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Dopamine Agonists Modify the Development of Brain D1 and D2 Receptor Responsiveness

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East Tennessee State University, 1990

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DOPAMINE AGONISTS MODIFY THE DEVELOPMENT OF BRAIN D1 AND D2 RECEPTOR RESPONSIVENESS

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

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

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by
Anwar Hamdi, M.D.
May, 1990

APPROVAL

This is to certify that the Graduate Committee of

Anwar Hamdi

met on the

27th day of November, 1989

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

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Signed on behalf of
the Graduate Council

Associate Vice-President for Research
and Dean of Graduate School

ABSTRACT

DOPAMINE AGONISTS MODIFY THE DEVELOPMENT

OF BRAIN D1 AND D2 RECEPTOR RESPONSIVENESS

by

Anwar Hamdi, M.D.

Dopamine (DA) agonist-induced behavioral supersensitivity in the adult rat has served as the standard model for certain of the motor and behavioral side effects associated with long-term exposure to DA agonists in humans. The mechanisms relating receptor events with behavior mediation, however, remain unclear.

The striatum of rats progresses through a prolonged and varied postnatal developmental period. In order to examine the relative contribution of D1 and D2 receptor-mediated mechanisms to behavioral changes which follow chronic dopamine agonist exposure, developing rats were treated daily from birth with a D1 agonist, SKF 38393 hydrochloride (3.0 mg/kg x 32d, i.p.), or a D2 agonist, LY 171555 hydrochloride (3.0 mg/kg x 32d, i.p.), and/or 6-OHDA (134 μg, i.c.v., at 3 d after birth). Following a drug-free interval, behavioral responses to selective DA agonists were evaluated.

The results indicate that (1) prolonged LY 171555 treatments in development produced a supersensitive animal model for yawning and eating behaviors. (2) Perioral movements of high frequency could be produced by a very low dose of the DA D2 antagonist spiroperidol in rats treated neonatally with 6-OHDA, thereby providing a useful animal model to study tardive dyskinesia. (3) The "priming" phenomenon described by Breese and co-workers which was thought to be produced by D1 agonists only has been found in this study to be produced by a D2 agonist as well. This model provides a means for studying specific stereotypic behaviors in animals. (4) [3H]SCH 23390 and [3H]spiroperidol binding to striatal tissue was not altered in rats treated in development with specific agonists or antagonists for the D1 and D2 receptors. A neonatal 6-OHDA lesion did not modify binding in any of the agonist- or antagonist-treated groups.

In conclusion, DA D1 and D2 agonist treatments during postnatal development are effective means of producing new animal models that are potentially useful for studying clinical disorders in man.

DEDICATION

This dissertation is dedicated to my wife, Hana, a wonderful woman who provided continued support and encouragement.

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CHAPTER 1

Introduction

Anatomical Review

Dopamine (DA), a catecholamine, is present as a neurochemical in one of the most studied neurotransmitter systems in the brain. Until 1958, DA had been thought to be only an intermediate precursor of norepinephrine and epinephrine synthesis (Carlsson et al., 1958; 1959). Largely on the basis of the fluorescence histochemical procedure (Dahlstrom and Fuxe, 1964) and later immunocytochemical procedure, DA-containing nerves were mapped in the brain. There are three major dopaminergic neuronal systems in the brain (Lader, 1983). These are as follows:

1. One group of dopamine-neurons originates in the ventral tegmental area and terminates in the nucleus accumbens, olfactory tubercle, nucleus interstitialis stria terminalis, hypothalamus, preoptic area, cingulate cortex, hippocampus, central amygdaloid nucleus, lateral septum, prefrontal and entorhinal cortices, midbrain, and ventromedial neostriatum. This so-called A10 group is commonly known as the mesolimbocortical DA system (Beckstead et al., 1979).

2. A more lateral group of DA cells, designated the A9 or mesostriatal DA system, originates in the substantia nigra pars compacta. These neurons project mainly to the neostriatum (caudate nucleus and putamen), amygdala, nucleus accumbens, bed nucleus of the stria terminalis, anteromedial cortex, entorhinal area, globus pallidus, subthalamic nucleus, and cingulate cortex.
3. A system consisting of short neurons arising mainly in the anterior part of the arcuate nucleus and the ventral periventricular nucleus of the hypothalamus (A12) terminates in the external layer of the median eminence (the tuberoinfundibular DA system), pars intermedia, and neural lobes of the pituitary (tuberohypophyseal DA system), and infundibular stalk (Nauta et al., 1978; Beckstead et al., 1979). Other short dopaminergic pathways are the incertohypothalamic, which links the hypothalamus and lateral septum, and the medullary periventricular nucleus in the periaqueductal grey matter.

Proposed Functions Of Dopamine Systems

The physiology and pharmacology of DA from the standpoint of its function as a neurotransmitter or modulator in the central nervous system (CNS) has been

established, and it is accepted now that the dopaminergic system is involved in:

1. Modulation of a variety of behaviors including: ambulation or locomotion (Ungerstedt and Arbuthnott, 1970; Pijnenburg et al., 1976), stereotyped behaviors (Creese and Iversen, 1973), self-stimulation behavior (Phillips and Fibiger, 1973), stimulus control behavior (Ho and Huang, 1975), conditioned avoidance responding behavior, feeding and drinking (for references see Kostrzewa and Jacobowitz, 1974; Kostrzewa, 1989), reward-seeking behavior (German and Bowden, 1974; Wise, 1978), latent inhibition in which prior exposure to a stimulus not followed by reinforcement retards subsequent conditioning to that stimulus (Weiner et al., 1984; 1987; Weiner and Feldon, 1987), as well as mechanisms controlling memory consolidation and retention (Routtenberg and Holzman, 1973; Major and White, 1978; Coulombe and White, 1980; White, 1988).
2. Neuroendocrine function (tuberohypophyseal dopaminergic system) including: hypothalamic secretion of releasing factors, gonadal secretion of steroids, and pituitary secretion of prolactin.

Dopamine Receptors

It is now well established on the basis of biochemical, anatomical, pharmacological, and behavioral characteristics that brain DA can act on at least two distinct subtypes of DA receptors (Kebabian, 1978; Kebabian and Calne, 1979; Creese et al., 1983; Stoof and Kebabian, 1984; Onali et al., 1985; Weiss et al., 1985). The D1 type DA receptor is coupled to an adenylate cyclase (AC) such that receptor occupancy by an agonist increases cyclic adenosine 3'5'-monophosphate (cAMP) formation (Hyttel, 1978). The D2 type DA receptor, on the other hand, is either unlinked or linked to adenylate cyclase in a negative manner such that receptor occupancy by an agonist decreases cAMP formation (Stoof and Kebabian, 1981; Onali et al., 1984; Battaglia et al., 1985).

The existence of these two receptors has been supported by the identification of agonists and antagonists selective for either of these receptors.

Studies Directed Specifically to Dopamine D1 and D2 Receptors

The increasing availability of drugs which act selectively on D1 or D2 DA receptors now permits a more detailed evaluation of their response to chronic blockade or stimulation.

The D2 DA receptors in the CNS have been labeled by using the selective D2 antagonist, [3H]spiroperidol (Creese

et al., 1977a; Leysen et al., 1978; Laduron et al., 1978; Creese, 1982). These autoradiographic studies show high densities of autoradiographic grains in the olfactory tubercles, nucleus accumbens, nucleus caudate-putamen, lateral septum, zona incerta, nucleus subthalamicus, arcuate nucleus, central nucleus of the amygdala, claustrum, prelateral and lateral mammillary nuclei of the hypothalamus, nucleus of the lateral optic tract, dorsal interpeduncular nucleus, ventral tegmentum, substantia nigra, superior and inferior colliculi, parts of periaqueductal grey, portions of the midline, cerebral cortex (Klemm et al., 1979), hippocampus, pineal gland, and the cerebellum (Bruinnk and Bischoff, 1986). The most dense aggregates are found in the striatum and nucleus accumbens (Gehlert and Wamsley, 1984).

D1 DA receptors have been studied by using the non-selective radioligands cis-[3H]piflupentixol and cis-(Z)-[3H]pifluthixol (Hyttel, 1978; 1981). A newer selective antagonist for D1 sites, SCH 23390, the 3-methyl 7-chloro analog of SKF 38393, [(R)-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepine-7-51] (Iorio et al., 1983; Hyttel, 1983), crosses the blood brain barrier and is subject to extensive first-pass metabolism (the ID50 of SCH 23390 given orally is about 100-fold higher than after intraperitoneal [i.p.] administration). This novel benzazepine exhibits nanomolar potency in inhibiting

dopamine stimulation of striatal adenylate cyclase activity (Onali et al., 1984; Plantje et al., 1984; Hyttel, 1984) and has a Ki value of approximately 0.5 nM for the striatal D1 DA receptors (Cross et al., 1983; Hyttel, 1983; Billard et al., 1984; Schulz et al., 1984). Treatment with SCH 23390 (0.50 mg/kg, s.c.) prior to peripheral administration of EEDQ (the protein-modifying reagent N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline) protects D1 DA receptors from irreversible blockade by EEDQ, while neither D2 DA nor S2 serotonin receptors are protected (Hess and Creese, 1986). Commercial [3H]SCH 23390 has a low level of nonspecific binding, high specific activity (Billard et al., 1984; Andersen et al., 1985), and specifically labels D1 sites both in vitro and in vivo (Billard et al., 1984; Schulz et al., 1984; 1985; Andersen et al., 1985; Andersen and Nielsen, 1986). Autoradiographic studies with [3H]SCH 23390 show that D1 DA receptors are present in abundance in the corpus striatum, olfactory tubercle, nucleus accumbens, dorsomedial prefrontal cortex, lateral preoptic area, globus pallidus, amygdala, lateral septum, parietal cortex, hippocampus, hypothalamus, olfactory bulb, cerebellum, and brainstem (Schulz et al., 1985). Very low numbers of D1 sites are observed in the ventral tegmental area (Dawson et al., 1986). Within the nigrostriatal system, D1 sites are found only postsynaptically within the striatum (Filloux et al., 1987; 1988) and presynaptically in the substantia nigra

(Altar and Hauser, 1987; Filloux et al., 1988).

Dopamine Receptor Distribution

The D1 and D2 DA receptors show different distribution and number in the brain, have different neuronal localization, and have different molecular size. In addition, their number changes with age, after long-term neuroleptic treatment, and after 6-hydroxydopamine (6-OHDA) lesions. The affinity of both neuroleptics and agonists for these receptors is very different.

Quantitatively, it has been hypothesized that there may be about four times as many D1 DA receptors as there are D2 DA sites in the striatum (Leff et al., 1984) and olfactory tubercles (Hyttel, 1978). More D1 than D2 sites are also found in the nucleus accumbens. The ordered ratio of D1/D2 sites in the brain is striatum> nucleus accumbens> olfactory tubercles.

Function and Interaction of DA D1 and D2 Receptors

The manner in which D1 and D2 DA receptors interact in the brain to carry out their function is still unclear, even though intensive studies have been directed toward understanding the relative contributions of D1 and D2 receptor subtypes in mediating physiological and behavioral phenomena. Available evidence suggests that D2 systems are linked to a particular mode of expression of behavior, such

as stereotypy. D1 receptors mediate a discontinuous, non-stereotyped activation of behavior characterized by episodes of a prominent grooming response interpolated among episodes of sniffing with some locomotion and rearing, repetitive mouth opening and clonic jaw movements (Rosengarten et al., 1983; 1986; Molloy and Waddington, 1984; 1985; 1987; 1988; Starr and Starr, 1986; Johansson et al., 1987; Murray and Waddington, 1989). D1 systems appear to have a greater role in influencing the intensity (enabling) of expression of the mode selected by D2 systems, with the full expression of dopaminergic behavior requiring concurrent stimulation of both receptor subtypes (Molloy et al., 1986; Mashurano and Waddington, 1986; Braun and Chase, 1986; Waddington, 1986; Arnt et al., 1987).

It is now accepted that D1 and D2 DA receptor-activated neuronal systems are not functionally isolated, but interact in their modulation of motor function:

1. Catalepsy induced by SCH 23390, a D1 antagonist, can be prevented by pretreatment with selective D2 agonists (Meller et al., 1985).
2. Behavioral effects of the D1 DA receptor agonist, SKF 38393, which include non-stereotyped sniffing, rearing, and locomotor responses, are partially reversed by the D2 DA receptor antagonist, metoclopramide (Molloy and Waddington, 1985).

3. Even though they occurs in unlesioned rats, behavioral responses to D2 agonists are not antagonized by SCH 23390, and responses to D1 agonists are insensitive to D2 antagonists in animals either lesioned with 6-OHDA or depleted of DA by treatment with reserpine or a-methyl-p-tyrosine (Arnt, 1985a; 1985b; Arnt and Hyttel, 1985; Herrera-Marschitz and Ungerstedt, 1985; Breese and Mueller, 1985; Breese et al., 1985a,b).

4. D2 DA receptors are not independently responsible for mediating effects of non-selective DA agonists such as apomorphine. Rather, a synergistic interaction between D1 and D2 DA receptor-regulated processes appears to be essential for the expression of behavioral phenomena typically associated with non-selective DA agonists. The same is true for increases in tonic single unit activity in the globus pallidus, evoked by systemic administration of these agents. The behavioral or physiological effects induced by selective D2 agonists apparently require the presence of endogenous DA concurrently stimulating the D1 receptors (Walters et al., 1987).

5. SKF 38393 stimulates the release of cAMP from superfused striatal tissue slices, and this effect is antagonized by LY 141865, a selective D2 DA

receptor agonist (Stoof and Kebabian, 1981).

6. Low doses of spiroperidol induce abnormal perioral movements in rats, which can be potentiated by SKF 38393 (Rosengarten et al., 1983; 1986).

7. Yawning induced by selective stimulation of DA D2 receptors is eliminated by the DA D1 receptor antagonist SCH 23390 (Serra et al., 1987).

8. In the dorsal striatum, the stimulation of DA D1 receptors facilitates the release of [3H]GABA, while stimulation of DA D2 receptors inhibits [3H]GABA release (Girault et al., 1986).

9. DA acting on D1 or on D2 receptors, respectively, inhibits or facilitates the veratridine-evoked release of cholecystokinin (Meyer and Krauss, 1983).

10. DA acting on D1 or on D2 receptors, respectively, increases or decreases the neurotensin level in striatum (Merchant et al., 1989).

11. DA acting on D1 or on D2 receptors, respectively, decreases or increases the substance P level in striatum (Sonsalla et al., 1984).

12. Electroencephalogram (EEG) synchronization and sedation, classically associated with neuroleptic treatment, does not depend upon the selective

blockade of either DA D1 or D2 receptors, but instead requires concurrent blockade of both subtypes of receptors (Bo et al., 1988).

13. In vitro studies have shown that haloperidol, at concentrations which appear to be selective for DA D2 receptor blockade, increases the release of [3H]acetylcholine (ACh) from superfused striatal tissue slices (Starke et al., 1983), and SCH 23390 is capable of inhibiting [3H]ACh release.

Consideration of the functional interaction between D1 and D2 receptors (Breese and Mueller, 1985; Breese et al., 1985a,b; Barone et al., 1986; Carlson et al., 1987) could have important implications in disorders such as Parkinson's disease and schizophrenia, where stimulation or blockade of postsynaptic DA receptors confers symptomatic benefit, and where the ratio of D1 to D2 receptor blockade may be an important determinant of drug action on functions mediated by these receptor subtypes.

6-OHDA and Dopamine Receptors

The specific catecholamine neurotoxin 6-OHDA produces a selective permanent destruction of catecholamine cell bodies and axon terminals in the brain (Ungerstedt, 1971; Uretsky and Iversen, 1970; Kostrzewa and Jacobowitz, 1974). In adult rats, the destruction of DA nigrostriatal fibers is associated with specific motor behavior dysfunctions; i.e.,

aphagia, adipsia, decreased body weight, akinesia (lower level of spontaneous exploratory activity), lack of self-grooming (Breese and Traylor, 1970; Evetts et al., 1970; Creese and Iversen, 1973), increase in the striatal DA receptors (Creese et al., 1977; Creese and Snyder, 1979; Breese et al., 1984), and hypermotility and oral stereotypy with a challenge dose of either D1 or D2 selective agonists (Arnt, 1985b).

While 6-OHDA treatment of neonates produces a nearly complete loss of brain catecholamines (Breese and Traylor, 1972), impairment in food intake is less than that seen in an adult lesioned rat.

