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Effects of dopamine D1 and D2 receptor inactivation on locomotor activity and sniffing in 11- and 17-day-old rats

Monja Mestlin

EFFECTS OF DOPAMINE D1 AND D2 RECEPTOR INACTIVATION ON LOCOMOTOR ACTIVITY AND SNIFFING IN 11- AND 17-DAY-OLD RATS

A Thesis

Presented to the

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California State University,

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In Partial Fulfillment

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ABSTRACT

Behavioral effects of dopamine receptors were assessed in 11- and 17-day-old rat pups using irreversible dopamine antagonist, EEDQ. In Experiment 1, the locomotor activity and sniffing behavior of 11- and 17-day-olds was assessed after treatment with the nonselective dopamine receptor agonist, NPA (0.00, 0.01, 0.1, 1.0, and 5.0 mg/kg). Testing sessions began 5-min after NPA treatment on three test days. In Experiment 2, 10- and 16-day-old rat pups received a single dose of EEDQ (7.5 mg/kg) or vehicle after dopamine receptors were left either unprotected or protected using a combination of sulpiride (100.0 mg/kg) and SCH 23390 (1.0 mg/kg). NPA (0.00, 0.01, or 5.0 mg/kg) was then administered to rat pups 24, 48, and 96 hours after EEDQ treatment. Results from Experiment 1 suggest that NPA did not increase activity in 11-day-old pups; whereas, 17-day-olds showed an increase in activity, with 0.01 mg/kg NPA producing the greatest effect. NPA produced a dose-dependent increase in sniffing in both aged pups. In Experiment 2, EEDQ did not affect the locomotor activity or sniffing of 11-day-old pups; whereas, 17-day-olds showed some enhancement of locomotor activity and sniffing after EEDQ treatment. Results indicate that the response of preweanling and adult rats to EEDQ is fundamentally different. Possible explanations are discussed.

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INTRODUCTION

Dopamine systems have been implicated in a number of important functions including attention, learning, and movement. Dopamine's role in the motor system has linked it to conditions involving excessive, exaggerated, or bizarre involuntary movements, such as in Huntington's chorea, tardive dyskinesia, epilepsy, and Parkinson's disease. L-Dopa, a precursor to dopamine, increases the synthesis and release of dopamine in Parkinson's patients, thereby alleviating symptoms of tremors, difficulty in initiating movement, loss of balance, and rigidity of limbs. Chronic administration of L-Dopa, as well as high doses of amphetamine, cocaine, and other dopamine agonists produce schizophrenic-like symptoms, such as hallucinations, delusions, and disorganized thinking. Drugs that block dopamine activity decrease symptoms of schizophrenia, suggesting that schizophrenia may be caused by an increase in dopaminergic functioning. Developmental disorders, such as Tourette's syndrome and Lesch-Nyhan disease have also been associated with dopamine systems (Calne, 1978, 1980; Clark & White, 1987; Cote & Crutcher, 1985; Mason, 1984).

The important roles of dopamine systems, as well as the severity of illnesses linked to dopamine systems, have prompted many researchers to investigate dopamine

functioning. Pharmacological studies have led to the discovery of many drugs which exert various effects on dopamine systems, drugs that help alleviate symptoms of disease and neurological disorders, as well as those that are used to provide an understanding of dopaminergic functioning. Ontogenetic studies also provide important information on dopamine systems and their development. Studies of the developing dopamine system contribute to an understanding of normal functioning as well as abnormal functioning. Information on how these systems mediate behavior in the developing animal also increase knowledge of dopaminergic systems, which is the focus of the current study.

In studying the ontogeny of the nigrostriatal dopamine system (a major ascending pathway in the CNS), several issues need to be addressed: 1. the basic biology of dopamine systems; 2. pharmacology of dopamine systems; and 3. behaviors mediated by dopamine systems.

Biology of Dopamine Systems

Biosynthesis of Dopamine

The synthesis of dopamine begins when the amino acid tyrosine is converted to 3,4-dihydroxyphenylalanine (DOPA) by tyrosine hydroxylase (Cooper, Bloom, & Roth, 1991). The next step involves the enzyme dopa decarboxylase (correctly called aromatic L-amino acid decarboxylase) which decarboxylates DOPA to dopamine in the cytoplasm of the nerve cell (Cooper et al., 1991). The dopamine is then stored in the nerve terminals until it is required for release. Feedback inhibition is apparent in this system, as tyrosine hydroxylase (rate-limiting substance) responds to high or low levels of dopamine in the nerve terminals (Roth, 1979). When dopamine levels are high, tyrosine hydroxylase is prevented from catalyzing the formation of dopamine (Roth, 1979).

