdrawal

1. Spontaneous oral activity

In order to determine whether the haloperidol-induced increased in spontaneous oral activity would persist in n6-OHDA-lesioned rats, nine of the n6-OHDA-lesioned rats receiving haloperidol continued to be observed after the
withdrawal of haloperidol. These rats were observed periodically (once a week) for 8 months. The high incidence of oral activity of these rats remained stable (range of 28.5 to 38.5 oral movements per session) for the duration of the experiment--8 months after the withdrawal of haloperidol (Fig. 13). This finding indicates that chronic haloperidol treatment produces a long-lived increase in spontaneous oral activity of n6-OHDA-lesioned rats. In order to assess the long-term effects of haloperidol on DA and 5-HT₁c receptors, it was necessary to take brain tissue within several days after the cessation of haloperidol. For that reason, and based on the previously reported rapid decay of spontaneous oral activity in intact rats after cessation of haloperidol treatment (Tamminga et al., 1990; Mithani et al., 1987), it was not able to observe this group after haloperidol withdrawal.

2. Effects of assorted receptor agonists and antagonists on spontaneous oral activity

In order to determine the possible neural mechanisms underlying the regulation of oral activity, the effect of a series of receptor-specific drugs was tested in the n6-OHDA-lesioned rats during the haloperidol-withdrawal period.

a. DA receptor antagonists

SCH 23390 (1.0 mg/kg) had no effect on spontaneous oral activity of n6-OHDA-lesioned rats after haloperidol withdrawal. The DA D₁ receptor antagonists spiperone (0.08
mg/kg, i.p.) and metoclopramine (5.0 mg/kg, i.p.) also had no effect on oral activity of these rats (Fig.14).

b. 5-HT receptor antagonists

Ketanserin (5.0 mg/kg), pindolol (3.0 mg/kg) and metoclopramine (5.0 mg/kg), respective 5-HT₁A/₁B, 5-HT₁A/₁B and 5-HT₁ receptor antagonists, had no effect on oral activity of n6-OHDA-lesioned rats after haloperidol withdrawal (Fig.15). These results suggest that 5-HT₁A/₁B, 5-HT₁A and 5-HT₁ receptors are not involved in regulating oral activity.

c. Drugs acting on other neuronal receptors

Drug acting on GABA receptors

Muscimol (3.0 mg/kg, i.p.), a GABA receptor agonist, significantly attenuated oral activity in n6-OHDA-lesioned rats after haloperidol withdrawal (P<0.01, Fig.16). Spontaneous oral activity of these rats was reduced from 28.1 ± 4.4 to 16.0 ± 3.9 movements per session after muscimol. These results support the view that GABA is one of the neuronal systems involved in the regulation of oral activity (Gunne et al., 1984; Morelli et al., 1985).

Drug acting on cholinergic receptors

Before withdrawing haloperidol, the muscarinic receptor antagonist scopolamine (0.1 mg/kg, i.p.) significantly attenuated spontaneous oral activity in both intact and n6-OHDA-lesioned rats receiving haloperidol treatment (Fig.12). However, after the withdrawal of haloperidol, the high incidence of oral activity in n6-OHDA-lesioned rats was not
attenuated by scopolamine. The spontaneous oral activity of these rats was $30.5 \pm 6.6$ and $23.5 \pm 3.0$ movements per session respectively before and after scopolamine ($P>0.05$, Fig. 16). The different effects of SCH 23390 and scopolamine on spontaneous oral activity before and after haloperidol withdrawal suggest that oral activity is regulated differently in these two phases. The failure of scopolamine to inhibit oral activity after haloperidol withdrawal indicates that the characteristics of oral activity are analogous to the oral activity in TD patients since it is usually agreed that scopolamine alleviate the early emergence of oral dystonia whereas cholinergic drug improve the symptoms of tardive dyskinesia (Rupniak et al, 1984; Waddington, 1990).

Drugs acting on adrenergic receptors

Phenoxybenzamine (10.0 mg/kg) and phentolamine (2.0 mg/kg), two $\alpha$-adrenoceptor antagonists, had no effect on spontaneous oral activity of n6-OHDA-lesioned rats after haloperidol withdrawal. Similarly, the $\beta$-adrenoceptor antagonist propranolol (20.0 mg/kg) had no influence on spontaneous oral activity of these rats (table 1). This implies that adrenergic systems do not have a prominent role in regulating oral activity of these rats.

Drugs acting on histamine and adenosine receptors

Cyproheptadine (0.3 mg/kg) and ranitidine (5.0 kg/mg), respective histaminergic $H_1$ and $H_3$ receptor antagonists, and
theophylline (20 mg/kg), an adenosine receptor antagonist, failed to attenuate spontaneous oral activity of the n6-OHDA-lesioned rats after haloperidol withdrawal (table 1).

**Drugs acting on NMDA and opioid receptors**

Ketamine (10.0 mg/kg), an NMDA receptor antagonist, and naloxone (1.5 mg/kg and 5.0 mg/kg), an opioid mu receptor antagonist, did not significantly alter oral activity in the n6-OHDA-lesioned rats after haloperidol withdrawal. At a dose of 0.3 mg/kg, MK-801, an NMDA receptor antagonist, significantly reduced the number of oral movement in these rats (table 1). However, treatment with MK-801 also caused prominent salivation and sedation in these rats. All rats were virtually immobile after MK 801 treatment. Therefore, the reduction of oral activity by MK-801 in these rats may be more reflective of general central nervous system depression.

**C. Changes of mRNA Levels of DA D<sub>1</sub> and 5-HT<sub>2C</sub> Receptors**

To correlate the functional changes with molecular mechanisms, the relative messenger RNA levels of DA D<sub>1</sub> and D<sub>2</sub> receptors, two isoforms of D<sub>1</sub> receptors generated by alternative splicing of the D<sub>1</sub> receptor gene, and the mRNA levels of 5-HT<sub>2C</sub> receptors were assessed by reverse transcription polymerase chain reaction (RT-PCR) using β-actin as an internal standard.

1. **Changes of mRNA levels of DA D<sub>2L</sub> and D<sub>2S</sub> receptors**

As shown in figure 17, the striatal mRNA levels of D<sub>2L</sub>
receptors in intact and n6-OHDA-lesioned rats receiving haloperidol were significantly increased as compared to the mRNA levels of rats that were not treated with haloperidol (Fig. 17, 1.10±0.24 and 1.27±0.26 vs. 0.24±0.04 and 0.36±0.11, P<0.01). However, 8 months after haloperidol withdrawal, the mRNA levels of n6-OHDA-lesioned rats returned to the level found in controls (Fig. 17, 0.44±0.13, P>0.05). The D2 mRNA levels of n6-OHDA-lesioned rats, regardless of whether haloperidol was administered was slightly but not significantly increased when compared to other treatment groups (Fig. 17). This may indicate that the changes of the D2 subtype of D2 receptor is more closely associated with the effect of long-term haloperidol treatment.