6-OHDA produces profound and permanent destructive effects, especially on the DA efferents arising from A9. This is evidenced by a near-complete loss of tyrosine hydroxylase (TH), the rate-limiting enzyme in the DA synthesis; a loss of TH-immunoreactive fibers in the striatum and cell bodies in the substantia nigra pars compacta; also a failure of intrastriatal horse radish peroxidase (HRP) injections to label cells within the substantia nigra. A 2 to 3-fold increase in the amount of striatal serotonin (5-HT) is detected at 2-5 months after neonatal 6-OHDA treatment of rats (Stachowiak et al., 1984; Breese et al., 1984). This effect is accompanied by a parallel increase in the uptake of [3H]5-HT into synaptosomes prepared from striatum (Stachowiak et al.,

1984) and an enlargement of the serotonergic projections from the raphe nucleus to the striatum, especially prominent within the rostral striatum (Berger et al., 1985; Snyder et al., 1986). Since DA- and 5-HT-containing neurons have similar effects on striatal interneurons (Davies and Tongroach, 1978), it will become clear why neonatal 6-OHDA treatment of rats could have some behavioral sparing. Permanent supersensitive behavioral responses to dopaminergic agonists, particularly the D1 receptor agonists, occur in these rats when observed as adults. The D1-induced behavioral responses include taffy pulling and self-mutilatory behaviors. Biochemically there is an inability to up-regulate D1 and D2 receptors in response to challenge doses of the respective antagonists in adults, suggesting that neurochemical adaptation occurs after neonatal 6-OHDA lesions, interfering with the capacity of specific antagonists to increase the respective DA receptor density (Duncan et al., 1987). Neonatally lesioned rats are subsensitive to the immobility effect typically produced by both DA D1 and D2 antagonists in rats lesioned as adults (Duncan et al., 1987). Rats that receive 6-OHDA lesions as adults do not show a progressive increase in response to repeated administration of DA D1 agonist, SKF 38393, as do the neonatally lesioned rats (Criswell et al., 1989). Also, there is no change in binding of [3H]SCH 23390 and [3H]spiroperidol to rat striatum and nucleus accumbens in

adulthood (Breese et al., 1984; 1985a,b; 1986; Duncan et al., 1987).

From the above it can be seen that the 6-OHDA lesions of neonatal rats produce a model characterized by DA D1 and D2 receptor supersensitivity in the absence of a change in receptor number.

Disturbances Of The Dopamine Systems In Human CNS

Several serious disorders are known to be related to disturbances in the function of the dopaminergic systems in the brain.

Parkinson's disease is associated with a loss of the A9 DA system (Hornykiewicz, 1966). Long term neuroleptic-treatment is associated with extrapyramidal side effects, including tardive dyskinesia (TD). Huntington's chorea and Gilles de la Tourette syndrome are characterized by progressive chorea, cognitive impairment, and emotional disturbance. These latter disturbances have been attributed to an increase in DA transmission (Butler et al., 1979; Baldessarini and Tarsy, 1980; Spokes, 1980; 1981; Marsden, 1982). The mesolimbocortical A10 DA system has been implicated in the pathophysiology of schizophrenia. Either excessive release of DA or altered receptor sensitivity has been implicated in the symptomatology of schizophrenia, which is treated with dopaminergic drugs (Matthysse, 1973; Stevens, 1973; Snyder, 1982; Chiodo and Bunney, 1983; White

and Wang, 1983a,b).

A complete understanding of the pathophysiologic role played by DA in such conditions is not possible without a knowledge of the development of the receptors which represent transducers of the signals generated by this neurotransmitter.

Antipsychotics and Dopamine Receptors

Antipsychotics or neuroleptics are the drugs of choice in the treatment of psychosis and other forms of schizophrenia. This class has also been used to treat the dystonic symptoms of Huntington's chorea and Gilles de la Tourette syndrome (Shapiro et al., 1978).

Using [3H]Haloperidol, an antipsychotic drug, and [3H]DA, Seeman et al. (1975; 1976) have provided the first direct evidence that neuroleptics compete with DA for stereospecific DA binding sites. The relative affinity for D2 sites is directly proportional to clinical potency.

Considerable evidence indicates that long term treatment with haloperidol results in the development of DA receptor supersensitivity. This is manifest as an enhanced behavioral response to DA agonists (Gianutsos et al., 1974; Bhargava and Ritzmann, 1980) and an increased number of [3H]neuroleptic/dopamine receptor binding sites (up-regulation) in the striatum (Burt et al., 1976; Muller and Seeman, 1977; Matwyshyn and Bhargava, 1983; Mackenzie and

Zigmond, 1985). The major side effect, tardive dyskinesia, which gradually develops in approximately 25% of schizophrenics receiving chronic neuroleptics, is characterized by uncontrollable purposeless and persistent movements of the mouth, tongue, and extremities (Baldessarini and Tarsy, 1980). This resembles the involuntary movements occurring in Parkinsonian patients treated with L-DOPA, and can be reduced either by lowering or raising the neuroleptic dose. The proliferation of neuroleptic/dopamine binding sites is observed after long term administration of either butyrophenones or phenothiazines (Clow et al., 1979; Wan et al., 1983).

Ontogeny of Dopaminergic Neurons

There is a progressive increase in the striatal concentration of DA and associated synthetic enzymes, such as TH, DA-stimulated AC, which at birth, represent only 20% of adult levels. TH and AC attain adult levels by the age of 3 to 4 weeks of postnatal life (Coyle and Axelrod, 1972; Coyle and Compochiaro, 1976), and this has been attributed to the proliferation and growth of axon terminals (Loizou and Salt, 1970; Coyle and Axelrod, 1972). Functional dopaminergic-cholinergic interaction does not occur until the second week of postnatal life (Coyle and Campiochiaro, 1976). There is no spontaneous single unit activity of striatal cells in eight-day-old rat pups. At 17 days

striatal single unit activity is substantially less diverse and of a lower frequency than that observed at 28 days. Haloperidol induces single unit activity of striatal cells at 28 days, but not at 17 days (Napier et al., 1985). Haloperidol induces a cataleptic response in 21 days rats, but not in a 14 days rats (Coyle et al., 1985). The growth of 5-HT axons into the striatum begins late in development (Lidov and Molliver, 1982) in comparison to the 5-HT innervation of other structures and DA innervation of the striatum (Specht et al., 1981). Marked stereotypy to apomorphine only appears at 21 days of age. Before that, the response is weak and variable. Islands of dopaminergic innervation, present in the striatum on day 5, nearly disappear by day 15 (Olson et al., 1972). In contrast, the DA receptors appear in a uniform pattern beneath the corpus callosum. This patch extends further into the striatum and is not as elongated as the fluorescent subcallosal streak. Receptors then diminish medially and there are no islands of receptors to correspond to the fluorescence (Murrin, 1982). Neuronal cell division in the striatum is mostly complete after the first few postnatal days in the rat (Das and Altman, 1970), and there is some evidence for neuronal cell death in the first two postnatal weeks, based on estimates of the total number of neurons present in the striatum (Fentress et al., 1981). At the first day after birth, the rat striatum contains only 12 and 24% of the adult level of

DA and DOPAC, respectively. The striatal DA content increases somewhat slowly thereafter, reaching adult levels by postnatal day 60. DA content remains constant through adulthood and senescence. In contrast, DOPAC levels develop more rapidly than DA levels, attaining adult values by 20 days after birth, a time when DA is less than 50% of adult level. DOPAC content remains unchanged through senescence. Basal AC activity increases 6-fold from birth to postnatal day 45. Concentration-response curves for DA-stimulated AC activity indicate that 100 uM DA induces a maximal stimulation of cAMP in rats of different ages. The effect of this concentration of DA increases 4-fold from birth to postnatal day 14 when the maximal responsiveness to DA is observed. The effectiveness of DA on AC production rapidly returns to adult levels thereafter. Striatal DA content reaches 50% of the adult value by postnatal day 35 (Giorgi et al., 1987). DA content of the brain rises significantly between 0-7 days after birth, with the greatest rise per unit time occuring between 7 and 18 days. Adult levels are attained at about 50 days. The most rapid increase in TH activity is between the 7th and 18th day.

The above findings indicate that DA-containing neurons gradually develop their functions during fetal life and the first 4-8 weeks of postnatal life.

Ontogeny of Dopamine D1 Receptors

The density of DA D1 receptors in rat striatum is only 9% of the adult value at birth and increases very rapidly thereafter, so that by postnatal day 35 a peak value is attained (Giorgi et al., 1987). The maturation of DA D1 receptors and DA-stimulated AC activity in the striatum (and other brain areas) precedes that of the presynaptic dopaminergic neuron markers (Spano et al., 1976; Hohn and Wuttke, 1979; Lamberts and Wuttke, 1981; Giorgi et al., 1987). DA D1 receptors increase in density more rapidly than DA D2 receptors in the first two weeks postnatally. The ratio of D1 to D2 receptors increases from 1.7 at 2 days of age to 3.3 at 14 days of age (Zeng et al., 1988).

Ontogeny of Dopamine D2 Receptor

In vivo and *in vitro* studies have shown that DA D2 receptors double in number between day 5 and day 15 after birth, and then gradually increase to adult levels by day 30 (Pardo et al., 1977; Murrin, 1982; Murrin and Zeng, 1986). Since DA plays an important trophic role in the development of a normal number of striatal DA receptors (Rosengarten et al., 1983a; Miller and Friedhoff, 1986), it has been suggested that the development of DA D2 receptors, and the responses that they mediate, are dependent upon the maturation of their corresponding presynaptic nerve terminals (Rosengarten and Friedhoff, 1979; Deskin et al.,

1981). Some reports do not support this view (Creese and Iversen, 1973; Pardo et al., 1977). Striatal presynaptic receptors, which are D2 receptors, may be just starting to function around the first week of life and may not attain full maturity until the second postnatal week (Shalaby et al., 1981).

Impairment Of Dopamine Receptor Ontogeny

Rosengarten and Friedhoff (1979) found that prenatal administration of haloperidol results in a persistent decrease in postnatal striatal DA D2 receptors (Rosengarten et al., 1983b). The critical period for this prenatal neuroleptic effect is between gestational days 15 and 18 (Rosengarten et al., 1983a). This treatment also affects muscarinic cholinergic activity in the striatum (increase in binding sites) (Miller and Friedhoff, 1986). On the other hand, giving haloperidol to nursing dams for the first 21 days after birth, results in a 40% increase in the number of striatal DA D2 receptors in the nursing littermates, two weeks after the last dose (Rosengarten and Friedhoff, 1979). Treatment of neonatal rats with penfluridol, on alternate days during the first postnatal week, does not affect the development of striatal DA D2 receptors, although there is a supersensitive response of these rats to the DA receptor agonist, apomorphine (Coyle et al., 1981).

Kostrzewa and Saleh (1989) have shown that

administration of the DA D1 receptor antagonist, SCH 23390 (0.3 mg/kg i.p.), or the DA D2 receptor antagonist, spiroperidol (1.0 mg/kg i.p.), to rats once a day for 32 successive days from birth results in a marked impairment of the development of striatal D1 receptors at 12 weeks (74% reduction in the specific binding) and marked impairment of the development of striatal D2 receptors at 12 weeks (74% reduction in the specific binding), respectively.

It can be conclude from the above results that the fetal and early postnatal periods in rats may be vulnerable stages in the functional maturation of the central dopaminergic system. Investigation of the development of this system might assist in understanding its physiological significance.

Dopaminergic System and Behavior

Animal behavioral assays are an important tool for elucidating actions of drugs used in the treatment of CNS disorders. The behavioral assays have been used to define the characteristic profiles of DA antagonists and DA agonists, in the hope of screening new drugs for therapeutic value. The behavioral assays used most commonly are as follows:

1. The rotational model, which usually includes unilateral 6-OHDA lesions of the substantia nigra or of the median forebrain bundle of rats. A

contralateral circling response (away from the lesioned side) is produced by DA agonists which preferentially stimulate supersensitive DA receptors on the denervated side. Conversely, an ipsilateral circling response is produced by dopaminomimetics which release DA from intact dopamine neurons on the non-lesioned side, resulting in postsynaptic stimulation of DA receptors on the intact side.

2. The stereotypy model, which includes observation of such behaviors as licking, gnawing, biting, chewing (oral stereotypy), sniffing, rearing, grooming, and locomotion in rats.

3. The catalepsy model is best described as a loss of locomotor behavior and an exaggeration of bracing responses to stimuli. This categorization is operationally defined, for example, by placing a rat's forepaws over a horizontal bar and observing time of immobility in the imposed posture. Catalepsy results from blockade of DA D1 and/or D2 receptors in the striatum. It is known that these receptive site are restricted to quinolinic acid-sensitive intrinsic neurons (Calderon et al., 1988).

4. The yawning response, which is elicited in experimental animals by DA agonists. This effect

is antagonized by the centrally acting DA antagonists. The receptors involved in this behavior have characteristics in common with the DA D2 autoreceptor (Mogilnicka and Klimek, 1977; Nickolson and Berendsen, 1980; Yamada and Furukawa, 1980; Gower et al., 1984; Stahle and Ungerstedt, 1984; Serra et at., 1983a,b; 1984; Dourish and Hutson, 1985; Yamada et al., 1986; Stoessl et al., 1987). However, according to some studies these receptors are located postsynaptically (Serra et al., 1986). Nevertheless, 6-OHDA lesions of DA neurons abolish the yawning response (Dourish and Hutson, 1985; Stoessl et al., 1987).

5. Perioral movements (abnormal mouth movements = oral dyskinesia). In humans, repetitious opening and closing of the mouth and high frequency clonic jaw movements are the most outstanding characteristic of tardive dyskinesia which results from prolonged neuroleptic treatment. Perioral movements in normal rats can be induced by selective stimulation of the DA D1 receptors or by selective blockade of D2 receptors with antagonists (Molloy et al., 1986; Rosengarten et al., 1986; Koshikawa et al., 1987; Molloy and Waddington, 1988). Rats with reduced numbers of

D2 receptors, resulting from strain difference, senescence, or pharmacological intervention in development demonstrate increased oral activity after D1 agonist treatment (Rosengarten et al., 1983c; 1986). Oral behavior is increased in rats treated chronically with several neuroleptics (Clow et al., 1979; Waddington and Gamble, 1980; Waddington et al., 1981). The incidence of oral activity is least when DA D2 as well as DA D1 receptors are stimulated (Rosengarten et al., 1986; Johansson et al., 1987).

Heterogeneity of the Striatum

The striatal internal organization of afferent and efferent projections is heterogeneous (Dray, 1979). The dorsal and ventral striatum are different in internal organization, embryonic origin, efferent and afferent projections (Joyce, 1983), and neurotransmitter content. More DA is present in the dorsal part and more 5-HT and norepinephrine are in the ventral part (Widmann and Sperk, 1986).

Dopaminergic drugs injected into different regions of the striatum may interact with different elements of the striatum and produce diverse behavioral effects (Costal et al., 1980; Joyce et al., 1981). The dorsal rostral region supports the rearing response; a more caudal and medio-

ventral region is involved in biting; and a rostro-ventral region sustains the hyperactivity response (Joyce, 1983). These findings emphasize the functional (behavioral) heterogeneity of the striatum (Dunnett et al., 1981a,b). After intraventricular 6-OHDA treatment, extensive loss of DA terminals occurs in medial portions of the striatum, while large numbers of terminals remain intact in more lateral areas (Onn et al., 1986).

Rationale

One factor that influences the clinical manifestation of any lesion in the CNS is the age of the brain at the time of the lesion. Although a lesion is static, its effect(s) on neurologic function is affected as the system matures. Since the striatum progresses through a prolonged and varied postnatal developmental period, DA receptor stimulation or blockade and/or dopaminergic fiber distruction during this interval is likely to alter development of receptor sites. The change may be quite different from that which is observed after a similar lesion in the adult.

Several drugs and neurotoxins administered to developing rats produce different biological consequences from those observed in adults. Chronic treatment of mature animals with neuroleptics leads to DA D2 receptor proliferation (Neve et al., 1985). Once the neuroleptic is withdrawn, the numbers of D2 receptors return to control

levels in a matter of days. However, when the dopaminergic system is altered during early development with prenatal treatment with DA D2 antagonists (Rosengarten and Friedhoff, 1979), or postnatal treatment with DA D1 or D2 specific antagonists (Kostrzewa and Saleh, 1989), DA receptor down-regulation is observed. The changes in the system are permanent, persisting through adulthood.

Breese et al. (1984) demonstrated that the behavioral responses to DA agonists are distinctly different in neonatal and adult 6-OHDA-lesioned rats. Other differences between adult and neonatal 6-OHDA-lesioned rats have also been described. Rats treated with the neurotoxin as adults exhibit a profound aphagia, adipsia, and akinesia (Zigmond and Stricker, 1973). In contrast, after neonatal 6-OHDA treatment, rats do not display such an acute behavioral syndrome (Duncan et al., 1987). Also, neonatal destruction of brain dopaminergic neurons with 6-OHDA results in a selective supersensitivity of D1 receptors in the absence of an altered number of receptors (Breese et al., 1984, 1985a,b, 1986). Whereas neonatal 6-OHDA-lesioned rats show a greater response to D1 receptor agonists than to D2 receptor agonists, adult 6-OHDA-lesioned rats show a greater sensitivity to D2 receptor agonists than to D1 agonists (Breese et al., 1985b). Chronic treatment with the specific DA D1 antagonist, SCH 23390, or DA D2 antagonist, haloperidol, produces a significant elevation in [3H]SCH

23390 or [3H]spiroperidol binding, respectively, in unlesioned and in 6-OHDA adult-lesioned rats. The same chronic treatment in adult animals that were lesioned as neonates with 6-OHDA did not result in an up-regulation of the respective receptors (Duncan et al., 1987).

Several measures of dopaminergic function in striatum and other brain areas are reduced in patients with Lesch-Nyhan syndrome, a disease associated with an inborn deficiency of the enzyme hypoxanthine-guanine phosphoribosyl transferase. Parkinson's disease is another syndrome in which brain dopamine is reduced. However, Parkinson's patients display tremor, bradykinesia, stiffness, and do not demonstrate the choreoathetoid movements, hypertonicity, the unique compulsive self-mutilation behavior (SMB) of digits and tissue about the mouth, or the elevation in serotonin in the striatum observed in children with Lesch-Nyhan disease. Apparent differences in these syndromes are the age of onset of the symptoms (the age at which dopaminergic pathways are destroyed) and the disordered purine metabolism in Lesch-Nyhan patients.