Storage of Dopamine

There is evidence to suggest that dopamine is stored in more than one pool (Groppetti et al., 1977). Two distinct pools with different rates of turnover have been described (Nissbrandt & Carlsson, 1987). There appears to be a soluble or free storage pool in the cytoplasm which is important for controlling dopamine synthesis by changing the activity of tyrosine hydroxylase. There is a second pool of dopamine which is stored in granules (McMillen, German, &

Shore, 1980).

Release of dopamine is regulated by exocytosis from the granule storage pool and is calcium dependent. Newly synthesized dopamine is preferentially released while previously synthesized dopamine is stored and released under aversive conditions only (McMillen et al., 1980). The free storage pool in the cytoplasm would first be transferred to the granules before being released. The substantia nigra in the rat seems to release dopamine from only one pool (Nissbrandt & Carlsson, 1987).

Dopamine release from the striatum is also regulated by cell firing rates and inhibitory autoreceptors that exist on the presynaptic neuron (Carlsson, 1975; Wolf & Roth, 1990).

Metabolism of Dopamine

The two main enzymes involved in the metabolism of dopamine are monoamine oxidase (MAO) and catechol-O-methyltransferase (COMT). MAO converts dopamine to 3,4-dihydroxyphenylacetaldehyde which is then converted to 3,4-hydroxyphenylacetic acid (DOPAC). DOPAC is converted to homovanillic acid (HVA) by COMT (Nissbrandt & Carlsson, 1987). COMT is particularly important for the extracellular degradation of dopamine (Gulderg & Marsden, 1975). Reuptake of dopamine into the presynaptic terminal, and not enzyme degradation, is the primary method of termination for dopaminergic action in the synapse (Baldessarini, 1975;

Iversen, 1978). The uptake of dopamine is mediated by a carrier mechanism in the membrane of the presynaptic terminal and cell body region (Geffen, Jessell, Cuello, & Iversen, 1976; Harris & Baldessarini, 1973). A second noncarrier mediated uptake system has been described accounting for approximately 10-30% of dopamine uptake (Krueger, 1990). The uptake system also functions as a receptor or binding site for many psychoactive compounds such as tricyclic antidepressants and the psychomotor stimulants (Bradford, 1986; Randrup & Braestrup, 1977).

Dopamine Receptors

Dopamine receptors have been divided into subtypes, the first two to be classified were: D1, which activates adenylyl cyclase when stimulated; and D2 which is thought to be uncoupled to adenylyl cyclase or coupled in an inhibitory manner (Kebabian, 1978; Kebabian & Calne, 1979; Spano, Govini, & Trabucchi, 1978). Further research has revealed D3, D4, and D5 receptors, although they have not yet been fully characterized (Sokoloff, Giros, Martres, Bouthenet, & Schwartz, 1990; Sunahara et al., 1991; Van Tol et al., 1991).

In the nigrostriatal system, most of the dopamine receptors lie on the dendrites or on the soma of neurons postsynaptic to dopamine containing neurons. In the striatum, the D1 receptors out number D2 receptors 3 to 1

(Boyson, McGonigle, & Molinoff, 1984; Rao, Molinoff, & Joyce, 1991).

Dopamine receptors have also been found presynaptically on dopamine containing neurons, on somatic-dendritic regions and on nerve terminals. These receptors are called autoreceptors and are important in the local regulation of nerve impulse flow, dopamine synthesis, and dopamine release (Wolf & Roth, 1987). Autoreceptors are more sensitive to dopamine agonists than postsynaptic receptors (Roth, 1979; Wolf & Roth, 1987). Dopamine autoreceptors are thought to be of the D2 receptor subtype (Wolf & Roth, 1987), however, preliminary evidence suggests that the D3 subtype may also exist as an autoreceptor. Dopamine autoreceptors also influence tyrosine hydroxylase activity by interfering with the phosphorylation of the enzyme (Wolf & Roth, 1987).