2. Changes of mRNA levels of 5-HT2C receptors

Since 5-HT2C receptors have an important role in regulating spontaneous oral activity of rats (Gong et al., 1992), striatal 5-HT2C mRNA levels were also assessed in our rats. In both groups (intact and n6-OHDA-lesioned rats) that received haloperidol treatment, there was a slight but not significant increase of 5-HT2C receptor mRNA level (Fig. 17, 13th and 14th bars vs 11th and 12th bars, P>0.05). Eight months after haloperidol withdrawal, the 5-HT2C receptor mRNA level was slightly reduced as compared to the level at the time of haloperidol withdrawal (Fig. 17, 14th bar vs 15th bar).
D. Changes of DA D2 Receptor Binding Parameters

As revealed by [3H]raclopride binding to homogenates of rat striatum, chronic haloperidol treatment produced a significant increase of D2 receptor density (Bmax) in rostral striatum of both intact and n6-OHDA-lesioned rats. The Kd was not significantly altered. Eight months after haloperidol withdrawal, the Bmax of n6-OHDA-lesioned rats returned to control levels (table 2).

E. Effects of n6-OHDA treatment on the levels of monoamines and their metabolites in the striatum

1. DA, DOPAC and HVA levels

Neonatal 6-OHDA treatment produced a marked reduction in the content of DA and DA metabolites in rat striatum. DA and HVA contents were reduced more than 96% in caudal striatum of n6-OHDA-lesioned rats, regardless of haloperidol treatment (table 3, P<0.01). DOPAC levels in the striata of n6-OHDA-lesioned rats were also profoundly reduced (>89%). Long-term haloperidol treatment produced a slight (about 12%) but significant reduction of DA, DOPAC and HVA levels in the striatum of intact rats (table 3, P<0.05 or 0.01).

2. 5-HT, 5-HT metabolites and NE levels

Long-term haloperidol treatment per se had no effect on striatal 5-HT content in intact and n6-OHDA-lesioned rats. However, there was a significant elevation of 5-HT content in the striatum of n6-OHDA-lesioned rats (table 3, P<0.01). The content of 5-HIAA was significantly increased in the n6-
OHDA-lesioned rats vs. the intact rats, regardless of the treatment of haloperidol (table 3). Therefore, n6-OHDA treatment significantly increased 5-HT and 5-HIAA contents in rat striata, whereas haloperidol had little effect. There was no changes in the content of NE in the striata of the different treatment groups (table 3). This indicates that desipramine pretreatment effectively protected noradrenergic neurons from n6-OHDA destruction. Accordingly, n6-OHDA produced a selective effect on the DA system.
### Table 1

Effects of assorted agonists and antagonists on spontaneous oral activity of rats during the 8 months following termination of long-term haloperidol treatment

<table>
<thead>
<tr>
<th>Test Substances</th>
<th>Dose (mg/kg)</th>
<th>Number of Oral Movement After Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Test Substance</td>
<td>vehicle</td>
</tr>
<tr>
<td>Cyproheptadine</td>
<td>0.3</td>
<td>27.9(5.0) 26.9(4.3)</td>
</tr>
<tr>
<td>Ketamine</td>
<td>10.0</td>
<td>27.9(2.8) 28.1(4.6)</td>
</tr>
<tr>
<td>Naloxone</td>
<td>1.5</td>
<td>37.7(7.4) 27.0(5.4)</td>
</tr>
<tr>
<td>Naloxone</td>
<td>5.0</td>
<td>31.7(3.8) 27.0(5.4)</td>
</tr>
<tr>
<td>Theophylline</td>
<td>20.0</td>
<td>32.4(4.2) 24.1(4.5)</td>
</tr>
<tr>
<td>Ranitidine</td>
<td>5.0</td>
<td>21.4(4.6) 23.9(4.3)</td>
</tr>
<tr>
<td>Phenoxybenzamine</td>
<td>10.0</td>
<td>13.7(2.1) 21.4(4.7)</td>
</tr>
<tr>
<td>Propranolol</td>
<td>20.0</td>
<td>24.7(6.2) 27.7(6.9)</td>
</tr>
<tr>
<td>Phentolamine</td>
<td>2.0</td>
<td>34.1(4.7) 30.1(5.4)</td>
</tr>
<tr>
<td>MK-801</td>
<td>0.3</td>
<td>5.4(2.4) 27.3(4.8)*</td>
</tr>
<tr>
<td>Morphine</td>
<td>1.0</td>
<td>33.4(3.5) 29.9(3.2)</td>
</tr>
</tbody>
</table>

Each value represents the mean(S.E.M.) number of vacuous chewing movements of 6 to 9 rats. Rats were first observed after i.p. or s.c. administration of vehicle, then after administration of the test substances. Doses refer to salt form of each substance.

*:P<0.01 when compared to vehicle treatment
Effects of n6-OHDA and chronic haloperidol treatment on [\(^3\)H]raclopride binding to rat striatum homogenates

<table>
<thead>
<tr>
<th>Group</th>
<th>(B_{max}) (fmol/mg protein)</th>
<th>(K_d) (pM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>nVehicle + tap water</td>
<td>203 (174-233) (^a)</td>
<td>657 (492-817)</td>
</tr>
<tr>
<td>n6-OHDA + tap water</td>
<td>261 (219-303) (^b)</td>
<td>809 (668-994)</td>
</tr>
<tr>
<td>nVehicle + Haloperidol</td>
<td>339 (259-418) (^c)</td>
<td>986 (776-1197)</td>
</tr>
<tr>
<td>n6-OHDA + Haloperidol</td>
<td>350 (301-399) (^d)</td>
<td>895 (822-966)</td>
</tr>
<tr>
<td>n6-OHDA + Haloperidol (withdrawn)</td>
<td>230 (172-287) (^e)</td>
<td>704 (524-873)</td>
</tr>
</tbody>
</table>

Rats received vehicle or 6-OHDA (100 µg each side, i.c.v., 20 mg/kg desipramine pretreatment, 1 hr) 3 days after birth and haloperidol (1.5 mg/kg, via drinking water) or tap water from 2 month for an 11 months period. Rostral striata were removed 9 days or 8 months after withdrawing haloperidol. Saturable curves for striatal homogenates were constructed, using binding data (n=5) from 10 concentrations of [\(^3\)H]raclopride (93pM to 8250pM). The \(B_{max}\) and \(K_d\) for \(^3\)H-raclopride binding was determined with a computer program (GraphPad Software, Inc., San Diego, CA). Data are expressed as mean (95% confidence interval)

\(^a\) vs. \(^c\) and \(^a\) vs. \(^d\): P<0.01
\(^e\) vs. \(^c\) and \(^e\) vs. \(^d\): P<0.05
Table 3

Effects of neonatal 6-OHDA and chronic haloperidol treatment on concentrations of monoamines and their metabolites in the striatum