The adult 6-OHDA-treated rats has been used as a neurochemical model of Parkinson's disease. Breese et al. (1984; 1984a) have suggested that the neonatal 6-OHDA-treated rat may serve as a model of central dysfunctions observed in childhood diseases with reduced brain dopamine. Lesch-Nyhan syndrome is one of those diseases.

Dopamine D2 agonists are useful in Parkinsonism and do not appear to initiate self-mutilation behavior in rats treated neonatally with 6-OHDA (Breese et al., 1985a). It is not known whether administration of the D2 agonist would be beneficial to the motor dysfunction in the Lesch-Nyhan syndrome, or would exacerbate the choreic symptoms as observed with L-dopa in Huntington's chorea (Breese et al., 1985a). One goal of the present experiment was to shed light on this important clinical question.

Agonist-induced behavioral supersensitivity has served as the standard animal model for certain of the motor and behavioral side effects associated with long-term exposure to DA agonists in humans. Its pharmacologic mechanism, however, remains unclear (Braun and Chase, 1988). In order to examine the relative contribution of D1 and D2 receptor-mediated mechanisms to the behavioral changes which follow chronic dopamine agonist exposure, either the R-enantiomer of SKF 38393 (2,3,4,5-tetrahydro-7,8-dihydroxy-1-phenyl-1H-3-benzazepine), the most selective, partial D1 agonist, (Pendleton et al., 1978; Setler et al., 1978; Watling and Dowling, 1981; Sibley et al., 1982) or the selective, full D2 agonist, LY 171555, (trans-4-4a,5,6,7,8,8a,9-octahydro-5-propyl-1H-pyrazolo(3,4-g) quinoline.), the (-)isomer of LY 141865 (Tsuruta et al., 1981; Gundlach et al., 1983; Stoof and Kebabian, 1984; Plantje' et al., 1985) was administered alone or in combination with 6-OHDA to rats during the

postnatal period. Following a drug-free interval, behavioral responses to the selective DA agonists were evaluated.

This study was undertaken with the objective of (1) assessing both striatal DA D1 and D2 receptors in rats after postnatal treatment with selective D1 or D2 agonists and antagonists alone and in combination with neonatal 6-OHDA treatment. Other aims were to determine whether the above treatments would (2) modify the ontogeny of the respective receptor populations in the striatum, (3) modulate the abnormal responses that occur following neonatal treatment with 6-OHDA, or (4) alter the behavioral response(s) to challenge doses of agonists or antagonists given in adulthood.

A comprehensive study of the dopaminergic system is needed in order to combine and compare the effects generated by 6-OHDA and the effects generated by specific agonists and antagonists on this system.

This study should lead to a better understanding of the dopaminergic system and better enable an animal model to be developed that can assist in the development of agents that can be useful in the treatment for DA receptor supersensitivity or subsensitivity caused by structural damage or functional alterations in the brain.

CHAPTER 2

Materials and Methods

This project was divided into two parts. The first series of experiments were conducted to determine the effect of chronic stimulation with specific agonists on the ontogeny of DA D1 and D2 receptors. The second series of experiments were undertaken to test the ability of specific postnatal treatment with D1 or D2 agonists or antagonists to modulate the changes produced by neonatal 6-OHDA treatment.

Animals and Treatment

Timed pregnant Sprague-Dawley albino rats were obtained from Charles River Labs (Research Triangle Park, NC) about 1 week before parturition, housed singly in plastic cages with food and water ad libitum, and were kept on a twelve hour light-dark cycle (on at 07:00 h) at 22+1 °C. Date of litter delivery was noted within 12 hours and the day of birth was considered day 0. At birth the pups from all litters were randomly assigned to the mothers, and litters were culled to a maximum of ten pups. All offspring used in the study were weighed once a day from birth until the end of treatment (32 days of age).

Starting at birth, all neonates were treated once a day for 32 successive days by intraperitoneal (i.p.) injection

with one of the following regimens: (a) saline (0.85%) plus saline containing tartaric acid (0.5%) and acetic acid (1.0 mM), (b) SCH 23390 HCl (0.3 mg/kg, free base; Research Biochemicals, Inc., Natick, MA) in saline (0.85%) plus saline-tartaric acid/acetic acid, (c) spiroperidol (0.3 mg/kg, Research Biochemicals) in saline-tartaric acid/acetic acid plus saline, (d) SKF 38393 HCl (3.0 mg/kg, Research Biochemicals), or (e) LY 171555 HCl (3.0 mg/kg, Lilly, Research Labs, Indianapolis, IN). At 3 days after birth, groups of rats receiving the above treatment were treated with 6-OHDA hydrobromide (67.5 μg intracerebroventricular, i.c.v., free base, in each side; Regis Chemical Co., Chicago, IL) or the diluent, saline (0.85%) with ascorbic acid (0.1%) to prevent the spontaneous oxidation of 6-OHDA. Rats were weaned at 4 weeks of age. At 38-41 days of age, rats from all the above groups were challenged with the specific DA D2 agonist, LY 171555, to study yawning behavior. At 44-47 or 49-51 days of age, rats were challenged with the DA D1 specific agonist, SKF 38393 (3.0 mg/kg) or the DA D2 specific agonist, LY 171555 (3.0 mg/kg), respectively, to study stereotypic behaviors. At 70 days of age, rats were challenged with the DA D2 specific antagonist, spiroperidol (40 μg/kg, i.p.), to study the oral dyskinesia.

For the binding studies, a separate group of rats untreated after 32 days was used. These rats were killed by

decapitation at 55 to 57 days. Brains were immediately removed and the striata were dissected free, frozen on dry ice, and stored at -60 °C.

One rat treated with 6-OHDA did have post-decapitation convulsions and was not used for assessment of D1 or D2 receptor number.

Tissue Preparation

At the time of assay, parts of striata from the same medio-lateral and/or dorso-ventral location were placed in 100 volumes of 50 mM Tris buffer (pH 7.4) containing 120 mM NaCl and 5 mM KCl. After homogenization (setting of 50, 20 s; Tekmar Tissumizer), samples were centrifuged at 48,000 x g for 25 min at 4 °C in a Beckman L5-75B ultracentrifuge. This step was repeated after resuspending the pellets in 100 volumes of fresh buffer. The final pellets were resuspended in the Tris-salt solution.

D1 Binding Assay

To assess DA D1 receptor binding, the method of Schulz et al. (1985) was employed. Briefly, samples of 0.2 ml of homogenate were added to 0.1 ml [3H]SCH 23390 (50 to 1500 pM, final conc.; Amersham, Monton Grave, IL) in Tris-salt solution containing 2 mM CaCl2. Samples (1 ml incubation mix) were incubated for 15 min at 37 °C in a shaking water bath and then rapidly filtered under partial vacuum on

Whatman GF/F glass fiber filters using a Millipore filtration unit. Filters were washed three times with 5 ml of ice-cold Tris-salt solution. After drying, filters were placed in 9 ml of Scintiverse E (Fisher Scientific, Atlanta, GA), and tritium activity was determined in a Beckman LS 9800 liquid scintillation spectrometer. Specific binding of [3H]SCH 23390 was defined as the difference in binding in the presence and absence of SCH 23390 (1 μM).

D2 Binding Assay

To assess DA D2 receptor binding, the method of Creese and Snyder (1979) was used. Aliquots of homogenate (0.2 ml) were incubated with 0.1 ml [3H]spiroperidol (50 to 1500 pM, final conc.; Amersham) in Tris-salt solution containing 0.5 mM MgCl2. Samples (1 ml incubation mix) were incubated for 15 min at 37 °C in a shaking water bath and then rapidly filtered under partial vacuum on Whatman GF/F glass fiber filters using a Millipore filtration unit. Filters were washed three times with 5 ml of ice-cold Tris-salt solution. After drying, filters were placed in 9 ml of Scintiverse E (Fisher Scientific), and tritium activity was determined. Specific binding of [3H]spiroperidol was defined as the difference in binding in the presence and absence of d-butaclamol (1 μM; Research Biochemicals, Inc.).

Since [3H]spiroperidol has been shown to bind to serotonin-2 receptors (S2) (Leysen et al., 1978; Pertoutka

and Snyder, 1979; Witby et al., 1980; Witby et al., 1981), ketanserin was added to block serotonin receptors (Murrin and Kuhar, 1979). Ketanserin, at a concentration of 27 nM, selectively binds to S2 sites allowing specific study of the D2 receptors (Leysen et al., 1981; 1982; Hamblin et al., 1984).

Equilibrium Binding Studies

In order to determine the maximal binding capacity (Bmax) and the apparent dissociation constant (Kd) for [3H]SCH 23390 and [3H]spiroperidol receptor binding, a double rectangular hyperbola model was used. This describes the interaction of a selective radioligand with two classes of non-interacting binding sites {B = (Bmax1)(L)/(L+Kd1) + (Bmax2)(L)/(L+Kd2) + EL}, where B is the amount of radioligand bound, Bmax1 and Bmax2 are the densities of the two classes of binding sites, L is the free concentration of radioligand, Kd1 and Kd2 are the dissociation constants of the binding sites for the radioligand, and E is the ratio of non-specific binding sites at the L concentration. Since the data could be fit by a single site model and since there was no significant improvement in fitting provided by a two site model, the Bmax2 was considered to be zero.

For [3H]SCH 23390 kinetic studies in rat striata, the [3H]SCH 23390 concentrations ranged from 50 to 1500 pM (8 concentrations, total).

For [3H]spiroperidol kinetic studies in rat striata, the [3H]spiroperidol concentrations ranged from 50 to 1500 pM (8 concentrations, total).

Behavioral Methods

In all behavioral studies rats were placed in separate cages and were allowed to accommodate to the behavioral testing laboratory for one hour prior to drug treatments. To avoid possible circadian effects, behavioral observations were conducted at the same time each day for the different tests.

1. Yawning. Yawning was studied by administering a challenge dose of the DA D2 specific agonist, LY 171555 (50 μg/kg, i.p.; Serra et al., 1987). Rats were then placed in small Perspex observation cages (48 x 26 x 18 cm) and observed individually. Starting 5 min after LY 171555 injection, the following behavior elements were scored over the next 25 min: a) number of yawns, b) number of penile erections, and c) duration of genital grooming. These sessions were carried out between 09:00 and 14:00 hr. in a quiet, well-lighted room.
2. Oral Dyskinesia. Oral dyskinesia (spontaneous chewing jaw movements) were assessed by administering a challenge dose of the specific DA D2 antagonist, spiroperidol (40 μg/kg, i.p.)

(Laduron et al., 1978; Leysen et al., 1978). One hour after spiroperidol treatment, the numbers of oral movements were recorded for 1 min every 10 min for 6 periods (Rosengarten et al., 1983; 1986). Chewing was recorded only if it appeared to be purposeless, that is, if it was not directed at any specific object.

3. Stereotypic Behavior. Behavioral observations were made as has been described by Breese et al. (1984; 1985a,b). Following a 60 min adaptation period in clear Perspex cages (48 x 26 x 18 cm) with wood chip bedding on the floor, drugs were administered. Behavioral assessment was made for a 1 min period once every 10 min for 90 min. Each min was divided into four 15 s periods and behaviors were scored as occurring or not occurring during each 15 s period. Therefore, the maximum score for any observed behavior in a 1 min period was 4 (i.e., once in each 15 sec interval). The behaviors observed were locomotion, rearing (both front legs off the floor of the cage), licking, sniffing (intense 8-10/sec directed towards the sides or floor of the observation cage), taffy pulling (coordinated movement of front paws toward the mouth and then away from the body), head nodding (head moving up-and-down or

side-to-side in a repetitive fashion), paw treading, jumping, digging, eating wood chips, grooming, and self-biting.

Data Analysis

Binding studies

The means of Bmax and Kd values in different groups were analyzed by the analysis of variance (ANOVA) followed by a post-ANOVA Newman-Keuls test, in order to test for significant differences between the treatment groups. A p value of <0.05 was considered to be the level for statistical significance.

Yawning and Oral Dyskinesia

The results were expressed as the mean number of responses per group $\pm$ S.E.M. to give an indication of variability. The statistical significance of the results was evaluated by the analysis of variance (ANOVA) followed by a post-ANOVA Newman-Keuls test. A p value of <0.05 was considered to be the level for statistical significance.

Stereotypic Behavior

Data are expressed as the mean total stereotypy score for 90 min $\pm$ S.E.M to give an indication of variability. The statistical significance of the results was evaluated by

the analysis of variance (ANOVA) followed by a post-ANOVA Newman-Keuls test. A p value of <0.05 was considered to be the level for statistical significance.

CHAPTER 3

Results

Enhanced Yawning Response to the DA D2 Specific Agonist LY 171555 in Rats Treated During Development with LY 171555

Rats treated daily during postnatal development with the D2 receptor agonist LY 171555 (3.0 mg/kg/d, i.p.) demonstrated an 89% increase in the number of yawns that were induced by a challenge dose of LY 171555 (50 μg/kg, i.p.). In rats that were tested at 38 to 41 days after birth, the incidence of LY 171555-induced yawning was 25.0 ± 3.9 during the 25 minute observation period vs. 13.2 ± 2.9 for that of the control group ($p < 0.05$, Figure 1 and Table 1). The yawning response rate for rats treated in development with spiroperidol (0.3 mg/kg/d, i.p.) was not different from control. When rats were treated neonatally with 6-OHDA (200 μg, salt form, i.c.v.), the enhanced response of the LY 171555-treated group was eliminated. The yawning response to a challenge dose of LY 171555 was approximately 3 yawns during the observation period for both the diluent and LY 171555 groups. However, neonatal 6-OHDA treatment did not alter the number of yawns in the rats treated with spiroperidol in development (Table 1, Fig. 1). Treatment during development with SKF 38393 (3.0 mg/kg/d,

i.p.) or SCH 23390 (0.3 mg/kg/d, i.p.) did not alter the yawning response to the challenge dose of LY 171555 when compared to the control.

The number of penile erections and the duration of genital grooming were not statistically different between the groups.

Enhanced Perioral Response to the DA D2 Specific Antagonist Spiroperidol in Neonatal 6-OHDA-Lesioned Rats

Rats treated at 3 days after birth with 6-OHDA (200 μg/kg, salt form, i.c.v.) demonstrated a 710% (7-fold) increase in the number of clonic jaw movements that were induced by a challenge dose of spiroperidol (40 μg/kg, i.p.). In rats tested at 70 days after birth, the incidence of spiroperidol-induced perioral movements (oral dyskinesia) was 45.4 ± 9.9 during the observation period (1 min every 10 min for 6 times) vs. 5.6 ± 2.0 for that of the control group ($p < 0.05$, Table 2, Figure 2). The clonic jaw response rate for rats treated in development with SCH 23390 (0.3 mg/kg/d, i.p.) or SKF 38393 (3.0 mg/kg/d, i.p.) alone was not different from control, but when combined with 6-OHDA, the enhanced response of the 6-OHDA-treated group was eliminated.

Table 1

Effects of Acute LY 171555 (50 μg/kg, i.p.) on Yawning in Rats Treated During Development with Dopamine Agonists or Antagonists

Developmental Treatment	No. Of Yawns	No. Of Penile Erections	Duration Of Genital Grooming (sec)
VEHICLE +			
DILUENT	13.2 ± 2.9	1.4 ± 0.8	7.4 ± 4.8
LY171555	25.0 ± 3.9[a,b,c]	2.2 ± 1.2	12.3 ± 5.0
SPIPERONE	12.6 ± 2.6	2.8 ± 0.5	19.4 ± 4.6
SCH23390	9.0 ± 2.0	1.0 ± 0.4	8.3 ± 3.4
SKF38393	12.6 ± 1.2	2.0 ± 0.5	14.2 ± 5.2
6-OHDA +			
DILUENT	3.0 ± 0.3	1.2 ± 0.2	9.4 ± 6.2
LY171555	3.3 ± 1.0	0.8 ± 0.8	5.0 ± 4.1
SPIPERONE	14.8 ± 3.1[b]	1.3 ± 0.3	12.3 ± 2.5
SCH23390	7.2 ± 2.1	1.6 ± 0.8	15.6 ± 8.2
SKF38393	1.3 ± 0.3	0.3 ± 0.3	3.3 ± 3.3

Rats were treated i.p., daily from the day of birth to 32 days after birth, with diluent, LY 171555 (3.0 mg/kg/d), spiroperidol (spiperone) (0.3 mg/kg/d), SCH 23390 (0.3 mg/kg/d) or SKF 38393 (3.0 mg/kg/d). All rats were also treated i.c.v. at 3 days after birth with 6-OHDA (200 μg, salt form) or diluent. At 38 to 41 d after birth, rats were challenged with LY 171555 (50 μg/kg, i.p.), and the numbers of yawns and penile erections, and the duration of genital grooming were recorded for 25 min, beginning 5 min after acute LY 171555 treatment.
Each value represents the Mean ± S.E.M. for 5 rats.
a, $p < 0.05$, compared to the 'Vehicle + Diluent' group.
b, $p < 0.05$, compared to the '6-OHDA + Diluent' group.
c, $p < 0.05$, compared to the 'LY 171555 + 6-OHDA' group.