Ontogeny of Dopamine Systems

The dopamine systems, with its many components, are typically present in the rat pup at birth. Dopamine is present at 12-13% of adult levels on postnatal day (PD) 0 in the striatum and slowly increases until adulthood (Coyle & Henry, 1973; Ribary, Schumlpf, & Lichtensteiger, 1986). Activity and conduction of dopaminergic neurons in the substantia nigra to the terminal regions of the striatum are evidenced on PD 0 (Tepper, Trent, & Nakamura, 1990). Neurons are near or at mature rates between the third and fourth

postnatal weeks (Tepper et al., 1990). Release mechanisms are mature at PD 10 (Walters, Chapman, & Howard, 1990). MAO and COMT, the degradative enzymes, can be detected at gestation day (GD) 15 in the striatum (Fiszman, Zuddas, Masana, Barker, & di Porzio, 1991). By GD 16, high affinity dopamine uptake mechanisms are functioning (Fiszman et al., 1991). Ontogenetic increases in uptake binding sites are seen as early as PD 3 when binding sites reach 22% of adult levels and adult levels are reached by PD 39 (Bonnet & Costentin, 1989).

Dopamine receptors are at a low level at birth, with D1 receptors at 9-14% of adult levels and D2 receptors at 9% (Murrin & Zeng, 1986; Rao et al., 1991). There is disagreement in the literature as to when these receptors reach adult levels. Several studies have shown D1 receptor levels to reach a peak between PD 28 and PD 40 and then decline to adult levels (Gelbard, Teicher, Faedda, Baldessarino, 1989; Giorgi et al., 1987; Rowlett, Rice, McDougall, Bardo, & Pedigo, 1991). However, another study suggests that adult levels for D1 receptors are reached by the end of the third postnatal week (Zeng, Hyttel, & Murrin, 1988). Rao et al. (1991) found that D1 receptors reached adult levels at PD 16, although adulthood was defined as 60 PD for this study; whereas, others used 70-120 PD as the criteria. Adult levels for D2 receptors are reached after

30 to 60 postnatal days, with adulthood defined as 60-day-olds (Murrin & Zeng, 1986; Rao et al., 1991). With D2 receptors there appears to be a linear increase from birth, although, other results indicate that D2 receptors peak at PD 40 and then decrease to adult levels (adults were defined as 120-day-olds).

It does not appear that rat pups have functional autoreceptors until PD 35. As shown by Shalaby & Spear (1980), attenuation of locomotor activity does not occur to low doses of apomorphine in rat pups under PD 35. Electrophysiological studies indicate that autoreceptors do not develop until at least four weeks after birth (Pitts, Freeman, & Chiodo, 1990).

Pharmacological Effects of Dopamine Systems

D1 and D2 receptors show different affinities for endogenous dopamine, with D1 having a ten times higher affinity than D2 (Hess & Creese, 1987; Seeman & Niznik, 1988). The recently classified dopamine receptor subtypes (i.e., D3, D4, and D5) also show different affinities for endogenous dopamine (Sokoloff et al., 1990; Sunahara et al., 1991; Van Tol et al., 1991).

Recently, the development of selective and non-selective dopamine-acting drugs has led to a rapid increase in knowledge about dopamine system functioning. Specific D1 receptor agonists (drugs that bind to and activate only D1 receptors) include SKF 82958 (O'Boyle, Gaitanopoulous, Brenner, & Waddington, 1989); whereas, partial agonists are: SKF 89641 and SKF 89145 (both theinopyridines derivatives), and SKF 38393 (a benzazapine compound) (Lorio, Barnett, Leitz, Houser, & Korduka, 1983; Sibley, Leff, & Creese, 1982). A selective antagonist for the D1 receptor (drugs that block D1 receptors) is SCH 23390. Specific agonists for D2 receptors are quinpirole and bromocriptine; antagonists include sulpiride and other benzamides (Clark & White, 1987).

Nonselective dopamine receptor agonists (drugs that bind to and activate the various dopamine receptor subtypes) include: N-propylnorapomorphine (NPA), apomorphine, and

ADTN; whereas, nonselective antagonists include: cis(Z)-Flupentixol, cis(Z)-Piflutixol, and cis(Z)-Clopentixol. Although apomorphine has been widely used in research, it has recently been shown to be more selective than NPA in activating D2 than D1 receptors (Goldman & Kebabian, 1984; Seeman & Grigoriadis, 1987). NPA and apomorphine have also been shown to have a greater potency at presynaptic sites than at postsynaptic sites (Meller, Goldstein, Friedhoff, & Schweitzer, 1988).