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>DA</th>
<th>DOPAC</th>
<th>HVA</th>
<th>5-HT</th>
<th>5-HIAA</th>
<th>NE</th>
</tr>
</thead>
<tbody>
<tr>
<td>nVehicle+</td>
<td>8</td>
<td>52.8±5.7</td>
<td>7.6±0.9</td>
<td>4.0±0.2</td>
<td>4.1±0.2</td>
<td>5.2±0.2</td>
<td>1.1±0.1</td>
</tr>
<tr>
<td>n6-OHDA+</td>
<td>9</td>
<td>1.6±0.5#</td>
<td>0.8±0.1#</td>
<td>0.1±0.1#</td>
<td>5.3±0.2#</td>
<td>6.4±0.2#</td>
<td>1.0±0.1</td>
</tr>
<tr>
<td>nVehicle+</td>
<td>8</td>
<td>46.0±3.7*</td>
<td>5.3±0.5*</td>
<td>2.7±0.3*</td>
<td>4.3±0.2</td>
<td>5.4±0.2</td>
<td>1.1±0.1</td>
</tr>
<tr>
<td>n6-OHDA+</td>
<td>8</td>
<td>1.9±0.3#</td>
<td>0.8±0.1#</td>
<td>0.1±0.1#</td>
<td>4.8±0.3</td>
<td>6.4±0.2#</td>
<td>1.2±0.1</td>
</tr>
</tbody>
</table>

Rats were treated at 3 days after birth with 6-OHDA HBr (100 μg each side, i.c.v., salt form; desipramine pretreatment, 1 hr) or its vehicle. From 2 months after birth, haloperidol (Hal) was given via drinking water (1.5 mg/kg/day, 2 days/week for 1 month, then daily for 10 months). Strata were removed for assay 8 or 9 days after the withdrawal of haloperidol. Values are mean nanomoles per gram of tissue ± S.E.M.. Eight samples from each group was detected.

*: p<0.05 and #:p<0.01 when compared to vehicle control group.
Figure 1. Representative saturation curves of [3H]raclopride binding to rostral striatal homogenates of rats. Aliquots of striatal homogenates were added to a graded series of 10 concentrations of [3H]raclopride (93-8245 pM). Curves for total, specific and nonspecific binding and the values for B_{max} and K_{d} were generated by fitting the data to a double rectangular hyperbola equation \[ Y = A \times X / (B + X) + C \times X / (D + X) + E \times X \] using a computer program (GraphPad Software Inc., San Diego, CA).
Effect of Haloperidol Treatment on Spontaneous Oral Activity of Intact and Neonatal 6-OHDA-Lesioned Rats

Fig. 2. Time-course effects of chronic haloperidol treatment on spontaneous oral activity of rats. Rats were treated at 3 days after birth with 6-OHDA HBr (200 μg, salt form, half in each lateral ventricle; desipramine pretreatment, 20 mg/kg, 1 hr) or vehicle. Haloperidol was added to the drinking water starting at 2 months after birth (1.5 mg/kg/day × 2 days per week for 1 month; then daily for 10 months). Spontaneous oral activity was observed at 1- or 2-week intervals during the 11 months of haloperidol administration, in 1-h observation sessions (observation of 1 min every 10 min for 60 min). Each point represents the mean number of spontaneous oral movements for each observation. Starting from the 15th week after haloperidol treatment, the spontaneous oral activity in both groups of rats that received haloperidol was significantly higher than before starting treatment (P < 0.01). The high oral activity in these rats persisted throughout the duration of the experiment (n=9).
Effects of Prolonged Haloperidol Treatment on Oral Activity of Intact and Neonatal 6-OHDA-Lesioned Rats

Fig. 3. Effect of long-term haloperidol treatment on oral activity of rats. Rats received the treatments and observations as described in Fig. 2. Bars represent the mean number of spontaneous oral movements (±SEM) one week before starting haloperidol administration (-1 week, n=9), 6 months (6 months, n=9) or 11 months (11 months, n=9) after haloperidol administration and 19 months (19 months, n=7) after discontinuing haloperidol.

*,**: Different labeled bars are significantly different from each other, P<0.01.
Fig. 4. Dose-effect curves for SKF 38393-induced oral activity of rats during the 12th week of haloperidol administration. Oral activity was observed 10 min after administration of SKF 38393 HCl (abscissa). Each point represents the mean number of oral movements (n=9) (ordinate).

*: P<0.05 vs. the oral activity induced by vehicle in the same group
SKF 38393 Dose-Effect Curves at 36 Weeks of Haloperidol Treatment

Fig. 5. Dose-effect curves for SKF 38393-induced oral activity of rats during 36th week of haloperidol administration. Legend ad in fig.4 (n=9)

*: P<0.01 vs. the oral activity induced by vehicle in the same group
Fig. 6. Dose-effect curves for m-CPP-induced oral activity of rats during the 12th week of haloperidol treatment. Oral activity was observed 10 min after administration of mCPP 2HCl. Legend as in fig. 4 (n=9).

*: P<0.01 vs. the oral activity induced by vehicle in the same group
m-CPP Dose-Effect Curves at 38 Weeks of Haloperidol Treatment

**Legend**
- Intact+Tap Water
- Intact+HAL
- 6-OHDA+Tap Water
- 6-OHDA+HAL

**fig.7.** Dose-effect curves for m-CPP-induced oral activity of rats during the 38th week of haloperidol treatment. Legend as in fig.4 (n=9).

*: P<0.01 vs. the oral activity induced by vehicle in the same group
Fig. 8. SCH 23390 attenuation of spontaneous oral activity of rats during haloperidol treatment. Rats received 6-OHDA and haloperidol treatment as described in fig. 2. Oral activity was observed 10 min after vehicle administration (saline, 1.0 ml/kg, i.p.) with or without SCH 23390 HCl (1.0 mg/kg, i.p., 1h) pretreatment. Bars represent the mean (± S.E.M.) number of oral movements (n=9).

*, **, ***: bars with different labels were significantly different from each other (P<0.01).
Effects of Mianserin on Oral Activities Induced by SKF 38393 and m-CPP

Fig. 9. Effect of mianserin on oral activity induced by SKF 38393 and m-CPP in rats. Rats received 6-OHDA and haloperidol treatments as described in fig. 2. Oral activity was observed 10 min after saline (1 ml/kg, i.p.), SKF 38393 HCl (1.0 mg/kg, i.p.) or m-CPP 2HCl (3.0 mg/kg, i.p.) challenge with or without mianserin pretreatment (1 mg/kg, i.p., 1h). Bars represent mean (± S.E.M.) number of oral movement (n=9).

*: P<0.05 vs. oral activity of the same group pre-treated with mianserin
Effect of Muscimol on Spontaneous Oral Activity of Rats During Haloperidol Treatment

Fig. 10. Effect of muscimol on spontaneous oral activity of rats during haloperidol treatment. Rats received 6-OHDA and haloperidol treatments as described in fig. 2. Oral activity was observed 10 min after vehicle administration (saline, 1.0 ml/kg) with or without muscimol (3.0 mg/kg, i.p., 1h) pretreatment. Bars represent the mean (± S.E.M.) number of oral movement (n=9).