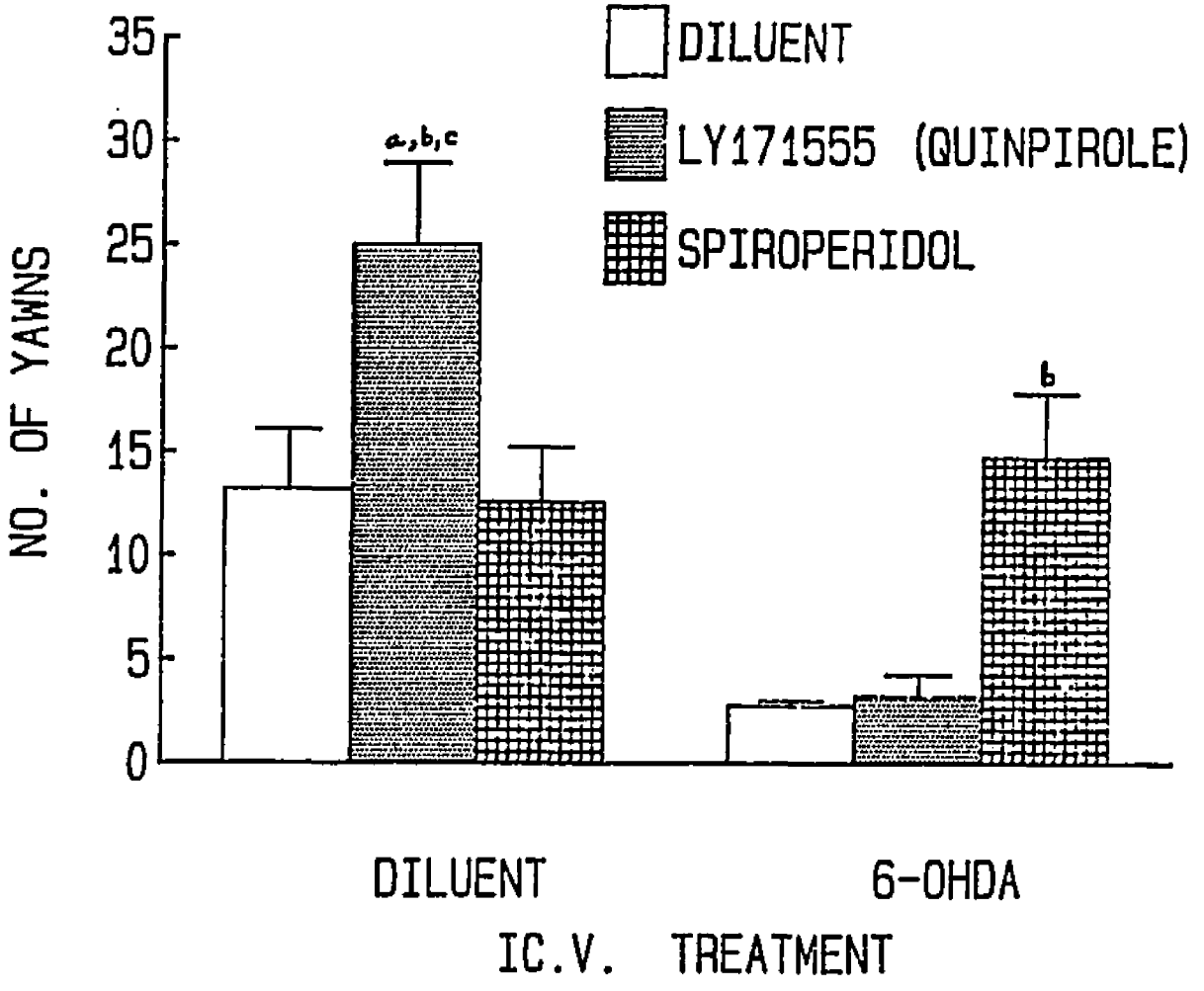

Figure 1. Effects of Acute LY 171555 (50 μg/kg, i.p.) on Yawning in Rats Treated During Development with Dopamine Agonists or Antagonists. Rats were treated i.p., daily from the day of birth to 32 days after birth, with diluent, LY 171555 (3.0 mg/kg/d) or spiroperidol (0.3 mg/kg/d). All rats were also treated i.c.v. at 3 days after birth with 6-OHDA (200 μg, salt form) or diluent. At 38 to 41 d after birth, rats were challenged with LY 171555 (50 μg/kg, i.p.), and the numbers of yawns was recorded for 25 min, beginning 5 min after acute LY 171555 treatment.
Each value represents the Mean ± S.E.M. for 5 rats.
a, $\underline{p} < 0.05$, compared to the 'Vehicle + Diluent' group.
b, $\underline{p} < 0.05$, compared to the '6-OHDA + Diluent' group.
c, $\underline{p} < 0.05$, compared to the 'LY 171555 + 6-OHDA' group.

Table 2

Effects of Acute Spiroperidol (40 μg/kg, i.p.) on the Incidence of Perioral Movements in Neonatal 6-OHDA-Lesioned Rats

Developmental Treatment	No. Of Repetitive Jaw Movements
VEHICLE +	
DILUENT	5.6 ± 2.0
SCH23390	4.8 ± 3.1
SKF38393	3.4 ± 0.9
6-OHDA +	
DILUENT	45.4 ± 9.9[a]
SCH23390	14.0 ± 3.9[b]
SKF38393	3.0 ± 1.7[b]

Rats were treated i.p., daily for the first 32 days from birth, with diluent, SCH 23390 (0.3 mg/kg/d), or SKF 38393 (3.0 mg/kg/d), in combination with i.c.v. 6-OHDA (200 μg, salt form) or its diluent at 3 days of age. Animals were challenged with spiroperidol (40 μg/kg, i.p.) at 70 days from birth, and were observed one hour after the challenge dose. Numbers of episodes of mouth movements (consisting of bursts of repetitive jaw movements) were summed over 6 one min periods, separated by 10 min intervals.
Results are expressed as the Mean ± S.E.M. for 5 rats.
a, $p < 0.05$, compared to the 'Vehicle + Diluent' group.
b, $p < 0.05$, compared to the '6-OHDA + Diluent' group.

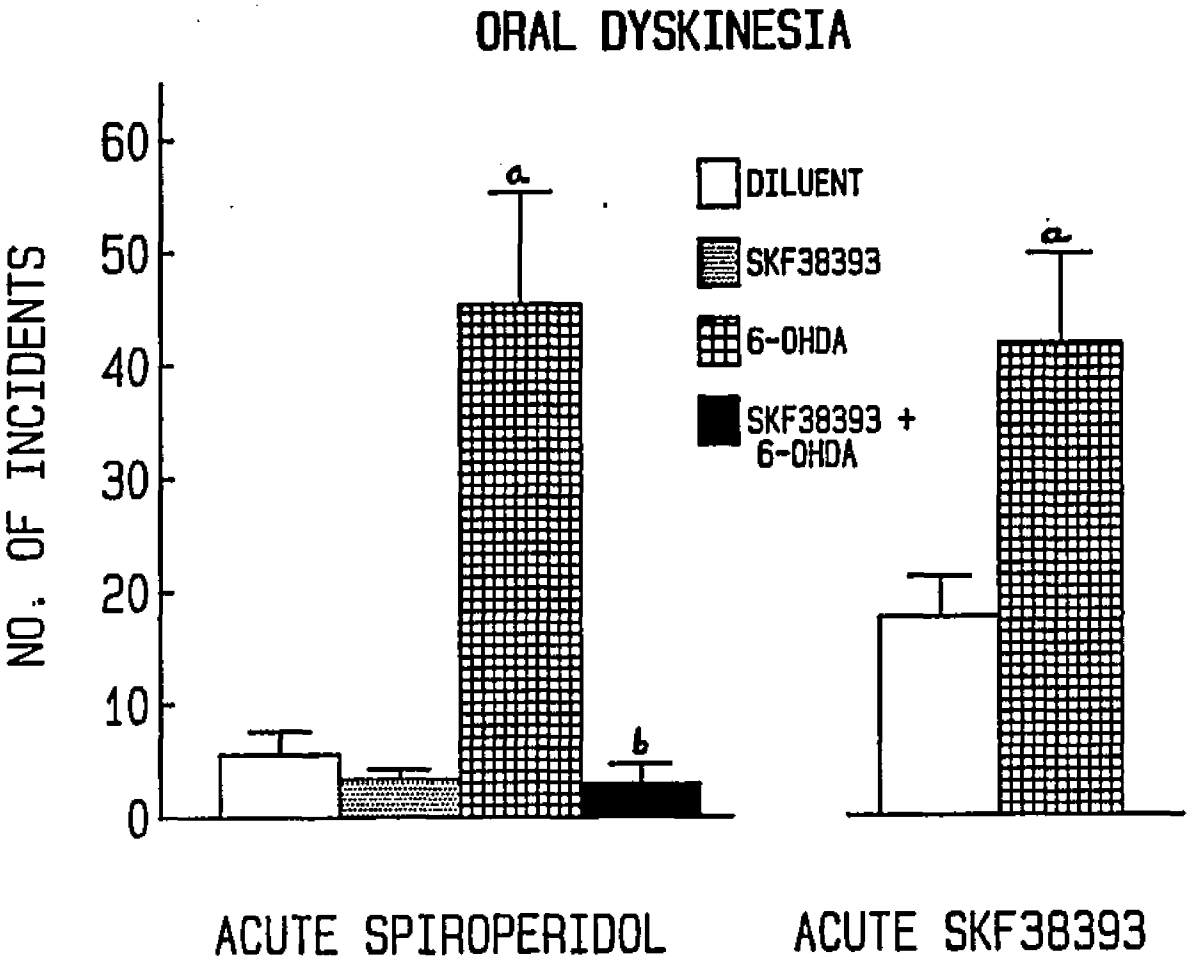

Figure 2. Effects of Acute Spiroperidol (40 μg/kg, i.p.) on the Incidence of Perioral Movements in Neonatal 6-OHDA-Lesioned Rats. Rats were treated i.p., daily for the first 32 days from birth, with diluent or SKF 38393 (3.0 mg/kg/d), in combination with i.c.v. 6-OHDA (200 μg, salt form), or its diluent at 3 days of age. Animals were challenged with spiroperidol (40 μg/kg, i.p.) at 70 days from birth or SKF 38393 (3.0 mg/kg, i.p.) at 77 days from birth, and were observed one hour after the challenge dose. Numbers of episodes of mouth movements (consisting of bursts of repetitive jaw movements) were summed over 6 one min periods, separated by 10 min intervals.
Results are expressed as the Mean ± S.E.M. for 5 rats.
a, $\underline{p} < 0.05$, compared to the 'Vehicle + Diluent' group.
b, $\underline{p} < 0.05$, compared to the '6-OHDA + Diluent' group.

Enhanced Stereotypic Response to the DA D1 Specific Agonist SKF 38393 in Rats Treated During Development with both SKF 38393 and 6-OHDA

Daily treatment of rats during postnatal development with the D1 receptor agonist SKF 38393 (3.0 mg/kg/d, i.p.) was not associated with remarkable changes in stereotypic responses to a challenge dose of SKF 38393 (3.0 mg/kg, i.p.) (Tables 3-6). The neonatal 6-OHDA-lesioned rats (200 μg, salt form, i.c.v.) also showed no remarkable changes in stereotypic responses to a challenge dose of SKF 38393 (Tables 3-6). In rats treated in development with both 6-OHDA and SKF 38393, there were marked changes in stereotypic responses to the challenge dose of SKF 38393 at 44 to 47 days. In this latter group there was a marked statistically significant increase over control in the score for licking (24.0 ± 3.2 vs. 5.2 ± 2.1), grooming (15.6 ± 4.4 vs. 3.4 ± 1.6), locomotion (26.8 ± 3.5 vs. 7.6 ± 2.7), jumping (8.0 ± 4.6 vs. 0.0), taffy pulling (16.8 ± 3.1 vs. 1.4 ± 0.9), and paw treading (14.0 ± 3.3 vs. 2.2 ± 1.3) (Tables 3-6 and Figures 3-8).

In the same group (SKF 38393 + 6-OHDA), the incidence of SKF 38393-induced sniffing, digging, gnawing, eating, rearing, head nodding, and self-biting was not different from that of the control group (Tables 3-6). A challenge dose of SKF 38393 at 44 to 47 days did not modify the above behaviors in rats treated in development with LY 171555 (3.0

mg/kg/d), spiroperidol (0.3 mg/kg/d), or SCH 23390 (0.3 mg/kg/d). Neonatal 6-OHDA (200 μg, salt form, i.c.v., at 3 days of age) did not modify the responses to SKF 38393 challenge in these groups (Tables 3-6).

Stereotypic Responses to the DA D2 Specific Agonist LY 171555 in Rats Treated During Development with both SKF 38393 and 6-OHDA

Daily treatment of rats with SKF 38393 (3.0 mg/kg/d, i.p.) during the postnatal development period was not associated with remarkable changes in stereotypic responses to a challenge dose of LY 171555 (3.0 mg/kg, i.p.) at 49 to 51 days of age (Tables 7-10). The neonatal 6-OHDA-lesioned rats (200 μg, salt form, i.c.v.) also showed no remarkable changes in stereotypic responses to a challenge dose of LY 171555 (3.0 mg/kg, i.p.) (Tables 7-10). However, in rats treated in development with both 6-OHDA and SKF 38393, there were marked changes in stereotypic responses to a challenge dose of the DA D2 specific agonist, LY 171555 (3.0 mg/kg, i.p.). The challenge dose of LY 171555 produced a marked increase over control, in the score for licking (20.6 ± 6.5 vs. 0.2 ± 0.2), grooming (20.0 ± 6.9 vs. 0.0), and digging (20.0 ± 3.2 vs. 0.4 ± 0.2) (Tables 7,8 and Figures 3,4,9). On the other hand, the challenge dose of LY 171555 reduced the score for gnawing (4.4 ± 1.5 vs. 23.6 ± 1.3) in this same group (SKF 38393 + 6-OHDA) (Table 8 and Figure 10). In

the same group (SKF 38393 + 6-OHDA), the incidence of LY 171555-induced sniffing, eating, locomotion, rearing, jumping, taffy pulling, paw treading, head nodding, and self-biting were not different from that of the control group (Tables 7-10). In rats treated i.p. from the day of birth to 32 days of age with spiroperidol (0.3 mg/kg/d) or SCH 23390 (0.3 mg/kg/d) and were challenged with LY 171555 (3.0 mg/kg, i.p.), there was no change in the incidence of stereotypic behaviors at age 49 to 51 days. Also, 6-OHDA did not affect the incidence of stereotypies in these latter groups (Tables 7-10).

Stereotypic Responses to the DA D2 Specific Agonist LY 171555 in Rats Treated During Development with LY 171555, with or without Neonatal 6-OHDA

When rats treated daily during postnatal development with LY 171555 (3.0 mg/kg/d, i.p.) were challenged with an acute dose of LY 171555 (3.0 mg/kg, i.p.) at 49 to 51 days from birth, there was a marked increase in the score for eating behavior (15.0 $\pm$ 4.2 vs. 4.6 $\pm$ 2.3) compared to that for the diluent control (Figure 11 and Table 8). Rats co-treated in development with LY 171555 and 6-OHDA had increased scores for digging behavior (15.2 $\pm$ 6.2 vs. 0.4 $\pm$ 0.2 for the control), in response to the challenge dose of LY 171555 (3.0 mg/kg, i.p.) (Figure 9). In the same group (LY 171555 + 6-OHDA), the incidence of LY 171555-induced

sniffing, licking, grooming, gnawing, eating, locomotion, rearing, jumping, taffy pulling, paw treading, head nodding, and self-biting was not different from the control group (Tables 7-10).

Development of Striatal D1 Receptors in Postnatally-Treated Rats

Administration of the DA D1 receptor agonist or antagonist to rats once a day for 32 successive days from birth had no effect on striatal DA D1 receptor number and D1 receptor affinity at 8 weeks of age (Table 11). In the group of rats treated in development with SKF 38393 (3.0 mg/kg/d, i.p.), the *in vitro* binding of [3H]SCH 23390 to striatal homogenates was not different from the control group (Bmax = 6.6 ± 2.2 fmol/mg tissue and Kd = 240.3 ± 53.3 pM vs. 19.1 ± 6.8 and 416.8 ± 101.5, respectively, for the diluent control group). Groups that were treated neonatally with 6-OHDA (200 µg, i.c.v., at 3 days of age) showed no difference in binding parameters from control. *In vitro* binding of [3H]SCH 23390 to striatal homogenates showed that the Bmax was 17.8 ± 5.6 fmol/mg tissue and the Kd was 600.0 ± 101.9 for the 6-OHDA group, vs. 19.1 ± 6.8 and 416.8 ± 101.5, respectively, for the diluent control group (Table 11). Rats treated alone with 6-OHDA (200 µg, i.c.v., at 3 days of age), in combination with SKF 38393 (3.0 mg/kg/d, i.p., for 32 days), or SCH 23390 (0.3 mg/kg/d, i.p., for 32

days) also were not statistically different from the control group.