EEDQ is a peptide coupling agent which noncompetitively binds to dopamine receptors and depletes both D1 and D2 receptors (Crawford, McDougall, Rowlett, & Bardo, 1992). It has been used to determine age-dependent and regional differences in dopamine receptor turnover and recovery for both D1 and D2 binding sites (Battaglia, Norman, & Creese, 1987; Fuxe, Agnati, Pich, Meller, & Goldstein, 1987; Leff, Gariano, & Creese, 1984; Norman, Battaglia, & Creese, 1987). EEDQ also inactivates alpha-adrenergic, serotonin and GABA receptors, however by protecting dopamine receptors, behaviors mediated by dopamine can be differentiated from behaviors mediated by other receptors. Receptors can be protected by using specific D1 and D2 antagonists such as SCH 23390 and sulpiride. These selective antagonists block D1 and D2 receptors, thereby eliminating EEDQ's ability to inactivate those specific receptor subtypes (Henry, Joseph,

Kochman, & Roth, 1987; Leff et al., 1984). Studies have shown that behavioral recovery is correlated with recovery of dopamine receptors (Hamblin & Creese, 1983).

Behaviors Mediated by Dopamine Systems

Adult Studies

Dopamine systems play a critical role in motor control. For example, when dopamine agonists are administered to rats, there is a decrease in locomotor activity at low doses and an increase in motor activity at high doses (Arnt, 1987). Stereotyped behaviors such as sniffing, rearing, and head movements, as well as oral dyskinesias are produced at even higher doses. A decrease in behavior is produced by dopamine antagonists, including suppression of conditioned responses (Arnt, 1987).

D1 receptors are involved in grooming, perioral activity, and perhaps non-stereotyped locomotor activities (Clark & White, 1987). D2 receptors are involved in locomotor activity, sniffing, yawning, and rearing (Arnt, Hyttel, & Perregaard, 1987; Dall' Olio, Gandolfi, Vaccheri, Roncada, & Montanaro, 1988; Longoni, Spina, & DiChiara, 1987; Molloy & Waddington, 1985). D1 and D2 receptors appear to interact to affect behavior. For example, grooming behaviors mediated by D1 receptors are attenuated by excessive D2 activation. In addition, D1 receptor systems appear to play a 'permissive' role for D2 agonist-induced behaviors, as D1 antagonists block the enhanced sniffing, yawning, and locomotor activity induced by D2 agonists (Arnt, Hyttel, & Meier, 1988; Longoni et al., 1987;

McDougall, Arnold, & Nonneman, 1990; Pugh, O'Boyle, Molloy, & Waddington, 1985; Serra, Collu, & Gessa, 1987). It also appears that a functioning D2 system may be necessary for activating D1 mediated grooming behavior (Gandolfi, Dall'Olio, Vaccheri, Roncada, & Montanaro, 1988; Murray & Waddington, 1989). Stereotyped behaviors such as: licking, biting, climbing, and gnawing are fully exhibited only after treatment with a combination of D1 and D2 agonists or mixed agonists (Arnt & Hyttel, 1985; Bordi & Meller, 1989; Davis, Jenner, & Marsden, 1990; Longoni et al., 1987). Additive effects are sometimes produced by antagonism of both D1 and D2 receptors (Arnt & Hyttel, 1985; McDougall, Nonneman, & Crawford, 1992; Waddington, 1989). Adult-like interactions of D1 and D2 receptors have also been shown in preweanling rats as young as 11-days-old (McDougall et al., 1990).

Ontogenetic Studies

Developmental studies of D1 and D2 receptors use similar compounds as those used for adult rats. Quinpirole, a D2 selective agonist, increases locomotor activity in both 11-and 17-day-old rat pups (McDougall et al., 1990). The selective D1 agonist, SKF 38393, produces enhanced grooming and, at high doses, increased locomotor activity (McDougall et al., 1990). Interactive effects of D1 and D2 receptors are found when SCH 23390, a selective D1 antagonist, blocks quinpirole-induced increases in locomotor activity

(McDougall et al., 1990). Moody and Spear (1992) assessed ontogenetic differences in neonatal (PD 3-4), preweanling (PD 10-11), and weanling (PD 21-22) rats. They found age-related responses to D1 and D2 receptor stimulation with separate and combined administration of D1 and D2 agonists. All ages showed increases in locomotor activity and sniffing to separate and combined administrations of SKF 38393 and quinpirole. Contrary to McDougall et al.'s results, adult-typical grooming responses were only seen in weanling aged rats in response to SKF 38393, due perhaps to a lower dosage of SKF 38393 used by Moody and Spear. Increases in vertical movements and licking were also seen only in the weanling aged rats in response to quinpirole or SKF 38393 combined or separately. Their results suggest that D1 and D2 receptors are functually operative after agonist stimulation at even early ages, although differences in blood brain barrier permeability, absorption rates, and other aspects of dopamine functioning do show ontogenetic changes (Moody & Spear, 1992).