**: P<0.01 vs. all other bars
Fig. 11. Effect of bicuculline on spontaneous oral activity of rats during haloperidol treatment. Rats received 6-OHDA and haloperidol treatments as described in fig. 2. Oral activity was observed as described in fig. 10 (n=9). Bicuculline did not attenuate the spontaneous oral activity of rats (P>0.05).
Effect of Scopolamine on Spontaneous Oral Activity of Rats During Haloperidol Treatment

![Bar graph showing the number of oral movements under different conditions.](image)

Fig. 12. Effect of scopolamine on spontaneous oral activity of rats. Rats received 6-OHDA and haloperidol treatments as described in Fig. 2. Oral activity was observed 10 min after vehicle administration (saline, 1 ml/kg) with or without scopolamine HCl (0.1 mg/kg, i.p., 1h) pretreatment. Bars represent mean (± S.E.M.) number of oral movement (n=9).

*,**: Different labeled bars are significantly different from each other.
Spontaneous Oral Activity of Haloperidol-Withdrawn Neonatal 6-OHDA-Lesioned Rats

Fig. 13. Spontaneous oral activity in 6-OHDA-lesioned rats after haloperidol withdrawal. The oral activity of 6-OHDA-lesioned rats was observed 10 min after injection of saline (1 ml/kg, i.p.), each week or in alternate week during haloperidol withdrawal. Oral activity of these rats remained at an elevated level, similar to that observed before haloperidol withdrawal. Each point represents the mean (± S.E.M.) number of oral movements (n=6-9).
Effects of DA Receptor Antagonists on Spontaneous Oral Activity of Haloperidol-Withdrawn n6-OHDA-Lesioned Rats

Fig. 14. Effects of DA receptor antagonists on spontaneous oral activity of n6-OHDA-lesioned rats after haloperidol withdrawal. Oral activity of n6-OHDA-lesioned rats was observed 10 min after vehicle administration (saline, 1 ml/kg, i.p.) with or without pretreatment with a DA receptor antagonists SCH 23390 HCl (1.0 mg/kg, i.p., 1h), spiperone HCl (0.08 mg/kg, i.p., 1h) or metoclopramide HCl (5.0 mg/kg i.p., 1h). Bars represent mean (± S.E.M.) number of oral movements (n=7-9). These DA antagonists did not attenuate the spontaneous oral activity of haloperidol-withdrawn n6-OHDA-lesioned rats (P>0.05).
Effects of 5-HT Receptor Antagonists on Spontaneous Oral Activity of Haloperidol-Withdrawn n6-OHDA-lesioned Rats

Fig. 15. Effects of 5-HT receptor antagonists on spontaneous oral activity of n6-OHDA-lesioned rats after haloperidol withdrawal. Oral activity of n6-OHDA-lesioned rats was observed 10 min after vehicle administration (saline, 1 ml/kg, i.p.) with or without pretreatment with the 5-HT antagonists pindolol (3.0 mg/kg, i.p., 1h), ketanserin tartrate (5.0 mg/kg, i.p., 1h) or metoclopramide HCl (5.0 mg/kg, i.p., 1h). Bars represent mean (± S.E.M.) number of oral movements (n=6-9). These 5-HT receptor antagonists did not attenuate the spontaneous oral activity of haloperidol-withdrawn n6-OHDA-lesioned rats (P>0.05).
Effect of Scopolamine and Muscimol on Spontaneous Oral Activity of Haloperidol-Withdrawn Neonatal 6-OHDA-Lesioned Rats

![Bar graph showing the effects of scopolamine and muscimol on spontaneous oral activity of n6-OHDA-lesioned rats after haloperidol withdrawal.](image)

Fig. 16. Effects of scopolamine and muscimol on spontaneous oral activity of n6-OHDA-lesioned rats after haloperidol withdrawal. Oral activity of n6-OHDA-lesioned rats was observed 10 min after vehicle administration (saline, 1 ml/kg, i.p.) with or without pretreatment with scopolamine HCl (0.1 mg/kg, i.p., 1h) or muscimol (3.0 mg/kg, i.p., 1h). Bars represent mean ± S.E.M. number of oral movements (n=6-9).

*: P<0.05 vs. the oral activity after vehicle stimulation
Messenger RNA levels of D\textsubscript{2L}, D\textsubscript{2S} and 5-HT\textsubscript{2C} receptors in rat striatum after long-term haloperidol treatment.

*P<0.05 when compared to the same receptor subtype of other groups.
Due to the difficulty in obtaining human tissues for investigation and the fact that available patients have usually received complex therapeutic regimens, a direct neurochemical basis underlying TD is difficult to be derived from TD patients (Casey, 1987). Therefore, animal models play a significant role in attempting to determine the pathophysiological basis of TD. Growing numbers of investigations have sought to develop animal models for TD (Crossman, 1987; Waddington, 1990). However, diversity, or even conflicting results, especially the time of occurrence, duration and persistence of spontaneous oral dyskinesias, exist in the current animal models of TD. It is important to establish an animal model that can reproduce the phenomenology of TD patients and thereby give rise to evidence related to the etiological alterations of TD (Waddington, 1990).

All primate models of TD were developed by long-term treatment with neuroleptics such as haloperidol (Gunne and Barany, 1976; Gunne et al., 1984) or fluphenazine (Kovacic and Domino, 1984; Gunne et al., 1984). Under the treatment by haloperidol (1-10 mg per kg, haloperidol decanoate, 3-week interval) or fluphenazine (4-10 mg per kg, fluphenazine
decanoate, 3-week interval) for a period of 1 to 3 years, Gunne and associates reported similar symptoms to those of TD observed in Cebus apella monkeys. The motor defects occurred during treatment and persisted in the dyskinetic monkeys for 1 to 6 years after cessation of neuroleptics (Gunne and Barany, 1976; Gunne et al., 1984). Kovacic and Domino (1984) reported that bi-weekly treatment with fluphenazine (0.1-3.2 mg/kg, IM) for 1 year did not produce symptoms resembling TD in Cebus apella monkey until a few months after the cessation of fluphenazine treatment. During the period of fluphenazine treatment, all monkeys displayed abnormal movements corresponding to the early appearing extrapyramidal symptoms of neuroleptic-treated patients. These abnormal movements were prevented and attenuated by the anticholinergic drug benztropine (0.2-0.6 mg/kg, IM). The distinct advantage of models developed in primates over those in sub-primates is that primate models exhibit a physical dyskinesia that is readily recognized. Nevertheless, primates react to chronic neuroleptic treatments in some aspects in a different way from humans. The acute motor abnormality of monkeys to chronic neuroleptic treatment and the alleviation of these acute reactions by anticholinergic drugs stands in contrast to what has been observed in TD patients (Crossman, 1987). These have led to a criticism of the validity of primate models of TD. In addition, the high cost of primates also
limits the availability of these animals for investigation.