Development of Striatal D2 Receptors in Postnatally-Treated Rats

When the DA D2 receptor agonist or antagonist was administered to rats once a day for 32 successive days from birth there was no effect on the development of striatal DA D2 receptors at 8 weeks of age (Table 12). In the group of rats treated chronically with LY 171555 (3.0 mg/kg/d, i.p.) the in vitro binding of [3H]spiroperidol to striatal homogenates was not different from that of the control group (Bmax = 14.2 ± 1.9 fmol/mg tissue and Kd = 57.5 ± 7.2 pM vs. 15.9 ± 2.0 and 73.3 ± 5.4, respectively). Groups treated neonatally with 6-OHDA (200 μg, i.c.v., at 3 days of age) showed no difference in binding parameters from the control. In vitro binding of [3H]spiroperidol to striatal homogenates showed that the Bmax was 20.0 ± 2.1 fmol/mg tissue and the Kd was 74.8 ± 5.3 in the 6-OHDA group, vs. 15.9 ± 2.0 and 73.3 ± 5.4, respectively, for the diluent control group (Table 12). In rats treated with 6-OHDA (200 μg, i.c.v., at 3 days of age), alone or in combination with LY 171555 (3.0 mg/kg/d, i.p., for 32 days) or spiroperidol (0.3 mg/kg/d, i.p., for 32 days), the binding parameters for D2 receptors were not different from the control group.

Dopamine D1 Receptor Equilibrium Binding Studies

Dopamine D1 receptor equilibrium binding studies were performed on striatal tissue taken from similar parts of the striatum at 8 weeks from birth. Rats were treated chronically with diluent, SKF 38393 (3.0 mg/kg/d, i.p.), or SCH 23390 (0.3 mg/kg/d, i.p.) in combination with 6-OHDA (200 µg, i.c.v. at 3 days of age) or its diluent. It was found that these treatments did not alter the Bmax or the Kd vs. the diluent control group (Table 11).

Dopamine D2 Receptor Equilibrium Binding Studies

Dopamine D2 receptor equilibrium binding studies were performed on striatal tissue taken from similar parts of the striatum at 8 weeks from birth. Rats were treated chronically with diluent, LY 171555 (3.0 mg/kg/d, i.p.), or spiroperidol (0.3 mg/kg/d, i.p.) in combination with 6-OHDA (200 µg, i.c.v. at 3 days of age) or its diluent. It was found that these treatments did not alter the Bmax or the Kd vs. the diluent control group (Table 12).

Table 3

Behavioral Effects Induced by SKF 38393 (3.0 mg/kg, i.p.) in Rats that Received Repeated Treatment with D1 and D2 Receptor Agonists or Antagonists During Postnatal Development

DEVELOPMENTAL TREATMENT	BEHAVIORAL SCORE		
	SNIFFING	LICKING	GROOMING
VEHICLE +			
DILUENT	24.2 ± 4.3	5.2 ± 2.1	3.4 ± 1.6
SKF38393	26.6 ± 6.2	8.6 ± 2.6	5.6 ± 1.7
LY171555	18.8 ± 2.0	9.6 ± 2.5	7.6 ± 3.1
SCH23390	18.6 ± 1.0	6.6 ± 1.6	6.0 ± 1.6
SPIPERONE	19.8 ± 5.3	5.4 ± 1.7	3.6 ± 1.6
6-OHDA +			
DILUENT	24.7 ± 3.8	5.2 ± 2.9	2.7 ± 1.6
SKF38393	31.8 ± 1.8	24.0 ± 3.2[a,b,c,e]	15.6 ± 4.4[a,b,e]
LY171555	27.8 ± 4.0	2.4 ± 1.0	2.0 ± 0.6
SCH23390	24.2 ± 5.2	8.0 ± 2.1	4.4 ± 1.4
SPIPERONE	28.3 ± 5.8	10.0 ± 3.8	2.0 ± 0.9

Rats were treated i.p., daily for the first 32 days from birth, with diluent, SKF 38393 (3 mg/kg/d), LY 171555 (3 mg/kg/d), SCH 23390 (0.3 mg/kg/d) or spiroperidol (spiperone) (0.3 mg/kg/d) in combination with i.c.v. 6-OHDA (200 μg, salt form, at 3 days of age) or its diluent. Animals were challenged with SKF 38393 (3 mg/kg, i.p.) at 44-47 days from birth, and were observed, beginning 10 min after the challenge dose. Numbers of episodes of each stereotypic behavior were summed over 9 one min periods, separated by a 10 min interval. Each min was subdivided into 15 sec sessions, and a score of 1 was assigned if a rat demonstrated a particular behavior during this interval. The maximum score for each min was 4. For the 90 min period, the maximum score was 36 for each behavior. Results are expressed as the Mean ± S.E.M. for 5 rats.
a, $p < 0.05$, compared to 'Vehicle + Diluent' group that were challenged with SKF 38393.
b, $p < 0.05$, compared to the corresponding control group.
c, $p < 0.05$, compared postnatal treatment with 6-OHDA vs. without 6-OHDA.
d, $p < 0.05$, compared to the same treatment with different challenge.
e, $p < 0.05$, compared to 'Vehicle + Diluent' group that were challenged with LY 171555.

Table 4

Behavioral Effects Induced by SKF 38393 (3.0 mg/kg, i.p.) in Rats that Received Repeated Treatment with D1 and D2 Receptor Agonists or Antagonists During Postnatal Development

DEVELOPMENTAL TREATMENT	BEHAVIORAL SCORE		
	DIGGING	GNAWING	EATING
VEHICLE +			
DILUENT	2.0 ± 0.7	8.0 ± 0.8	1.0 ± 0.8
SKF38393	2.8 ± 1.2	15.8 ± 2.4	2.6 ± 1.1
LY171555	0.4 ± 0.2	8.0 ± 1.9	1.0 ± 0.5
SCH23390	1.8 ± 0.4	11.0 ± 1.4	0.4 ± 0.4
SPIPERONE	2.4 ± 0.7	6.2 ± 1.4[c]	2.0 ± 1.1
6-OHDA +			
DILUENT	2.5 ± 1.6	11.3 ± 2.2	1.5 ± 1.2
SKF38393	13.4 ± 4.9[c]	11.2 ± 4.1	1.2 ± 0.4
LY171555	2.4 ± 0.9	6.0 ± 2.4[c]	0.6 ± 0.4
SCH23390	2.8 ± 0.8	8.4 ± 2.4	1.2 ± 0.8
SPIPERONE	2.8 ± 1.3	12.3 ± 5.1	2.5 ± 0.9

Legend as in Table 3

Table 5

Behavioral Effects Induced by SKF 38393 (3.0 mg/kg, i.p.) in Rats that Received Repeated Treatment with D1 and D2 Receptor Agonists or Antagonists During Postnatal Development

DEVELOPMENTAL TREATMENT	BEHAVIORAL SCORE		
	LOCOMOTION	REARING	JUMPING
VEHICLE +			
DILUENT	7.6 ± 2.7	8.0 ± 3.4	0.0 ± 0.0
SKF38393	12.4 ± 3.2	13.0 ± 4.0	0.2 ± 0.2
LY171555	3.0 ± 0.8	2.4 ± 1.1	0.0 ± 0.0
SCH23390	5.8 ± 2.4	3.6 ± 1.4	0.0 ± 0.0
SPIPERONE	5.8 ± 2.3	3.4 ± 1.4	0.2 ± 0.2
6-OHDA +			
DILUENT	10.5 ± 3.8	10.0 ± 4.6	0.0 ± 0.0
SKF38393	26.8 ± 3.5[a]	18.0 ± 3.4	8.0 ± 4.6[a,b,c,d,e]
LY171555	16.2 ± 6.1	12.8 ± 7.3	5.6 ± 3.7
SCH23390	11.0 ± 4.5	10.8 ± 4.3	0.2 ± 0.2
SPIPERONE	16.8 ± 3.6	17.8 ± 6.4	0.3 ± 0.3

Legend as in Table 3

Table 6

Behavioral Effects Induced by SKF 38393 (3.0 mg/kg, i.p.) in Rats that Received Repeated Treatment with D1 and D2 Receptor Agonists or Antagonists During Postnatal Development

DEVELOPMENTAL TREATMENT	BEHAVIORAL SCORE		
	TAFFY PULLING	PAW TREADING	HEAD NODDING
VEHICLE +			
DILUENT	1.4 ± 0.9	2.2 ± 1.3	3.6 ± 1.7
SKF38393	3.6 ± 1.3	2.0 ± 1.1	8.6 ± 3.4
LY171555	2.8 ± 1.4	0.0 ± 0.0	6.0 ± 1.1
SCH23390	1.8 ± 0.5	0.8 ± 0.8	6.4 ± 1.9
SPIPERONE	1.8 ± 1.1	0.0 ± 0.0	7.6 ± 3.9
6-OHDA +			
DILUENT	2.2 ± 1.4	8.0 ± 3.6	5.0 ± 2.6
SKF38393	16.8 ± 3.1[a,b,c,d,e]	14.0 ± 3.3[a,c,e]	2.6 ± 0.8
LY171555	1.2 ± 0.5	11.2 ± 6.9	5.4 ± 3.9
SCH23390	4.0 ± 1.3	6.0 ± 2.6	9.8 ± 3.5
SPIPERONE	1.0 ± 0.7	9.8 ± 3.9	5.3 ± 2.1

Legend as in Table 3

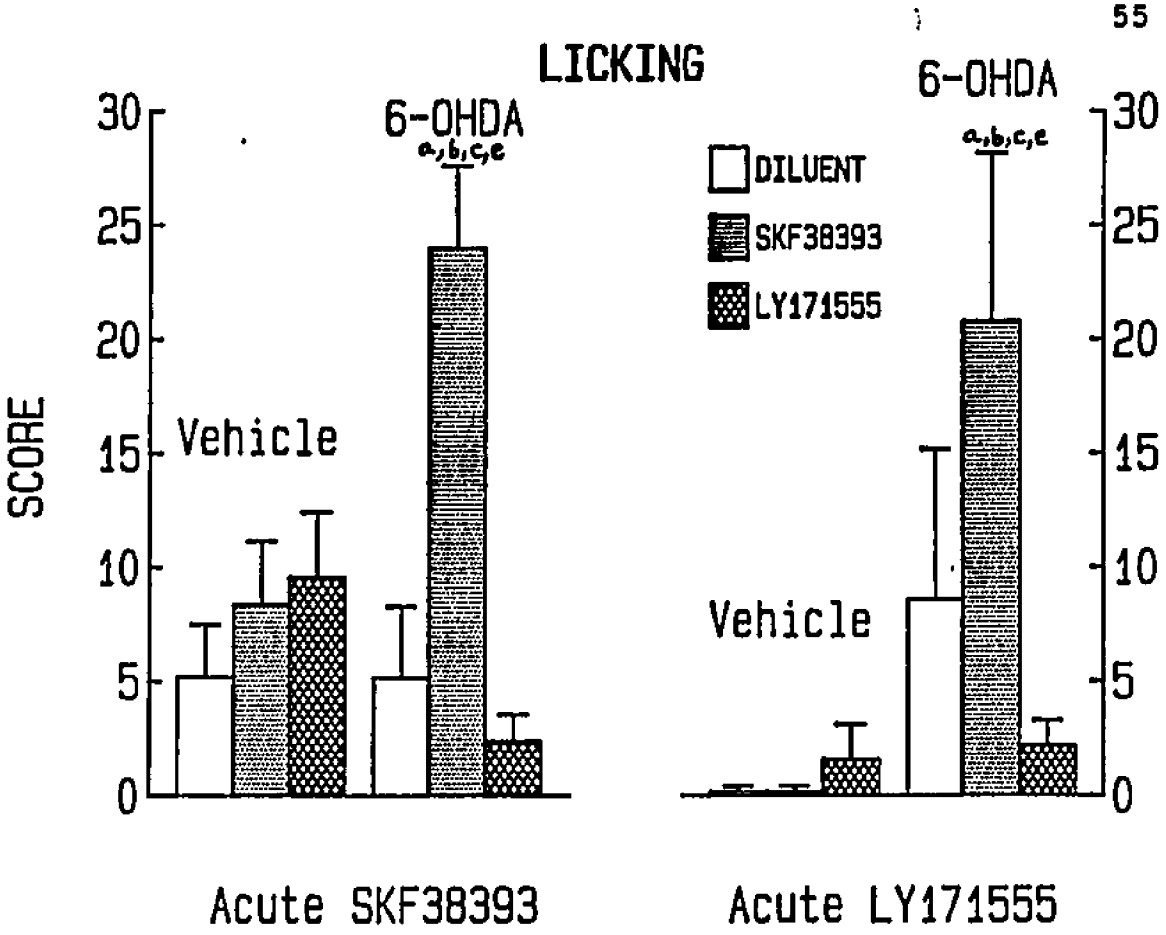

Figure 3. Behavioral Effects Induced by SKF 38393 (3 mg/kg, i.p.) in Rats that Received Repeated Treatment with D1 or D2 Receptor Agonists During Postnatal Development. Rats were treated i.p., daily for the first 32 days from birth, with diluent, SKF 38393 (3 mg/kg/d), or LY 171555 (3 mg/kg/d) in combination with i.c.v. 6-OHDA (200 μg, salt form, at 3 days of age) or its diluent. Animals were challenged with SKF 38393 (3 mg/kg, i.p.) at 44-47 days from birth, and were observed, beginning 10 min after the challenge dose. Numbers of episodes of each stereotypic behavior were summed over 9 one min periods, separated by a 10 min interval. Each min was subdivided into 15 sec sessions, and a score of 1 was assigned if a rat demonstrated a particular behavior during this interval. The maximum score for each min was 4. For the 90 min period, the maximum score was 36 for each behavior. Results are expressed as the Mean $\pm$ S.E.M. for 5 rats.
a, $p < 0.05$, compared to 'Vehicle + Diluent' group that were challenged with SKF 38393.
b, $p < 0.05$, compared to the corresponding control group.
c, $p < 0.05$, compared postnatal treatment with 6-OHDA vs. without 6-OHDA.
d, $p < 0.05$, compared to the same treatment with different challenge.
e, $p < 0.05$, compared to 'Vehicle + Diluent' group that were challenged with LY 171555.

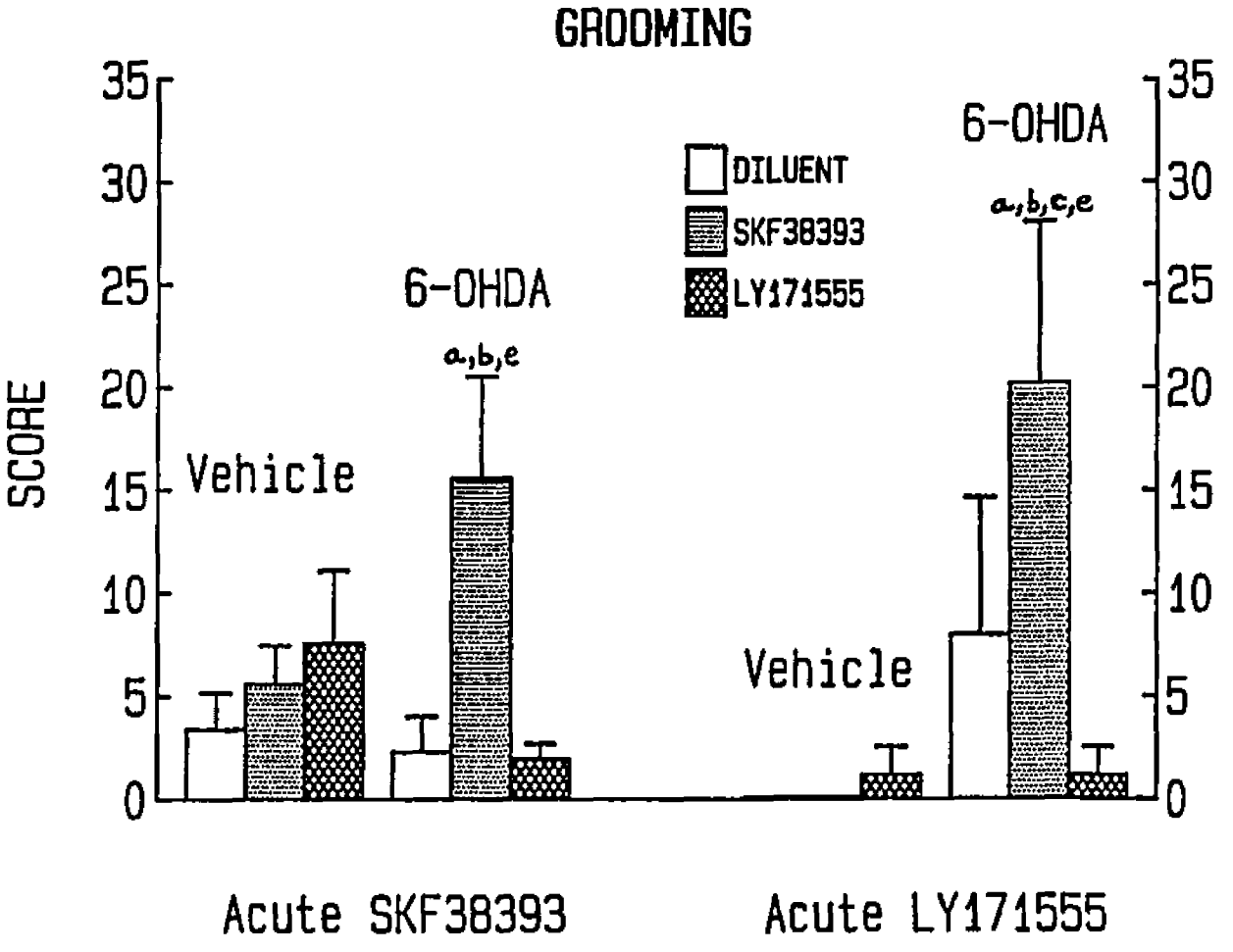

Figure 4.
Legend as in Figure 3.