Learning studies in young rats have also provided information on ontogenetic differences in response to dopamine receptor agonists and antagonists. Response suppression learning of younger (11- and 13-day-old) and older (17- and 19-day-old) rat pups was compared after D2 receptor activity was blocked with the antagonist sulpiride

(McDougall & Nonneman, 1989). The researchers found differences in responding between the 11-and 17-day-old rats, as sulpiride had no effect on the 11-day-olds punished responses; whereas, the punished responding of 17-day-old rats was disrupted by sulpiride. These results suggest that some maturation of the D2 system may occur between 11- and 17-days of age accounting for differences in response to sulpiride (McDougall & Nonneman, 1989).

Recent studies have shown that rat pups do not show drug sensitization until after 21 PD (Kolta, Scalzo, Ali, & Holson, 1990). Habituation is a decrease in response to a novel environment. Drug sensitization occurs when there is an overresponiveness to a drug after repeated administration. Drug sensitization and habituation research on 17-day-old pups showed stable levels of activity when treatment consisted of successive NPA treatment for three testing days (NNN) (McDougall, Crawford, & Nonneman, 1992). However, rats given saline for two days and NPA (SSN) on the third testing day did exhibit higher activity levels than saline subjects (SSS) and less than those in the NNN group (McDougall, Crawford, & Nonneman, 1992). Habituation to the chamber decreased the number of activity counts, although the pattern of responding was similar for both nonhabituated and habituated groups (McDougall, Crawford, & Nonneman, 1992).

Mature rats and preweanling rats differ in their responses to EEDQ. McDougall, Crawford, and Nonneman (1992) found that EEDQ does not attenuate the typical dopamine agonist induced behaviors such as grooming and locomotor behavior in young rats. Crawford et al. (1992) tested D1 and D2 binding sites for depletion after EEDQ treatment and found that 17-day-old rats showed a smaller relative decrease of receptors (69%) when compared to adult rats (86% depletion rate). In a recent experiment with 11- and 17-day-old rat pups, it was found that although depletion of receptors was similar after EEDQ treatment, full recovery did not occur for the 11-day-olds even after eight days (Crawford, Rowlett, McDougall, Elkins, & Bardo, 1991).

These results indicate that there are interesting ontogenetic differences in the responses of various aged rats to EEDQ. First, the behaviors of 90-day-old rats, but not 17-day-olds, are affected by EEDQ (11-day-old rats have never been tested behaviorally after EEDQ). Second, 17- and 90-day-old rats show full receptor recovery after EEDQ treatment, but 11-day-olds do not. Thus, it is unclear what effect EEDQ should have on the behaviors of 11-day-old rats. This proposal will further assess the behavioral effects of EEDQ on 11- and 17-day-old rats.

The age-dependent differences in receptor neurochemistry between 11- and 17-day-old rat pups leads to

the question of whether 11-day-olds should respond differently to EEDQ. In studies involving learned behaviors, striking age-dependent behavioral differences have been observed after treatment with dopamine-acting drugs (McDougall & Nonneman, 1989); however, when unlearned behaviors were assessed using similar drugs, few age-dependent differences have been reported (McDougall et al., 1990). Since EEDQ produces profound neurochemical differences between 11- and 17-day-old rats, it is expected that this will be reflected behaviorally. Since no behavioral data has been obtained on 11-day-old pups over time, the present investigation will establish a dose response curve in 11- and 17-day-olds, as well as test NPA in the 11-day-olds for the first time. Experiment 1 will involve testing various NPA dosage levels on both 11- and 17-day-old rat pups. Sniffing is maximally produced by joint D1 and D2 stimulation and should be fully observed at high doses of NPA. Locomotor activity reflects increased D2 receptor activation, and should be observed after lower doses of NPA.

As stated previously, EEDQ's behavioral effects are different for 17- and 90-day-old rats, as EEDQ does not affect the locomotor activity and grooming of NPA-treated 17-day-old rats (McDougall, Crawford, & Nonneman, 1992). It is possible that the lack of an EEDQ-induced effect in the

17-day-old rats is because the behaviors assessed require only a small percentage of available dopamine receptors. Therefore, in Experiment 2, a high dose of NPA will be used, which should produce a stereotyped sniffing response. This behavior requires a full complement of dopamine receptors, thus an EEDQ-induced decrease in sniffing should, therefore, be observed in the 17-day-old rats. Because full receptor recovery is not apparent in EEDQ treated 11-day-old rats, it is uncertain how they will respond behaviorally after EEDQ treatment.