Most studies of TD animal models have been carried out in rodents, almost exclusively in rats (for review, see Waddington, 1990). All of the current TD animal models were produced by long-term administration of neuroleptics such as haloperidol, fluphenazine, trifluoperazine and metoclopramine to intact rats. The duration of drug treatment ranges from 1.5 months to 13 months with durations of withdrawal up to 5 months. There were marked inconsistencies among the rats models developed by comparable treatments. Some studies reported late-onset oral movement (Ellison and See, 1989) whereas others reported early emergence of similar movement (Rupniak et al., 1985a) or even lack of any drug effect (Levy and Ellison, 1987). There were also conflicting results concerning whether the altered oral activity can persist in rats, a phenomenon that is found in TD patients. Although it has been reported that oral activity was able to persist for up to 2.5 months in trifluoperazine-treated rats (Waddington et al., 1983), many other studies reported a gradual diminution of oral activity during 2.5 to 5 months of drug-withdrawal (Mithani et al., 1987; Gunne and Haggstrom, 1983; Gunne et al., 1982). It is difficult to draw firm evidence toward the pathophysiology basis of TD from current animal models because of the diversity in outcomes of the present models. Therefore, an improved TD
animal model which can appropriately reproduce the phenomenology and pathophysiology changes in human is urgently needed (Waddington, 1990; Huang and Kostrzewa, 1994b).

The drugs being administered and the subjects receiving such treatment are two major factors in the development of a TD animal model (Waddington, 1990). Since neuroleptics induce abnormal oral activity in rodents, it is likely that rodents and humans response to these drugs in a similar manner. Therefore, it appears to be appropriate to administer neuroleptics to rats when developing a TD animal model. However, TD occurs when neuroleptics are given to those human who have already had neuropsychiatric disorders rather than to healthy people. Therefore, intact rats may not be appropriate subjects for animal models of TD. Some factors other than chronic neuroleptic treatment, perhaps some extent of central nervous system damage, may contribute to the emergence of TD syndromes in either TD patients or rats. In fact, more and more evidence suggests that central dysfunction contributes to the vulnerability of TD (Kane et al., 1985; Owens, 1985; Waddington et al., 1989; Waddington, 1990). There are closer associations between abnormal orofacial dyskinesias and central dysfunctions (such as produced by frontal cortex damage). It was also proposed that TD is the result of loss of a discrete subpopulation of striatal efferent neurons (probably
striatonigral) which release GABA as their transmitter (Fibiger and Lloyd, 1984). It seemed to us that oral dyskinesias are more likely to occur when rats with endogenous or exogenous central dysfunctions, are under the influence of chronic neuroleptic treatment. Therefore, rats with selective central DA neuronal destruction may serve as a better substrate as an animal model for neuroleptic-induced tardive dyskinesias.

It is well known that n6-OHDA treatment produces supersensitization of DA receptors in rats. In n6-OHDA-lesioned rats, doses of L-dihydroxyphenylalanine (L-DOPA), SKF 38393 and A77636 [(1R,3S)-3-(1'-adamantyl)-1-aminomethyl-3-4-dihydro-5,6-dihydroxy-1H-2-benzopyran] that have little effect on intact rats, produce great changes in stereotyped, locomotor or oral activities in n6-OHDA-lesioned rats (Breese et al., 1984, 1985a, 1985b; Hamdi and Kostrzewa, 1991; Huang and Kostrzewa, 1994a; Kostrzewa, 1993, 1995; Kostrzewa and Brus, 1991). Serotonin 5-HT₂ receptor-mediated oral activity is also enhanced in these rats (Gong and Kostrzewa, 1991). In both the earlier DA receptor supersensitization hypothesis (Klawans and Hitri, 1978; Chiu et al., 1981; Crossman, 1987) and the current multi-neurotransmitter hypothesis of TD (Waddington, 1990), the DA neuronal system is considered to be a major element. Because chronic haloperidol treatment and neonatal 6-OHDA treatment each sensitize DA receptors in rats, we believed
that chronic neuroleptic-treated n6-OHDA-lesioned rats would represent an improved TD animal model. This provided the basis of our study.

Neonatal 6-OHDA treatment markedly decreases the striatal content of DA (Breese and Traylor, 1971, 1972; Breese et al., 1984) and elevates 5-HT content (Breese et al., 1984; Stachowiak et al., 1984). The findings of our study are in agreement with those reports (table 3). There is >96% reduction of DA and HVA as well as 89% reduction of DOPAC in the caudal striata of the n6-OHDA-lesioned rats, regardless of whether rats were treated with haloperidol. The concentrations of HVA and DOPAC in striatum are slightly but significantly reduced in intact rats treated by long-term haloperidol (table 3). In agreement with the earlier report that HVA concentration and tyrosine hydroxylase (TH) activity are significantly decreased by chronic (2 mg/kg/day for 21 days) haloperidol treatment, reductions in the levels of TH activity, DOPAC and HVA concentrations suggest a reduced turnover of DA (Rastogi et al., 1982). Rastogi and associates (1982) did not find any change in the level of DA in striatum in their haloperidol-treated rats, whereas DA level in caudal striata of our haloperidol-treated rats was significantly reduced. This may due to the differences in the length of treatment duration. Meanwhile, as expected, the striatal concentration of 5-HT and 5-HIAA were significantly elevated by n6-OHDA treatment. The unaltered
levels of NE in the striatum indicate that selective depletion of DA system with n6-OHDA treatment was well-achieved by desipramine pretreatment of rats (table 3).

In the present study the incidence of spontaneous oral activity in intact and n6-OHDA-lesioned rats remained at stable and low levels throughout the entire duration of the study. A neonatal 6-OHDA-lesioned per se did not cause a significant increase in spontaneous oral activity (Fig. 2; Fig. 3 1st, 2nd, 5th, 6th, 9th and 10th bars). The dose of haloperidol (1.5 mg/kg/day) used in this study has been shown to produce a plasma haloperidol level equivalent to that observed in TD patients (Tamminga et al., 1990). A disproportionate increase of spontaneous oral activity following chronic haloperidol treatment was found in rats lesioned as adults with 6-OHDA (Gunne et al., 1982). There were no early emergence of any form of abnormal behavior in our rats during haloperidol treatment. However, after 15 weeks of haloperidol treatment the level of oral activity in both intact and 6-OHDA-lesioned rats was markedly increased, when compared to the respective base lines of oral activity. Chronic haloperidol treatment produced a greater effect in n6-OHDA rats vs. intact rats (Fig. 2). In the present study, when tested during the 12th, 36th and 38th weeks of haloperidol treatment, the oral activity of n6-OHDA rats was markedly elevated by the DA D1 receptor agonist SKF 38393 and 5-HT1 receptor agonist m-CPP (Figs. 4-7). These
findings are in agreement with many others (Gong and Kostrzewa, 1991; Kostrzewa and Gong, 1991; Huang and Kostrzewa, 1994a) and indicate that there is a supersensitization of DA D_1 and 5-HT_1 receptors that are involved in oral response in n6-OHDA-lesioned rats. If the process of DA receptor supersensitivity partly accounts for increased oral activity in TD patients (Chiu et al., 1981; Klawans and Hitri, 1978; Crossman, 1987), the effects produced by chronic haloperidol treatment could be either additive or synergistic with the receptor sensitization produced by a neonatal 6-OHDA lesion. This could account for the 2-fold higher oral activity in n6-OHDA-lesioned rats vs. intact rats receiving haloperidol (Fig.2 and Fig.3).