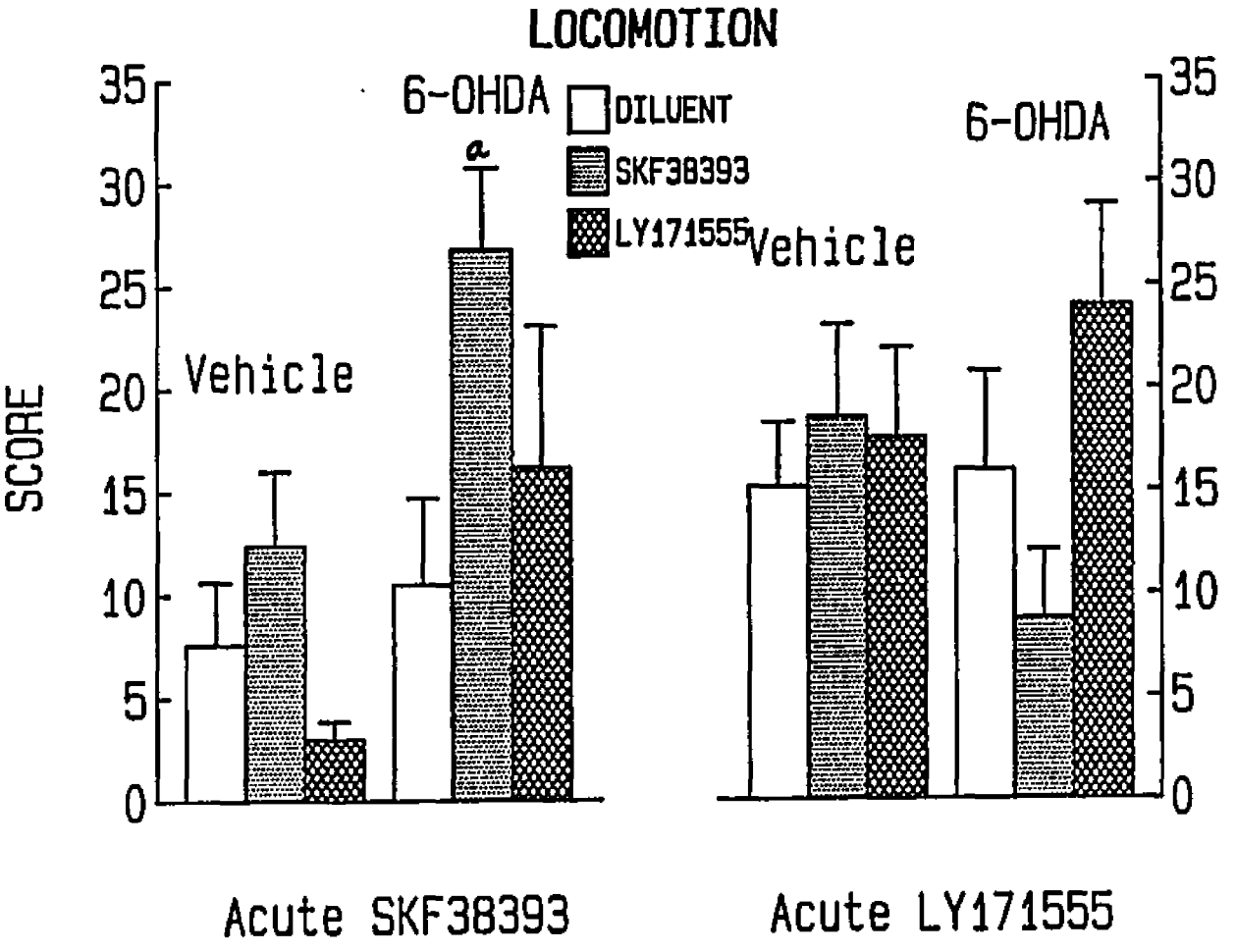

Figure 5.
Legend as in Figure 3.

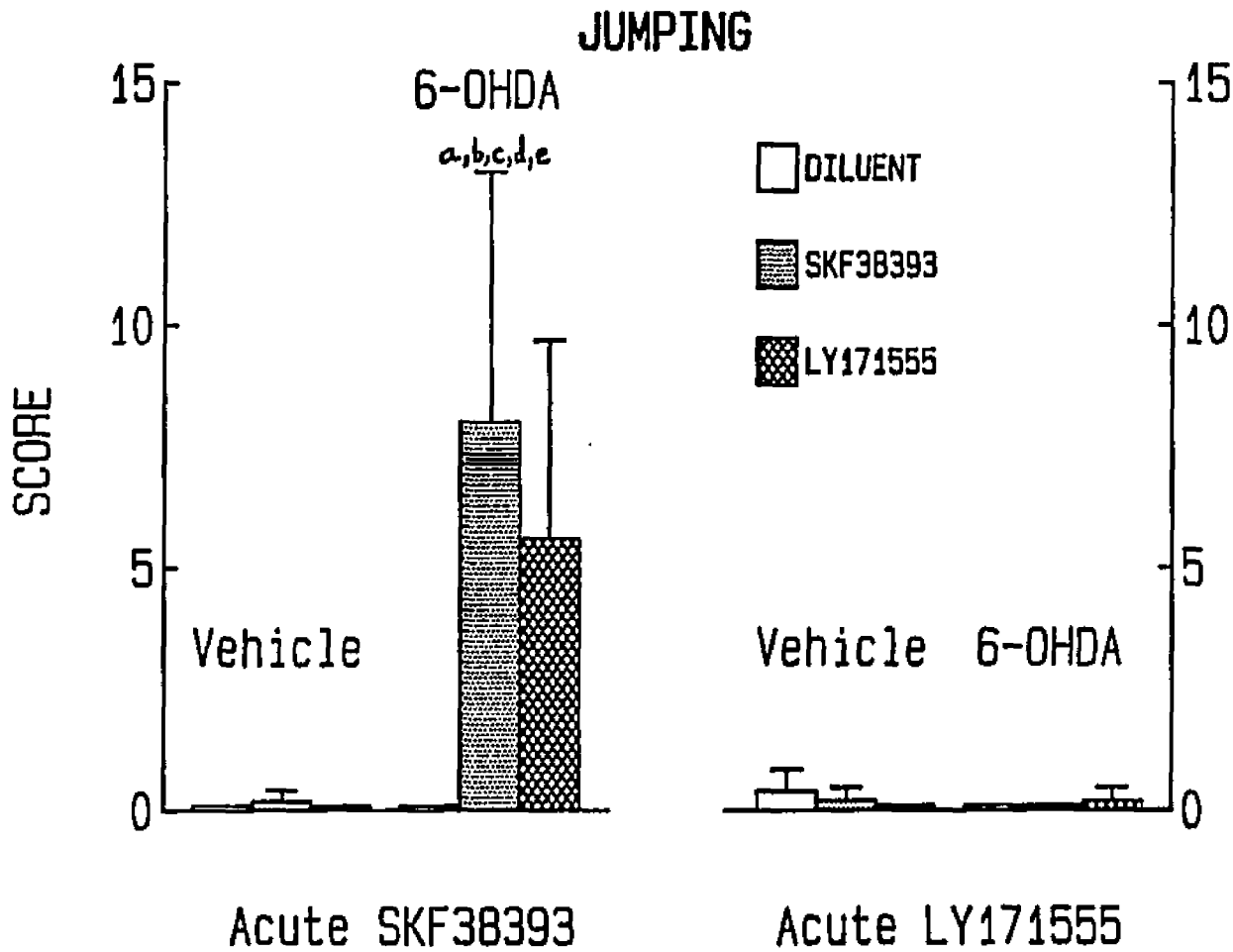

Figure 6.
Legend as in Figure 3.

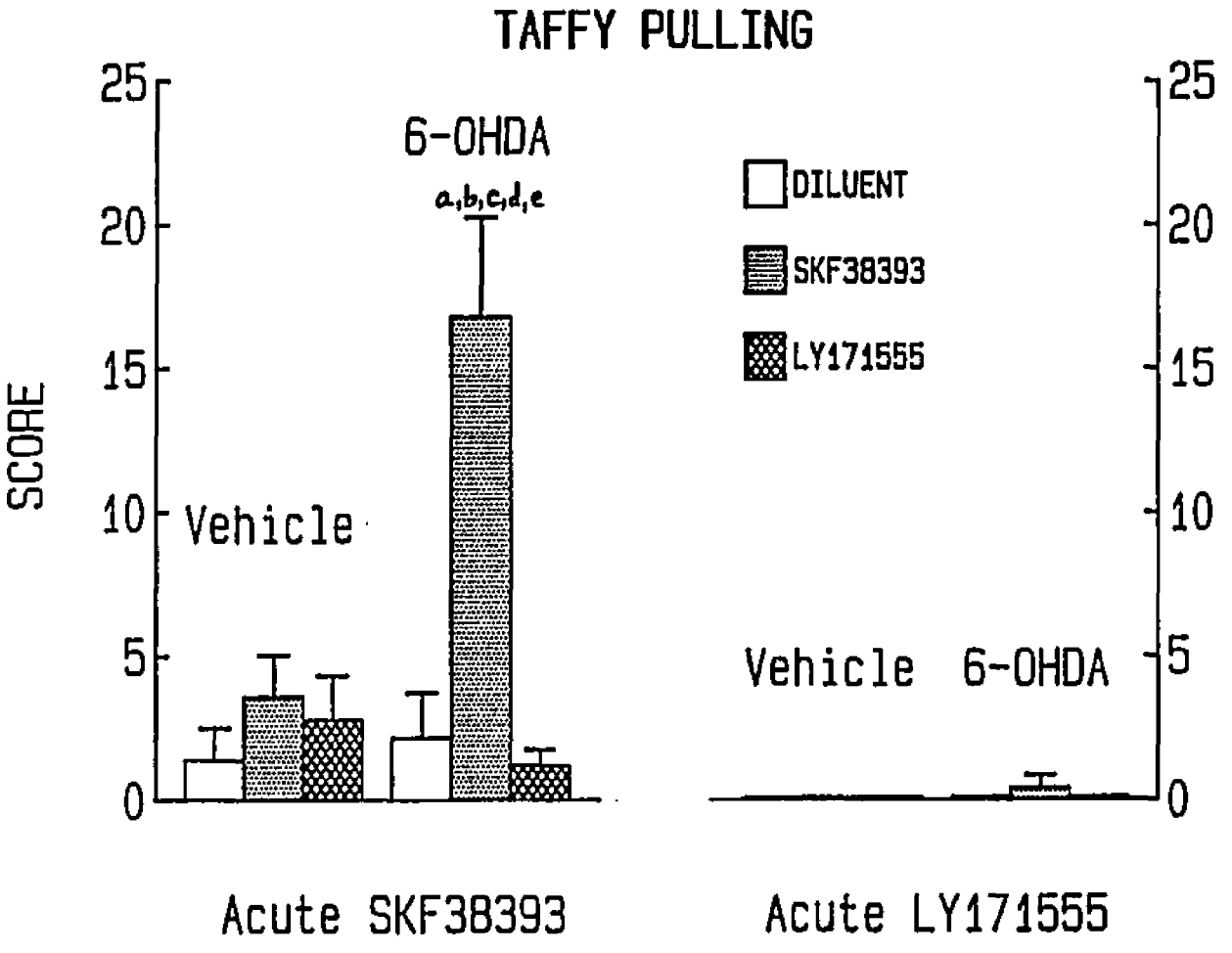

Figure 7.
Legend as in Figure 3.

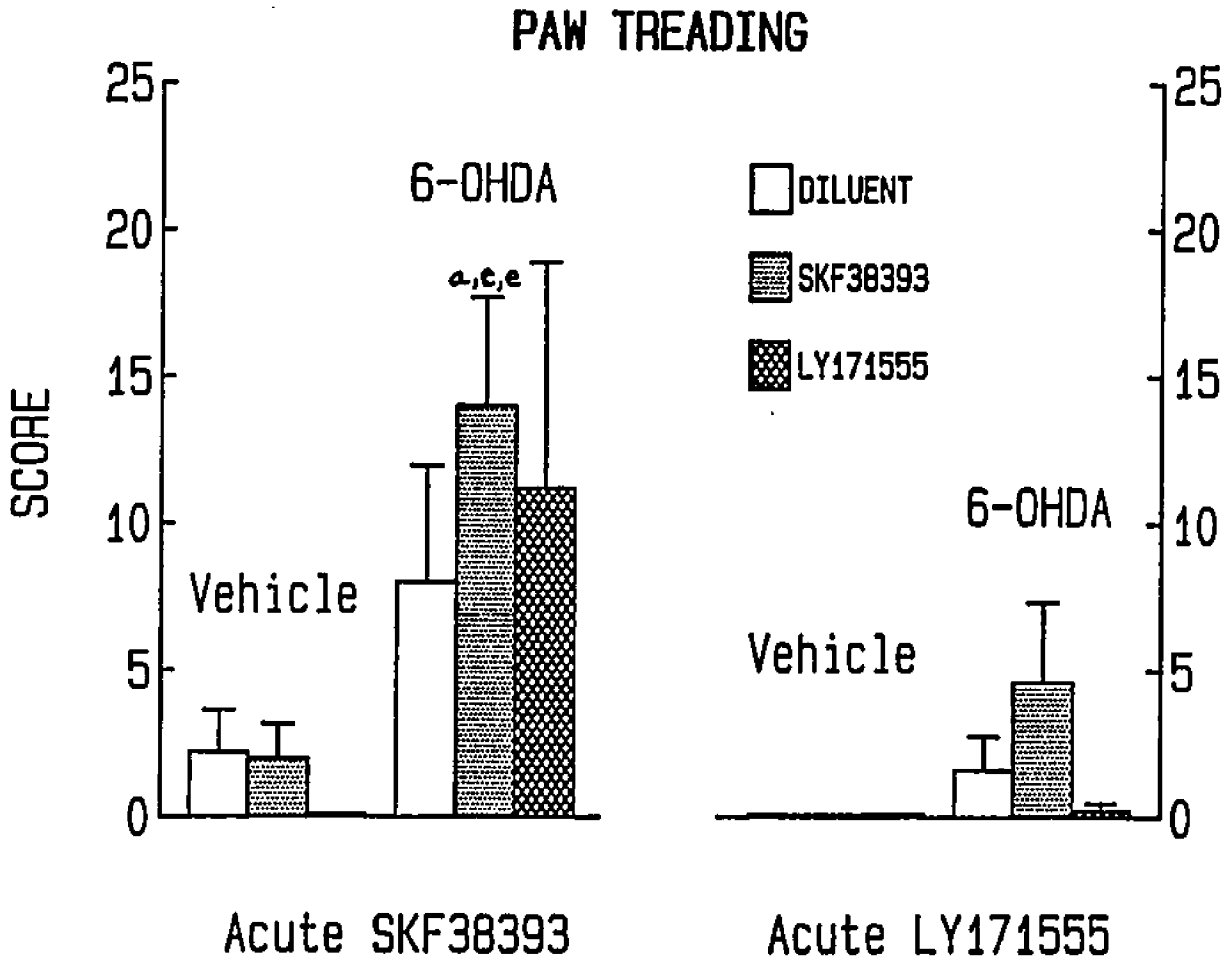

Figure 8.
Legend as in Figure 3.

Table 7

Behavioral Effects Induced by LY 171555 (3.0 mg/kg, i.p.) in Rats that Received Repeated Treatment with D1 and D2 Receptor Agonists or Antagonists During Postnatal Development

DEVELOPMENTAL TREATMENT	BEHAVIORAL SCORE		
	SNIFFING	LICKING	GROOMING
VEHICLE +			
DILUENT	31.0 ± 1.7	0.2 ± 0.2	0.0 ± 0.0
SKF38393	32.2 ± 0.9	0.2 ± 0.2	0.0 ± 0.0
LY171555	32.6 ± 1.3	2.2 ± 1.3	1.2 ± 1.2
SCH23390	30.8 ± 1.2	0.4 ± 0.2	0.0 ± 0.0
SPIPERONE	29.4 ± 1.5	0.0 ± 0.0	0.0 ± 0.0
6-OHDA +			
DILUENT	31.6 ± 2.2	8.6 ± 5.9	8.0 ± 5.9
SKF38393	21.8 ± 5.5	20.6 ± 6.5[a,b,c,e]	20.0 ± 6.9[a,b,c,e]
LY171555	34.0 ± 0.8	2.2 ± 0.9	1.2 ± 1.2
SCH23390	28.8 ± 3.3	3.8 ± 2.5	2.4 ± 1.5
SPIPERONE	30.3 ± 2.3	4.5 ± 2.0	4.0 ± 2.0

Rats were treated i.p., daily for the first 32 days from birth, with diluent, SKF 38393 (3 mg/kg/d), LY 171555 (3 mg/kg/d), SCH 23390 (0.3 mg/kg/d), or spiroperidol (spiperone) (0.3 mg/kg/d) in combination with i.c.v. 6-OHDA (200 μg, salt form, at 3 days of age) or its diluent. Animals were challenged with LY 171555 (3 mg/kg, i.p.) at 49-51 days from birth, and were observed, beginning 10 min after the challenge dose. Numbers of episodes of each stereotypic behavior were summed over 9 one min periods, separated by a 10 min interval. Each min was subdivided into 15 sec sessions, and a score of 1 was assigned if a rat demonstrated a particular behavior during this interval. The maximum score for each min was 4. For the 90 min period, the maximum score was 36 for each behavior. Results are expressed as the Mean ± S.E.M. for 5 rats.
a, $p < 0.05$, compared to 'Vehicle + Diluent' group that were challenged with SKF 38393.
b, $p < 0.05$, compared to the corresponding control group.
c, $p < 0.05$, compared postnatal treatment with 6-OHDA vs. without 6-OHDA.
d, $p < 0.05$, compared to the same treatment with different challenge.
e, $p < 0.05$, compared to 'Vehicle + Diluent' group that were challenged with LY 171555.