Experiment 1

Method

Subjects. Animals were 80 male and female rats of Harlan Sprague-Dawley descent, born and reared at California State University of San Bernardino. The day of birth was defined as day 0. Litters were culled to 8-10 pups at three days of age. Pups were caged with dam except during experimental procedures. Assignment of subjects was random with eight animals selected in each group. The colony room was maintained at 28°C and kept under a 12:12 hr light:dark cycle. Testing was conducted during the light phase of the cycle.

Apparatus. Behavioral testing was done in activity chambers made of plywood (25 X 25 X 18 cm) with a wood floor and an open top. Walls and floor were painted gray. The floors were divided by black lines into four equal quadrants. The testing chambers were housed in a large glass-topped incubator maintained at 31 $\pm$ 1°C. Clear Plexiglas heated holding cages with hardwood chip bedding were used to house pups before behavioral testing.

Procedure and Drugs. Different doses of NPA (0, 0.01, 0.1, 1.0, and 5.0 mg/kg) were administered 5 minutes prior to observation of 11- and 17-day-old rats. Each pup was tested at only one age and each pup received only one drug sequence. Each subject was individually habituated to the

testing chamber for 20 minutes prior to injection of the agonist. Locomotor activity (number of line crossings) and sniffing behavior were assessed for a 20 minute testing session (Test Day 1). Behaviors of the same rats were then assessed after drug treatment on two subsequent test days (Test Days 2 and 4). A single line-crossing was defined as the rat pup placing two front paws into an adjacent quadrant. Sniffing was measured every 20 seconds during the session using a time sampling technique. Sniffing was defined as a head down movement, in which the nose and whiskers visibly moved. Eight subjects were used in each group.

All drugs were injected intraperitoneally (i.p.) and were given at a volume of 5.0 ml/kg. NPA was obtained from Research Biochemicals INC. (USA) and dissolved in saline prior to injection.

Statistics. Two 5 X 2 univariate analyses of variance (ANOVAs) with repeated measures were used for statistical analysis of locomotor activity and sniffing data. The three test days were analyzed as a repeated variable and dose (0, 0.01, 0.1, 1.0, and 5.0) was analyzed as a between factor. One-way ANOVAs were used to further examine significant interactions. Newman-Keuls' tests supplemented ANOVAs when appropriate ($p < 0.05$).

Results

Locomotor Activity. A significant two-way interaction between dosage level and day was found for both 11- and 17-day-old rat pups ($F(8,70) = 7.87$, $p < 0.001$ and $F(8,70) = 6.39$, $p < 0.001$, respectively) (see Figure 1). On Day 2, 11-day-old pups were significantly less active after treatment with 5.0 mg/kg NPA, as compared to the saline-treated pups ($p < 0.05$). The effects of dose varied significantly on Days 2 and 4 for 11-day-old pups, with the NPA-treated animals being in general less active; $F(4,39) = 3.286$, $p < 0.05$ and $F(4,39) = 7.503$, $p < 0.001$, respectively. All dosage levels of NPA (0.01, 0.1, 1.0 and 5.0 mg/kg) significantly decreased locomotor activity in 11-day-olds on Day 4 ($p < 0.05$). Saline-treated rats showed a progressive increase in locomotor activity across days, due possibly to maturational effects (i.e. opening of eyes), $F(4,39) = 99.72$, $p < 0.001$. Surprisingly, NPA-treated pups did not show a day-dependent effect.

Seventeen-day-old pups also displayed significant drug-dependent differences in activity on Days 2 and 4 ($F(4,39) = 8.243$, $p < 0.001$ and $F(4,39) = 19.284$, $p < 0.001$, respectively). When assessed across days, saline-treated 17-day-old rats showed a significant decline in locomotor activity, perhaps due to habituation effects ($p < 0.05$). In contrast all doses of NPA increased activity significantly from Day 1 to Day 2 ($p < 0.05$), then leveled off.