The elevated level of oral activity that was induced by chronic haloperidol treatment persisted unabated in n6-OHDA-lesioned rats during the 8 months after haloperidol withdrawal (Fig.13). This is the longest withdrawal period that has ever been described in neuroleptic-treated rats. Considering the life span of rats, the persistence of enhanced oral activity in these rats seems to represent a permanent effect, akin to the oral dyskinesia accompanying TD in humans. It has been observed that increased oral activity persists in about 65% of TD patients, long after the cessation of neuroleptic treatment. The greater the duration of neuroleptic treatment, the more likely is the abnormal oral activity to persist after neuroleptic
withdrawal (Jeste and Wyatt, 1982a; Kane and Smithe, 1982). The long-live spontaneous oral dyskinesias of our rats contrast with the rapid or gradual decay of this behavior in intact rats that were withdrawn from haloperidol. In 3 different strains of rats that received chronic haloperidol in tap water at the same daily dose as in the present study (1.5 mg/kg/day) there was a rapid decay in the frequency of spontaneous oral activity during the first 2 weeks after haloperidol withdrawal, to a level about half of that during the haloperidol phase (Tamminga et al., 1990). In a similar experiment in which haloperidol was administered in tap water for 300 days, there was also a rapid decay in spontaneous oral activity of rats in the first month after haloperidol withdrawal (Gunne et al., 1986). Mithani et al. (1987) reported that a similar decay in spontaneous oral activity occurred in rats 2-8 weeks following withdrawal from haloperidol decanoate implants. The interval following haloperidol withdrawal is an opportune one for testing drugs for their potential to attenuate oral dyskinetic behavior. There was no decrement in spontaneous oral activity in the lesioned rats following haloperidol withdrawal, indicating that there is a stable baseline for testing the ability of drugs to attenuate spontaneous oral activity. Although numerous trials have been carried for drug testing, presently no drug have been proven to be both safe and effective for TD treatment. In rats the DA D_1 receptor
antagonist SCH 23390 (0.01-0.25mg/kg) and DA D_2 receptor antagonist sulpiride (4-100 mg/kg) reduced vacuous chewing movements in a dose-dependent manner (Levin et al., 1989). In monkeys that were withdrawn from chronic haloperidol treatment for 1.5 years, chronic SCH 23390 (9 weeks) treatment did not change the baseline of oral dyskinesia, whereas raclopride (3 weeks) increased the oral activity of these monkeys (Lublin et al., 1993). The acute effect of SCH 23390 on the oral dyskinesias of these monkeys was biphasic. That is, SCH 23390 increased oral activity of these monkeys at a low dose (0.02 mg/kg) and reduced their oral activity at a high dose (0.2 mg/kg). Raclopride had no effect on oral activity of these monkeys (Peacock et al., 1990). Therefore, the effectiveness of DA receptor antagonists on oral dyskinesias is unresolved. In the present study, the effect of SCH 23390 on oral activity was tested before and after the cessation of haloperidol treatment. SCH 23390 (1.0 mg/kg) significantly attenuated the oral activity of haloperidol-treated rats before haloperidol withdrawal (Fig.8, 5th bar vs. 6th bar, P<0.05; 7th bar vs. 8th bar, P<0.01) but had no effect after haloperidol withdrawal (Fig.14, 1st and 2nd bars). The DA D_2 receptor antagonists spiperone and metoclopramide had no effect on oral dyskinesias after haloperidol withdrawal. These results may relate to the view that an imbalance of D_1 and D_2 receptor sensitivity in favor of D_1 receptor is
associated with oral dyskinesia (Peacock et al., 1990).

The involvement of 5-HT systems in the behavioral regulation was described almost 2 decades ago. One of the earliest reports regarding 5-H and behavior was that a distinctive and highly reproducible motor syndrome was elicited by 5-H precursors [L-5-hydroxytryptophan (5-HTP) and L-tryptophan] (Jacobs, 1976). Patterned motor activities such as chewing, biting and licking behaviors are associated with 5-H in adult animals (Jacob and Fornal, 1993; Wallis, 1994). Specifically, 5-Hₐ and 5-Hₐc receptors are linked to the initiation of motor pattern (Cazalets et al., 1992). It was proposed that the major function of 5-H neurons in the central nervous system is to initiate and modulate motor outputs and to inhibit sensory information processing. These functions can be attenuated when 5-HT systems are inhibited. Therefore, central 5-H systems are important for maintaining continuous output in motor systems (Jacob and Fornal, 1993; Wallis, 1994). It is also known that 5-H systems, particularly 5-Hₐc receptors, play important roles in regulating oral activity in rats (Gong et al., 1992; Gong and Kostrzewa, 1992; Brus et al., 1994). Atypical neuroleptics have higher affinity to 5-H receptors and produce much less extrapyramidal side effects (such as TD) as compared to classical neuroleptics. Therefore, while DA D₄ receptor blockade may be essential for the treatment of psychosis, blockade of 5-HT receptors
may reduce the incidence of extrapyramidal side effects. It is also quite possible that atypical neuroleptics modulate DA function via 5-HT₃ receptors (Meltzer and Nash, 1991). 5-HT₃ receptor antagonists have been suggested as novel neuroleptics with fewer side effects (Leysen et al., 1993).

Presently, in support with our previous findings (Gong et al., 1992), we find that the 5-HT₁C receptor antagonist mianserin attenuates both m-CPP- and SKF 38393-induced oral activity in rats before haloperidol withdrawal (Fig.9). After haloperidol withdrawal, the 5-HT₁A/₁B receptor antagonist pindolol, 5-HT₂ receptor antagonist ketanserin and 5-HT₄ receptor antagonist metoclopramine fail to attenuate spontaneous oral activity of the haloperidol-withdrawn rats (Fig.15). We had previously found that disruption of 5-HT fibers by neonatal 5,7-DHT (5,7-dihydroxytryptamine) treatment attenuates SKF 38393- and pilocarpine-induced oral activity in n6-OHDA-lesioned rats. It was thereby proposed that multiple sites of interactions involving 5-HT fibers and 5-HT receptors, take place between 5-HT and DA systems in the brain. Drugs acting on 5-HT receptors may be beneficial in preventing or attenuating oral dyskinesias in TD patients (Brus et al., 1994).