Table 8

Behavioral Effects Induced by LY 171555 (3.0 mg/kg, i.p.) in Rats that Received Repeated Treatment with D1 and D2 Receptor Agonists or Antagonists During Postnatal Development

DEVELOPMENTAL TREATMENT	BEHAVIORAL SCORE		
	DIGGING	GNAWING	EATING
VEHICLE +			
DILUENT	0.4 ± 0.2	23.6 ± 1.3	4.6 ± 2.3
SKF38393	6.2 ± 2.4	23.6 ± 4.6	9.4 ± 4.5
LY171555	4.6 ± 1.4	26.2 ± 2.7[a,d]	15.0 ± 4.2[a,b,d,e]
SCH23390	5.6 ± 2.6	26.0 ± 3.5[a]	9.6 ± 2.3
SPIPERONE	2.2 ± 2.2	19.0 ± 6.1	14.0 ± 4.9[a,d]
6-OHDA +			
DILUENT	2.2 ± 1.0	19.4 ± 2.3	4.2 ± 2.1
SKF38393	20.0 ± 3.2[a,b,c,e]	4.4 ± 1.5[e]	1.0 ± 0.8
LY171555	15.2 ± 6.2[a,b,c,d,e]	16.2 ± 3.7	5.6 ± 1.4
SCH23390	4.4 ± 2.3	27.0 ± 3.8[a,d]	10.4 ± 3.6
SPIPERONE	8.0 ± 4.5	23.0 ± 5.8	6.3 ± 2.8

Legend as in Table 7

Table 9

Behavioral Effects Induced by LY 171555 (3.0 mg/kg, i.p.) in Rats that Received Repeated Treatment with D1 and D2 Receptor Agonists or Antagonists During Postnatal Development

DEVELOPMENTAL TREATMENT	BEHAVIORAL SCORE		
	LOCOMOTION	REARING	JUMPING
VEHICLE +			
DILUENT	15.2 ± 2.8	0.4 ± 0.2	0.4 ± 0.4
SKF38393	18.6 ± 4.0	3.8 ± 2.6	0.2 ± 0.2
LY171555	17.6 ± 3.9	2.6 ± 1.9	0.0 ± 0.0
SCH23390	15.6 ± 2.9	0.4 ± 0.2	0.2 ± 0.2
SPIPERONE	10.2 ± 3.2	4.4 ± 2.2	0.6 ± 0.6
6-OHDA +			
DILUENT	16.0 ± 4.3	4.8 ± 3.8	0.0 ± 0.0
SKF38393	8.8 ± 2.9	7.0 ± 2.7	0.0 ± 0.0
LY171555	23.8 ± 4.4	3.6 ± 2.2	0.2 ± 0.2
SCH23390	14.0 ± 4.2	5.6 ± 4.2	1.0 ± 1.0
SPIPERONE	13.8 ± 2.3	2.0 ± 1.2	0.3 ± 0.3

Legend as in Table 7

Table 10

Behavioral Effects Induced by LY 171555 (3.0 mg/kg, i.p.) in Rats that Received Repeated Treatment with D1 and D2 Receptor Agonists or Antagonists During Postnatal Development

DEVELOPMENTAL TREATMENT	BEHAVIORAL SCORE		
	TAFFY PULLING	PAW TREADING	HEAD NODDING
VEHICLE +			
DILUENT	0.0 ± 0.0	0.0 ± 0.0	1.4 ± 0.6
SKF38393	0.0 ± 0.0	0.0 ± 0.0	1.0 ± 0.6
LY171555	0.0 ± 0.0	0.0 ± 0.0	2.2 ± 1.4
SCH23390	0.0 ± 0.0	0.0 ± 0.0	1.2 ± 0.5
SPIPERONE	0.0 ± 0.0	0.0 ± 0.0	0.6 ± 0.2
6-OHDA +			
DILUENT	0.0 ± 0.0	1.6 ± 1.0	0.8 ± 0.8
SKF38393	0.4 ± 0.4	4.6 ± 2.4	0.4 ± 0.2
LY171555	0.0 ± 0.0	0.2 ± 0.2	2.2 ± 2.2
SCH23390	0.0 ± 0.0	0.4 ± 0.2	0.4 ± 0.2
SPIPERONE	0.0 ± 0.0	0.0 ± 0.0	0.8 ± 0.8

Legend as in Table 7

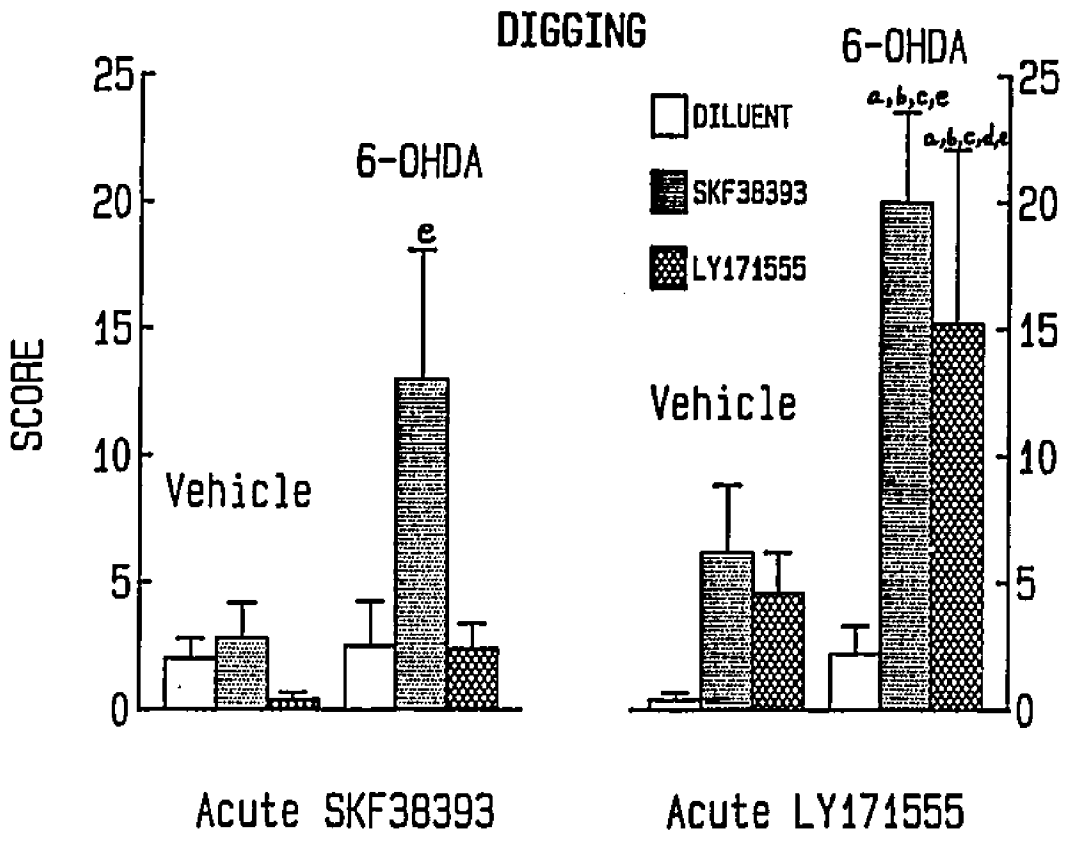

Figure 9. Behavioral Effects Induced by LY 171555 (3.0 mg/kg, i.p.) in Rats that Received Repeated Treatment with D1 or D2 Receptor Agonists During Postnatal Development. Rats were treated i.p., daily for the first 32 days from birth, with diluent, SKF 38393 (3 mg/kg/d), or LY 171555 (3 mg/kg/d) in combination with i.c.v. 6-OHDA (200 μg, salt form, at 3 days of age) or its diluent. Animals were challenged with LY 171555 (3 mg/kg, i.p.) at 49-51 days from birth, and were observed, beginning 10 min after the challenge dose. Numbers of episodes of each stereotypic behavior were summed over 9 one min periods, separated by a 10 min interval. Each min was subdivided into 15 sec sessions, and a score of 1 was assigned if a rat demonstrated a particular behavior during this interval. The maximum score for each min was 4. For the 90 min period, the maximum score was 36 for each behavior.
Results are expressed as the Mean ± S.E.M. for 5 rats.
a, $\underline{p} < 0.05$, compared to 'Vehicle + Diluent' group that were challenged with SKF 38393.
b, $\underline{p} < 0.05$, compared to the corresponding control group.
c, $\underline{p} < 0.05$, compared postnatal treatment with 6-OHDA vs. without 6-OHDA.
d, $\underline{p} < 0.05$, compared to the same treatment with different challenge.
e, $\underline{p} < 0.05$, compared to 'Vehicle + Diluent' group that were challenged with LY 171555.

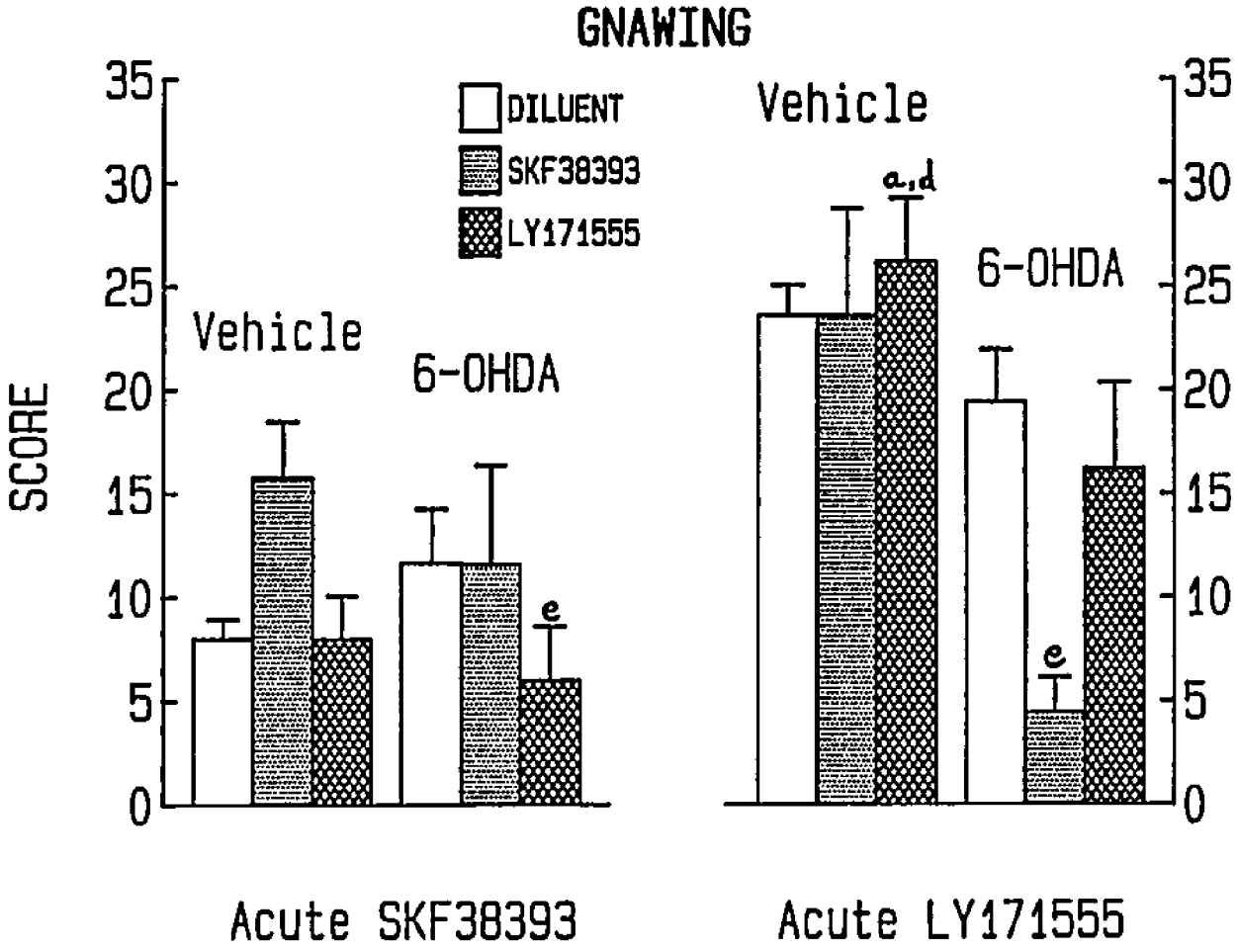

Figure 10.
Legend as in Figure 9.

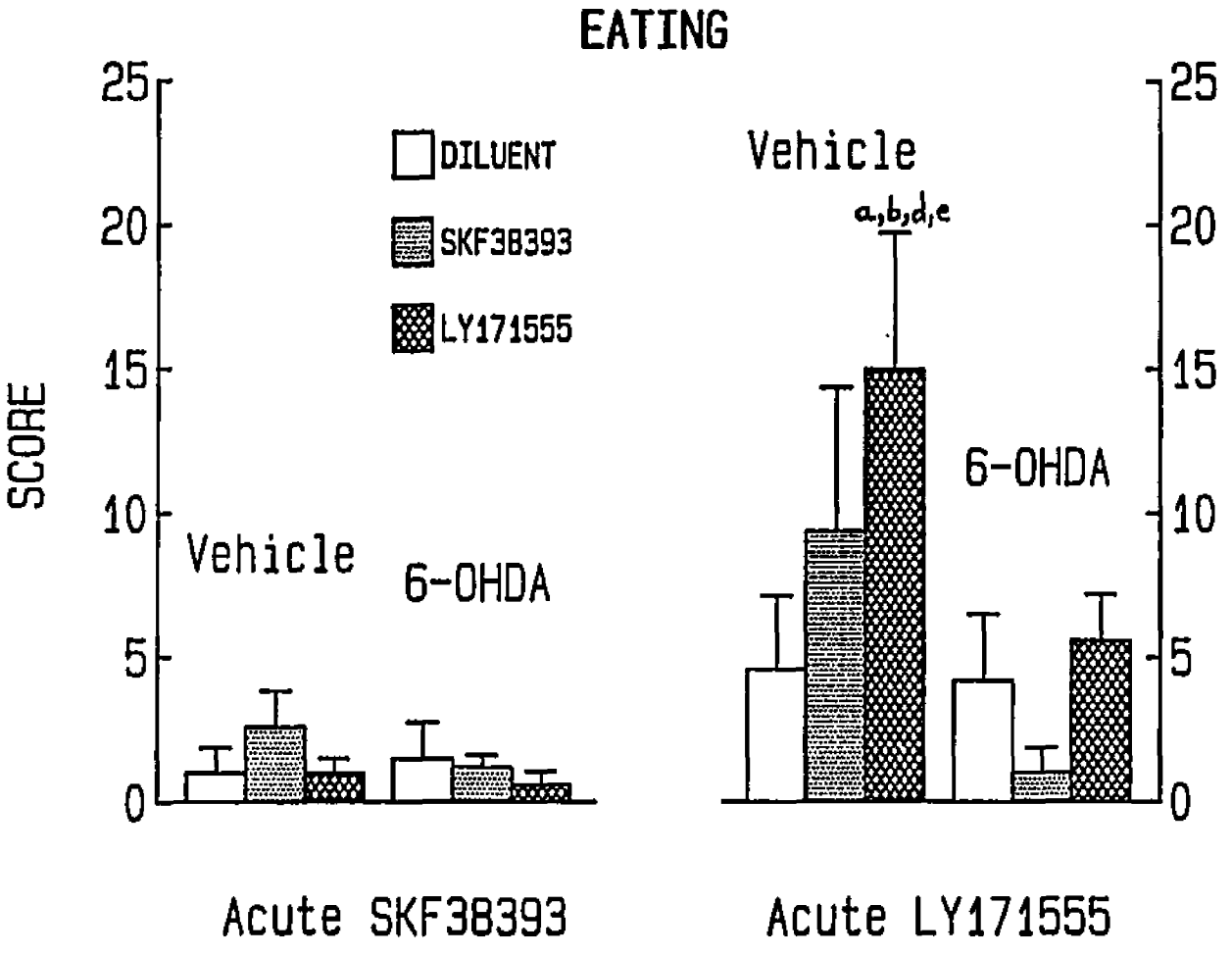

Figure 11.
Legend as in Figure 9.