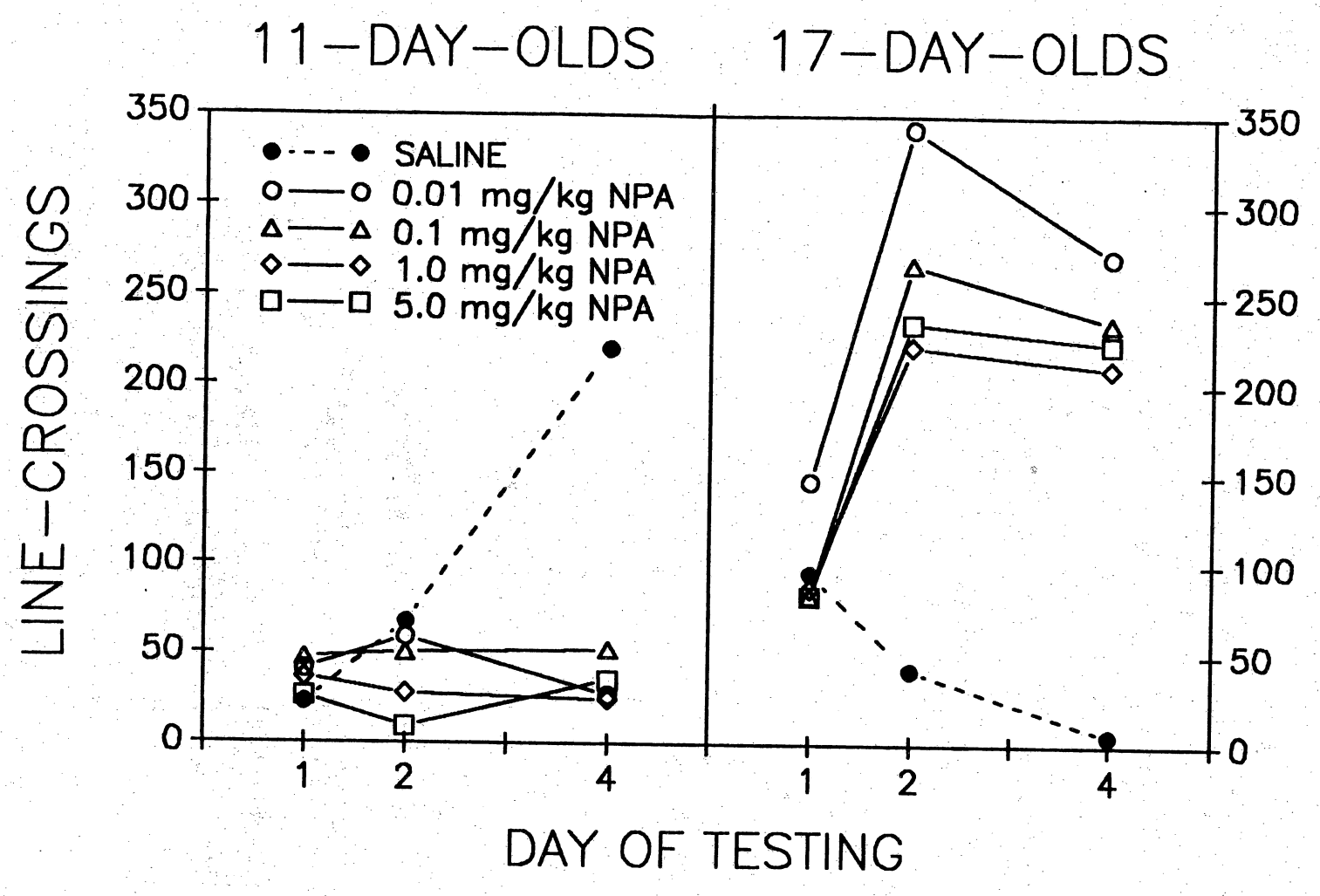

FIGURE 1 Mean number of line-crossings for 11- and 17-day-old rat pups in Experiment 1 by NPA dosage on each test day

Sniffing. Significant main effects for day and dosage were found for both 11- and 17-day-olds ($F(8,70) = 5.30$, $p < 0.001$ and $F(8,70) = 2.77$, $p < 0.010$, respectively) (see Figure 2). In all cases, NPA produced significantly higher sniffing counts than saline; moreover, the greater doses of NPA (5.0 and 1.0 mg/kg) produced significantly higher counts of sniffing than the lower doses (0.01, and 0.1) for both 11- and 17-day-old rats ($p < 0.05$). For 11-day-old pups, sniffing in the 5.0 mg/kg NPA group increased significantly over the three testing days; whereas, all other doses showed significant increases from Day 1 to Day 2 only ($p < 0.05$).

Summary. The purpose of Experiment 1 was to determine the doses of NPA which produced maximal sniffing and locomotor activity. Results indicated that 0.01 mg/kg NPA led to the most pronounced increase in locomotor activity in 17-day-olds. Since NPA-treated 11-day-olds were significantly less active on Days 2 and 4, compared to saline-treated pups, it is more difficult to ascertain an appropriate dosage level. Therefore, 0.01 mg/kg NPA was chosen as the low dose in Experiment 2. Sniffing for both 11- and 17-day-olds was significantly enhanced by 5.0 mg/kg NPA leading to its choice as the high dose for Experiment 2.