The involvement of central cholinergic systems are also known to be involved in regulating oral activity in animals (Rupniak et al., 1983, 1985a,1985b; Salamone et al., 1990; Kostrzewa and Neely, 1993). However, the role of
cholinergic drugs on TD is still unclear. In animals, acute or co-administration of anticholinergic drug scopolamine were found to decrease (Rupniak et al., 1983, 1985a; Salamone et al., 1990; Steinpreis et al., 1993) or have no effect (Stoessel et al., 1989) on oral activity induced by chronic neuroleptic treatment. Clinically it was found that cholinomimetics are much more effective than cholinolytics in reducing the severity of dyskinetic movements of TD. In many cases anticholinergic drugs aggravate TD symptoms (Jeste and Wyatt, 1982a; Casey, 1987; Klawans and Rubovits, 1974). It is generally agreed that cholinolytics alleviate the early emergence of oral dystonias, while cholinomimetics improve the symptoms of late-onset oral dyskinesias (Klawans and Rubovits, 1974; waddington, 1990). The response to scopolamine has been used as one criterion to assess cholinergic involvement in animal models of TD (Klawans and Rubovits, 1974; Rupniak et al., 1983; Casey, 1987). With this in mind, we tested the effects of scopolamine on the oral activity of our rats. Scopolamine significantly attenuated the elevated oral activity of haloperidol-treated rats during the phase of haloperidol treatment (Fig. 12) but had no effect during the phase after haloperidol was withdrawn as a treatment (Fig.16). The different responses of oral activity to scopolamine and SCH 23390 before and after haloperidol withdrawal indicate that different neuronal interactions exist before and after the withdrawal
of haloperidol. It is also possible that at the time when the effect of scopolamine was tested during the haloperidol treatment phase, the nature of oral activity of the rats was more closely related to dystonia, and that the oral activity after haloperidol withdrawal might more closely represent TD.

Data from human and animal studies reveal that GABA agonists such as muscimol and progabide are effective in diminishing oral dyskinesias. GABA levels in cerebrospinal fluid (CSF) were found to be significantly decreased in TD patients (Tamminga et al., 1979). When administered to neuroleptic-treated and neuroleptic-free TD patients, muscimol at an oral dose of 5 to 9 mg consistently attenuated involuntary oral movements and improved TD symptoms (Tamminga et al., 1979; Cassady et al., 1992). Acute challenge and co-administration of progabide with neuroleptics markedly reduced neuroleptic-induced oral dyskinesias (Mithani et al., 1987; Kaneda et al., 1992). Intrastriatal injection of muscimol (25 and 50 ng/0.2μg) demonstrated that GABAergic inhibition of apomorphine (0.2mg/kg, i.v.)-induced oral movement is regionally specific. Muscimol injected into the ventral striatum, but not the dorsal striatum significantly inhibited oral movement induced by apomorphine (KiKuchi de Beltran et al., 1993). Although it is difficult to separate the antidyskinetic effects of GABA agonists from their sedative
and muscle relaxant effects and there is still no convincing
evidence to suggest that GABA agonists exert a specific
antidyskinetic effect (Jeste and Wyatt, 1982a), the
involvement of impaired GABAergic mechanisms in TD have been
widely accepted (Mithani et al., 1987; Cassady et al.,
1992). In the present study, the GABA agonist muscimol, at
a dose of 3.0 kg/mg, significantly attenuated oral activity
during and after haloperidol treatment. In contrast,
bicuculline, a GABA receptor antagonist, does not have any
effect on the oral activity of haloperidol-treated rats
(Fig.10; Fig.11 and Fig.16).

Other neuronal systems such as opioid (Cancela et al.,
1988; Patrick et al., 1993) and neurokinin systems (Liminga,
1993) have been reported to be involved in the regulation of
oral activity. With this in mind we tested a variety of
agents acting at different neuronal systems. As listed in
table 1, no significant effect on oral activity was found
with adrenergic receptor antagonists phenoxybenzamine (α),
phentolamine (α) and propranolol (β), or histamine receptor
agonists cyproheptadine (H1) and ranitidine (H2), or NMDA
(N-methyl-D-aspartate) receptor antagonist (ketamine),
adrenosine receptor antagonist (theophylline), opioid
receptor antagonist (naloxone) and agonist (morphine). The
NMDA receptor antagonist MK-801 significant reduced the oral
activity of the rats. However, severe sedation and
immobility were also observed in these rats. This may mask
the effect of MK-801 on oral activity. Therefore, it is unlikely that these systems had a prominent role in regulating oral activity of rats.

Chronic treatment with neuroleptics was shown to cause a reversible increase in the \( B_{\text{max}} \) for DA \( D_2 \) receptors in rat striatum (Duncan et al., 1987; Marin and Chase, 1993; Laruelle, et al., 1992). Clow et al. (1980) reported an increased \( B_{\text{max}} \) and \( K_d \) for DA \( D_2 \) receptors in rat striatum and mesolimbic area after chronic trifluoperazine treatment. The increased \( B_{\text{max}} \) and \( K_d \) persisted for 3 months and 2 weeks respectively after neuroleptic withdrawal. Dewey and Fibiger (1983) similarly reported that the elevated \( B_{\text{max}} \) for DA \( D_2 \) receptors induced by chronic neuroleptic treatment, returned to the control level 6 weeks after cessation of neuroleptic. Chronic neuroleptic treatment also increased DA \( D_2 \) density in rat caudate putamen (Jiang et al., 1990; See et al., 1989). The increase of \( D_2 \) receptor density in rat striatum and caudate putamen was dissociated from DA agonist-induced behavioral supersensitivity (Marin and Chase, 1993; Jiang et al., 1990). There are conflicting reports regarding neuroleptic-induced alterations in the \( B_{\text{max}} \) for DA \( D_2 \) receptors in other parts of brain. Rupniak et al. (1985b) found that DA \( D_2 \) receptors in nucleus accumbens (mesolimbic system) are no longer blocked after 3-6 months of neuroleptic treatment. However, Laruelle and associates found similar changes of \( D_2 \) receptor binding parameters in
both rat striatum and nucleus accumbens (Laruelle et al., 1992). Neonatal 6-OHDA treatment (bilateral icv, 50 µg each side) at 3 days after birth was reported to increase [\(^3\)H]raclopride binding to the substantia nigra, all parts of the caudal striatum and most parts of rostral striatum (Radja et al., 1993). In contrast, intrastriatal injection of 6-OHDA (4, 8 or 20 µg, each side) at 0 to 2 days after birth did not cause significant changes in DA \(D_1\) receptor binding characteristics in rat striatum. The lack of change of DA \(D_1\) receptors may be due to the absence of DA \(D_1\) receptors in the striatum at the time in ontogeny when rats were lesioned (Neal and Joyce, 1992; Neal-Beliveau and Joyce, 1993). Duncan and colleagues (1987) reported that 15 days of chronic haloperidol treatment caused a significant increase in the \(B_{max}\) for DA \(D_1\) receptors in nucleus accumbens of intact and adult 6-OHDA-lesioned rats but not in n6-OHDA-lesioned rats. In the striatum, this treatment also led to a significant increase of \(B_{max}\) in intact and adult 6-OHDA-lesioned rats. However, changes in the \(B_{max}\) for DA \(D_1\) receptors in the striatum of n6-OHDA-lesioned rats was not determined in their study (Duncan et al., 1987). In our study (table 2), in agreement with the previous findings, DA \(D_1\) receptor density (\(B_{max}\)) was significantly increased in the intact rats receiving chronic haloperidol treatment while \(K_d\) was not significantly altered. Neonatal 6-OHDA treatment itself does not cause any change in DA \(D_1\) binding
parameters. However, there is a marked increase of the $B_{\text{max}}$ for DA $D_2$ receptors in the striatum of n6-OHDA-lesioned rats receiving haloperidol. This finding conflicts with the report in which the $B_{\text{max}}$ for DA $D_2$ receptors was found to be unaltered in the nucleus accumbent of n6-OHDA-lesioned rats after 15 days of haloperidol treatment (Duncan et al., 1987). The reason for this apparent discrepancy is unknown. However, chronic neuroleptic-induced upregulation of $D_1$ receptors in nucleus accumbens is transient. DA $D_2$ receptor density increased temporary and returned to control level in 3-9 months after starting chronic neuroleptic treatment (Clow et al., 1980; Rupniak et al., 1985b). Eight months after haloperidol withdrawal, the $B_{\text{max}}$ for striatal DA $D_2$ receptors in n6-OHDA-lesioned haloperidol-treated rats is at control levels whereas the elevated spontaneous oral activity still persists (table 2). These results support the view that behavioral changes in haloperidol-treated rats is dissociated from changes in DA $D_2$ receptor number (Jiang et al., 1990; Marin and Chase, 1993).