Table 11

Characteristics of Striatal D1 Receptors in Postnatally-Treated Rats, as Determined by *In Vitro* Binding of [3H]SCH 23390

Treatment	Bmax (fmol/mg tissue)	Kd (pM)
Vehicle +		
Diluent	19.1 ± 6.8	416.8 ± 101.5
SKF38393	6.6 ± 2.2	240.3 ± 53.3
SCH23390	15.9 ± 3.8	466.4 ± 79.6
6-OHDA +		
Diluent	17.8 ± 5.6	600.6 ± 101.9
SKF38393	10.7 ± 2.6	439.4 ± 63.5
SCH23390	10.2 ± 2.3	508.8 ± 103.2

Each value is the Mean ± S.E.M. of 5 tissues.

Table 12

Characteristics of Striatal D2 Receptors in Postnatally-Treated Rats, as Determined by In Vitro Binding of [3H]spiroperidol

Treatment	Bmax (fmol/mg tissue)	Kd (pM)
Vehicle +		
Diluent	15.9 ± 2.0	73.3 ± 5.4
LY171555	14.2 ± 1.9	57.5 ± 7.2
Spiroperidol	26.0 ± 2.3	96.0 ± 8.1
6-OHDA +		
Diluent	20.0 ± 2.1	74.8 ± 5.3
LY171555	22.6 ± 4.9	104.7 ± 16.5
Spiroperidol	27.6 ± 4.1	100.7 ± 12.0

Each value is the Mean ± S.E.M. of 5 tissues.

CHAPTER 4

Discussion

Enhanced Yawning and Eating Responses to the DA D2 Specific Agonist LY 171555 in Rats Treated During Development with LY 171555

Yawning behavior appeared to be a well-suited model to study the function of the dopaminergic system. It provided a functional means for evaluating potential new dopamine agonists. Yawning has been associated with agonist activation of DA D2 receptors (Nickolson and Berendsen, 1980; Yamada and Furukawa, 1980; Gower et al., 1984; Stahle and Ungerstedt, 1984; Serra et at., 1983a,b; 1984b). Low doses of DA agonists have been reported to reduce schizophrenic morbidity and symptoms of hyperkinetic disorders (see references in Stahle and Ungerstedt, 1989). In view of these clinical findings, it is important to establish experimental models for effects of low doses of DA agonists and to elucidate the underlying pharmacological mechanisms. Yawning is one of the candidate behaviors.

Yawning is also of clinical interest since it has been reported that psychotics rarely yawn, and yawning is symptomatic in a wide range of CNS disorders such as brain lesions, tumors, hemorrhage, motion sickness, chorea, and encephalitis (see references in Laping and Ramirez, 1988).

Yawning is considered to be therapeutic in preventing post-operative respiratory complications and in adjusting the air pressure in the middle ear (see references in Provine et al., 1987).

It is now well accepted that yawning involves DA D2 receptors, which have characteristics in common with the D2 autoreceptor (Gower et al., 1984). This hypothesis is mainly based on the observation that the minute doses of DA receptor agonists needed to produce yawning are within the same range as those needed to stimulate DA autoreceptors. Higher doses of DA agonists, which stimulate postsynaptic DA receptors as well, produce locomotor hyperactivity and stereotypy and suppress yawning. The presynaptic origin of the yawning response to DA agonists is further suggested by the fact that this behavior is abolished by bilateral 6-OHDA-induced denervation of the striatum (Dourish and Cooper, 1985) and olfactory tubercles (Protais et al., 1983), and by the fact that yawning is elicited by DA agonists such as 3-(3-hydroxyphenyl)-N-n-propylpiperidine (3PPP) (Gower et al., 1984; Stahle and Ungerstedt, 1984) and TL-99 (Mogilnicka et al., 1984), which are considered to preferentially or selectively stimulate DA autoreceptors. On the basis of these observations it has been proposed by various authors (Mogilnicka and Klimek, 1977; Nickolson and Berendsen, 1980; Yamada and Furukawa, 1980; Gower et al., 1984; Stahle and Ungerstedt, 1984; Serra et at., 1983a,b;

1984b) that yawning is a behavioral consequence of DA autoreceptor-mediated inhibition of DA transmission. This hypothesis, however, has been challenged by the fact that yawning appears about 9 days postnatally, before maturation of presynaptic dopamine receptors (Arnt, 1987), and by the fact that DA agonist-induced yawning is not due to a decrease in the extracellular levels of DA (Stahle and Ungerstedt, 1989). DA agonists can induce yawning when DA neurotransmission is apparently enhanced (Stahle and Untgerstedt, 1989).

It has recently been proposed that DA agonists induce yawning by actions outside the striatum, namely by releasing oxytocin in the CNS, possibly in the paraventricular nucleus (PVN) of the hypothalamus. This nucleus contains the cell bodies of DA neurons of the A14 group which constitute the so-called incerto-hypothalamic DA system together with those of the A11 and A13 groups. DA D2 receptors have been identified in these regions (Argiolas et al., 1987; Melis et al., 1986; 1987).

Results of this study revealed that rats treated during postnatal development with the DA D2 agonist LY 171555 exhibited a marked increase in LY 171555-induced yawning at 5 to 6 weeks after birth. This enhanced behavioral response was not accompanied by an increased binding of [3H]spiroperidol to DA D2 receptors at 55-57 days from birth. It was not clear whether these results indicated

that [3H]spiroperidol binds to a site (striatum) which did not represent the action of LY 171555 or whether other mechanisms (supersensitivity) were responsible for the enhanced responses of this DA D2 agonist in the LY 171555 group of rats.

The yawning rate in the control group corresponded to that at the reported peak of the LY 171555 dose-response curve (Serra et al., 1987), while the yawning response rate in the LY 171555 group was greater than in any previous report, to the researchers knowledge, suggesting that the enhanced response was not a shift in the dose-response curve. These results suggested that LY 171555 treatment in development produced a supersensitive animal model for yawning behavior.

Yawning has been suggested as a behavior subserving arousal when attention is decreasing in front of a danger. Yawning might then indicate a low degree of arousal mediated by the activation of the most sensitive population of DA receptors. Further activation of DA transmission might progress to full arousal, exploratory behavior, and then to stereotyped sniffing, licking, and gnawing. This hypothesis offers a logical explanation for the fact that yawning and stereotypy are mutually exclusive. Namely, when the latter is elicited, the former is suppressed (Protais et al., 1983). Here it was proposed that the DA D2 agonist LY 171555 produced yawning by stimulating DA D2 receptors in

the PVN of the hypothalamus which may represent the most sensitive group of D2 receptors. Greater stimulation by the agonist or increased sensitivity in the striatal DA receptors may lead to stimulation of the DA receptors in the striatum or activation of these receptors even with the same degree of the stimulus, respectively. Neonatal 6-OHDA-lesioned rats demonstrate a supersensitivity to DA D1 agonists (Breese et al., 1984; 1985a,b; 1986). Furthermore, supersensitivity can be primed (increased) by repeated administration of specific DA D1 agonist SKF 38393. Those observations seem to be in accord with the low number of yawns that was observed in the group treated with 6-OHDA plus SKF 38393.

Dopaminergic mechanisms may be involved in the regulation of feeding behavior. Electrical stimulation of the lateral hypothalamus can elicit feeding in food-satiated animals. This stimulation-induced feeding is disrupted by neuroleptic treatment (Streather and Bozarth, 1987). Selective DA D2 receptor antagonists decrease the sham intake of sucrose in food-deprived rats (Schneider et al., 1986). DA D2 receptor-selective agonists increase consumption of food pellets in rats (solid diets) which are blocked by D2 antagonists or by D1 antagonists, while a D1 receptor selective agonist SKF 38393 decreases intake and produces anorexia (Martin-Iversen and Dourish, 1988).

Yawning, as mentioned, is considered a behavior

subserving arousal when attention is decreasing in front of a danger, so it might be considered to indicate a low degree of arousal, mediated by the activation of the most sensitive population of DA receptors. On the other hand, stress- or stimulation-induced eating could reflect a general "arousal" effect with specific behaviors determined by salient environmental stimuli (Martin-Iversen and Dourish, 1988).

Results of this study showed that rats treated during postnatal development with a DA D2 agonist LY 171555 exhibited a marked increase in LY 171555-induced eating at 7 weeks after birth. This enhanced behavioral response was not accompanied by an increased binding of [3H]spiroperidol to DA D2 receptors at 7-8 weeks from birth. It was not clear if these results were suggesting that [3H]spiroperidol is binding to a site (striatum) which did not represent the action of LY 171555 to elicit eating or whether other mechanisms (supersensitivity) were responsible for the enhanced responses of this DA D2 agonist in LY 171555 treated rats.

In conclusion, because of the fact that both yawning and eating behaviors were enhanced in the group that was treated during the postnatal period with a specific DA D2 agonist LY 171555 and because these behaviors reflected a degree of "arousal," it was suggested that D2 agonist treatment during development produced a supersensitivity or a change in number of DA D2 receptors which mediated these

behaviors. Further studies are needed to determine the relationship between these behaviors and the numbers of DA D2 receptors in the hypothalamus and to determine the possible usefulness of D2 agonist-treated rats as models to study mechanisms associated with eating behavior.

Enhanced Perioral Response to the DA D2 Specific Antagonist Spiroperidol in Neonatal 6-OHDA-Lesioned Rats

Oral dyskinesia represent an excess number of involuntary perioral movements. This behavior appears with increasing frequency during continued stable therapy in Parkinsonian patients, perhaps reflecting increasing denervation supersensitivity of DA receptors (Marsden, 1982). Oral dyskinesia, the most outstanding characteristic of tardive dyskinesia (TD), is observed in rats treated chronically with several neuroleptics (Clow et al., 1979a,b; Waddington and Gamble, 1980; Waddington et al., 1981). These movements are most pronounced when DA D1 receptors are stimulated or when DA D2 receptors are inhibited (Molloy et al., 1986; Rosengarten et al., 1986; Koshikawa et al., 1987; Molloy and Waddington, 1988) or reduced in number following prenatal drug intervention, strain difference, or in senescence (Rosengarten et al., 1983c; 1986). When DA D2 as well as DA D1 receptors are stimulated, these movements are least prominent (Rosengarten et al., 1986; Johansson et al., 1987), an observation that may prove relevant to the

treatment of TD and Tourette's syndrome. These movements can be suppressed by the selective DA D1 antagonist SCH 23390 or the cholecystokinin octapeptide (Stoessl et al., 1989). The molecular and/or neural mechanism(s) involved in the receptor modulation in TD are unknown.

An important finding in the present study was that perioral movements could be produced in neonatal 6-OHDA-treated rats in high frequency when a very low dose of the DA D2 antagonist spiroperidol was administered. This animal model might be of importance for studying mechanisms involved in the mediation of oral dyskinesia in humans.

The availability of this neurochemical model with increased susceptibility for perioral movements may permit screening of a variety of pharmacological agents that could minimize the TD syndrome observed after long-term neuroleptic administration. The demonstration that neonatal 6-OHDA-treated rats have D1 dominance (supersensitivity) without alteration in the number of binding sites in vitro for [3H]SCH 23390 and [3H]spiroperidol to striatal homogenates is compatible with the findings of Breese and co-workers (1984, 1985a, 1986, 1987; Duncan et al., 1987). The findings in this study suggested that the mechanisms involved in this disorder were at a site beyond the receptor complex. It has been suggested that clinical psychopathological disorders in humans, such as schizophrenia and TD, represent conditions in which the

defect is a developmental or drug-induced permanently altered state. With the ability of neonatal 6-OHDA-lesions to produce permanent effects on the dopaminergic system, it can be seen that the present behavioral model might more closely reflect the clinical situation. Future investigations in the neonatal 6-OHDA-treated rats will test the usefulness of this proposed model.

Enhanced Stereotypic Response to the DA D1 or D2 Specific Agonists SKF 38393 or LY 171555 in Rats Treated During Development with both SKF 38393 and 6-OHDA

The study of animal behaviors induced by centrally-acting dopaminergic agents is a classical means of investigating the pharmacology of the DA system and the pathophysiology of human neuropsychiatric diseases. So it was sought first to characterize the behavioral consequences of independent D1 receptor stimulation in rats with an intact dopaminergic system and other rats with a 6-OHDA lesioned-system.

Following neonatal, but not adult 6-OHDA lesions, rats exhibit greater behavioral supersensitivity to a DA D1 agonists than to DA D2 agonists (Breese et al., 1985a,b, 1986). The sensitivity of DA D1 receptors increases with repeated exposure to a DA agonist in the neonatally 6-OHDA-treated rats (priming). Supersensitivity to DA agonists develops during the first days after denervation and reaches

a steady state depending on the number of treatments, the dose of drug used and the time after lesion. The priming is effective as a facilitatory factor for the expression of DA receptor supersensitivity and provides a mechanism for the strong enhancement of the effectiveness of the agonist or for making otherwise ineffective doses of agonist effective in producing an effect. The changes induced by the primer do not involve the DA receptor per se, and stimulation of DA receptors could trigger some changes in neurons located downstream from the DA receptor itself (Morelli et al., 1989). This priming phenomenon has not been associated with DA D2 agonists (Breese et al., 1985b) and can be prevented by the D1 antagonist SCH 23390 (Criswell et al., 1989). The priming phenomenon, which appears to be permanent (Criswell et al., 1989), suggests that DA D1 receptors may possess a degree of plasticity that could have important implications for understanding long-term changes in behavior associated with repeated drug use or the basis of symptoms of childhood disorders secondary to a central DA deficiency.

In this study priming of DA D1 receptor response was reflected by an increase in the frequency of several behaviors elicited by a challenge dose of the DA D1 agonist SKF 38390 including licking, grooming, locomotion, jumping, taffy pulling, and paw treading. Priming of DA D1 receptor responses was reflected by the increase in frequency of several behaviors elicited by a challenge dose of the DA D2

agonist LY 171555 including licking, grooming, and digging. While priming of the DA D2 receptor response has not been observed with repeated administration of the DA D2 agonist at weekly intervals (Criswell et al., 1989), this treatment results in priming of responses to the DA D1 agonist in neonatal 6-OHDA-lesioned rats (Criswell et al., 1989). Present results show that priming of the DA D2 receptor response is present and is reflected by an increase in the frequency of digging behavior elicited by a challenge dose of the DA D2 agonist LY 171555. At present, it is not known whether the time between treatments, the dose, and/or the length of the treatment are/is an important determinant of DA D2 receptor priming.

Although functional responses to SKF 38393 or LY 171555 are enhanced in rats treated developmentally with SKF 38393 or LY 171555 plus neonatal 6-OHDA, respective binding of [3H]SCH 23390 or [3H]spiroperidol to striatal membranes from these rats is not altered.

The absence of an association between the enhancement of DA agonist-induced behavior and DA antagonist binding suggests that there are deficiencies in the present understanding of DA receptor mechanisms. Change(s) in the second or third messengers linked to these receptors or change(s) in systems modulating DA-mediated effects may explain this. It is evident, however, that if any prolonged treatment produces no change in the number of a receptor

population, it cannot necessarily be concluded that there is no effect on the kinetics of the processes determining steady-state receptor levels (Norman et al., 1987). Future studies might investigate a molecular mechanism for the enhanced behavioral responses observed in the absence of changes in receptor number.

DA D1 receptors may play a role in the transition from levels of arousal associated with behavioral efficiency to states of hyperarousal associated with behavioral disorganization. At low levels of DA D1 receptor stimulation (i.e., that produced by endogenous DA), DA D2 receptor activation elicits complex motor responses such as locomotion and exploratory activity which are part of the animal's endogenous behavioral repertoire. This is seen, for example, when normal animals are placed in a novel environment. These behaviors are purposeful, goal-oriented, and associated with efficient motor responsiveness to a variety of sensory stimuli (Braun et al., 1986). In contrast, higher levels of DA D1 receptor stimulation are associated with diminished responsiveness to environmental stimuli and in the appearance of stereotypic behaviors which represent fragmentation and purposeless repetition of elements of these same behaviors (Braun et al., 1986). Acute mania, attention deficit disorders with hyperactivity in children, Lesch-Nyhan disease, Gilles de la Tourette syndrome, and certain types of schizophrenia have been

characterized as states of hyperarousal associated with attention deficits and behavioral disorganization. Priming of the DA D1 receptor responsiveness in the neonatal 6-OHDA-lesioned rat represents a model of a long-term change in neural function associated with repeated activation of a chemically defined receptor system. As such, the underlying neurochemical mechanisms that lead to this increased sensitivity may be representative of changes in other central processes requiring a "permanent" neural message (Criswell et al., 1989).

CHAPTER 5

Summary

The present investigation demonstrated the following:

1. The DA D2 agonist LY 171555, when given chronically to rats in the postnatal period, produced supersensitive behavioral responses, namely yawning and eating, when these rats were challenged as adults with the same D2 agonist.
2. [3H]SCH 23390 and [3H]spiroperidol binding to striatal tissue was not altered in rats treated in development with specific agonists or antagonists for the D1 and D2 receptors. A neonatal 6-OHDA lesion did not modify binding in any of the agonist- or antagonist-treated groups.
3. Neonatal 6-OHDA-lesioned rats may serve as a useful animal model of tardive dyskinesia.
4. The ability of DA D2 receptors to be primed, as demonstrated for the first time in this study, may provide a means for studying particular stereotypic behaviors in animals.

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Honors and Awards

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