The molecular basis of DA $D_2$ receptor upregulation after chronic haloperidol has been studied by many investigators. It is generally accepted that chronic neuroleptic treatment increases the number of DA $D_2$ receptors in the brain (Creese and Fraser, 1987). In most studies chronic haloperidol treatment increased striatal mRNA of DA $D_2$ receptors (Bernard et al., 1991; Buckland et
Although it has also been reported that chronic neuroleptic treatment did not influence the mRNA of DA D<sub>1</sub> receptors (Van Tol et al., 1990), the DA D<sub>1</sub> receptor gene gives rise to two variants of mRNA by alternate splicing. These two variants of mRNA code for two D<sub>1</sub> receptor isoforms termed D<sub>L</sub> and D<sub>S</sub>, with sequences of 444 and 415 amino acids respectively. D<sub>L</sub> protein has 29 more amino acids than D<sub>S</sub> in the third intracellular loop of the receptor (Giros et al., 1989; Monsma et al., 1989; Selbie et al., 1989; Martres et al., 1992). These two isoforms have the same ligand-binding properties but it has been proposed that they may be coupled differently to G proteins in signal transduction processes (Monsma et al., 1989). It was reported that 16 days (4mg/kg per day, i.p.) or 32 days (1.5 mg/kg/injection, i.p. twice a day) treatment of haloperidol significantly increased striatal mRNA levels of both D<sub>L</sub> and D<sub>S</sub> receptors (Fishburn et al., 1994; Buckland et al., 1993). Rogue and associates reported an increase of striatal total D<sub>L</sub> and D<sub>S</sub> receptor mRNA levels after chronic haloperidol treatment (Rogue et al., 1991). In contrast, Srivastava et al. (1990) reported no change in D<sub>L</sub> and D<sub>S</sub> receptor mRNA levels of rat striatum after chronic haloperidol treatment. Similarly, both striatal D<sub>L</sub> and D<sub>S</sub> receptor mRNA levels were found unaltered after chronic haloperidol (Matsunaga et al.,
Therefore, the increase of mRNA levels may not be critical for $D_2$ receptor up-regulation after long-term haloperidol. It is possible that post-transcriptional mechanisms regulate the haloperidol-induced increase of $D_2$ receptor numbers (Srivistava et al., 1990). In our study, in agreement with what was reported by Rogue and colleagues (1991), we found that long-term haloperidol treatment increased the mRNA levels of $D_2_L$ receptors in both intact and n6-OHDA-lesioned rats whereas $D_2_S$ receptor mRNA levels remained unchanged (Fig. 17). These findings imply that different transcriptional mechanisms regulate the expression of mRNA for two $D_2$ isoforms under the influence of chronic haloperidol treatment. The mechanism underlying this regulation is yet to be determined.

A change in 5-HT receptor mRNAs after chronic haloperidol treatment has not been reported. Because of the interactions between central DA and 5-HT receptors, especially 5-HT$_{3C}$ receptors (Gong et al., 1992; Brus et al., 1994), we determined the levels of 5-HT$_{3C}$ receptor mRNA in all rats after haloperidol treatment. Levels of 5-HT$_{3C}$ mRNA were slightly but not significantly increased in both groups of rats receiving haloperidol, as compared to the rats without haloperidol treatment and the rats withdrawn from haloperidol (Fig. 17). Our findings suggest that the interactions of DA and 5-HT systems in regulating oral activity under chronic haloperidol treatment are not at the
transcriptional level. It is possible that the interactions at post-transcriptional or receptor levels contribute to the cross-talk of these two systems.

The present study utilized for the first time, chronic haloperidol-treated n6-OHDA-lesioned rats as an animal model of TD. Long-term haloperidol treatment produces a greater incidence of oral dyskinesias in n6-OHDA-lesioned rats vs. intact rats. Higher densities of DA D$_1$ receptors and increased DA D$_2$ receptor mRNA levels in rostral striatum were found in all rats that were treated by haloperidol. Eight months after discontinuing haloperidol, the elevated D$_1$ receptor densities and D$_2$ receptor mRNA levels returned to control levels whereas the oral dyskinesia still persisted. The dissociation of oral dyskinesia, D$_1$ receptor density and mRNA levels indicates that D$_1$ receptor upregulation is not important for the occurrence of oral dyskinesias. Instead, oral dyskinesias appear to be related to an imbalance of multineuronal systems. An advantage of the present model of TD is the persistence of a stable elevated baseline of oral activity after haloperidol withdrawal, providing a means for dose-response testing and even retesting of agents that have the potential to attenuate dyskinetic oral activity.
Chapter 5
Summary

The finding of the present study are summarized as following:

a. Chronic haloperidol treatment produced a greater incidence of oral activity on neonatal 6-OHDA-lesioned rats vs. intact rats.

b. The elevated oral activity in n-6OHDA-lesioned rats persisted for at least 8 months after withdrawal of haloperidol treatment.

c. The interval during haloperidol withdrawal is useful for studying potential-useful drugs for treatment of TD.

d. Chronic haloperidol treatment significantly increased dopamine D2L receptor mRNA levels in rostral striatum of both intact and neonatal 6-OHDA-lesioned rats.

e. Chronic haloperidol treatment significantly increased dopamine D3 receptor density but not affinity in rostral striatum of both intact and neonatal 6-OHDA-lesioned rats.

d. The increase of D3 density and D2L mRNA returned to control level after 8 months of haloperidol termination.


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