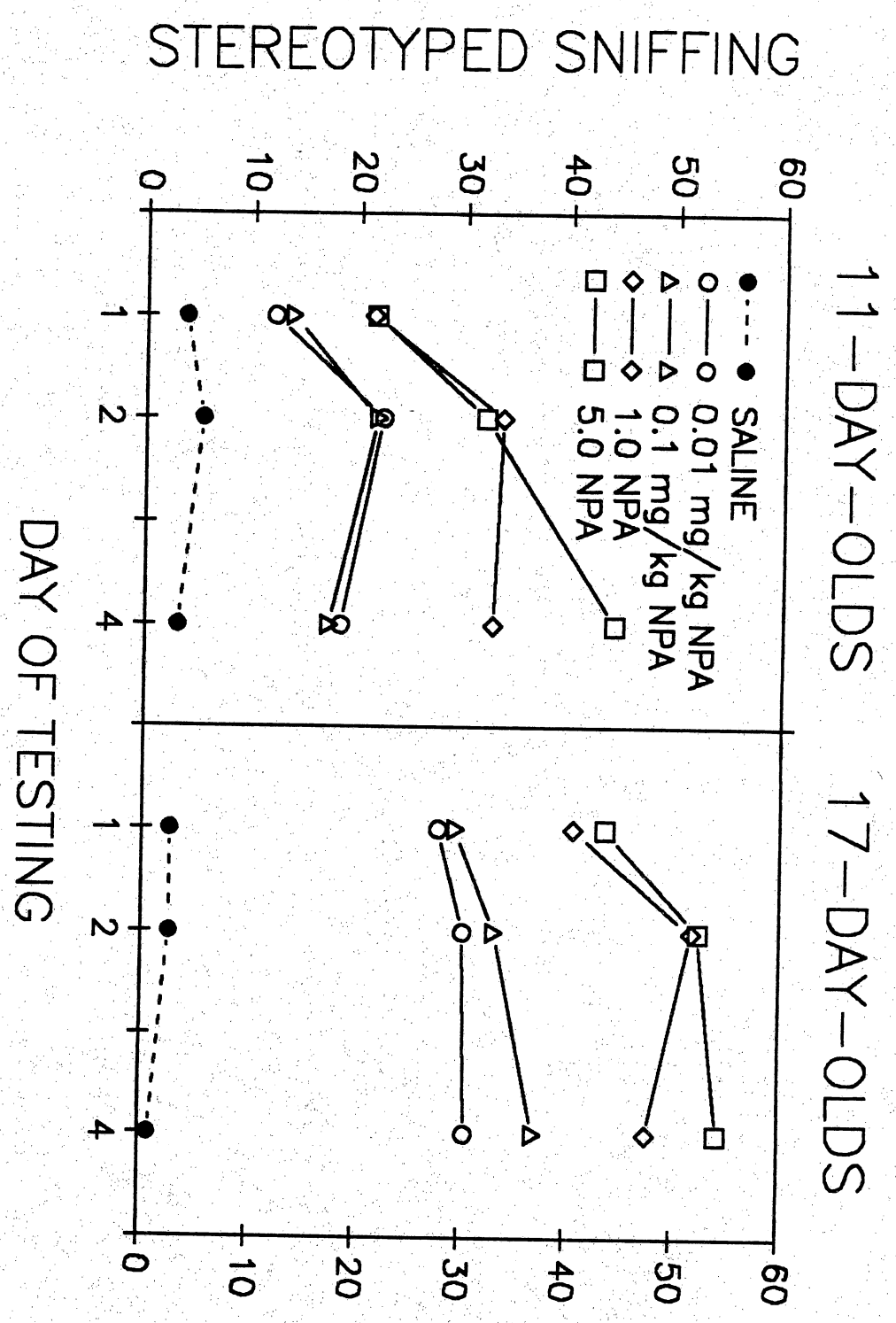

FIGURE 2 Mean number of sniffing counts for 11- and 17-day-old rat pups in Experiment 1 by NPA dosage on each test day.

Experiment 2

It has previously been shown that EEDQ does not affect NPA-induced locomotor activity in 17-day-old rat pups (McDougall, Crawford, & Nonneman, 1992). It is quite possible that this paradoxical effect is due to a large number of spare receptors in the 17-day-old rat. Thus, although EEDQ inactivates at least 65% of striatal dopamine receptors in 17-day-olds (Crawford et al., 1992), it is possible that a sufficient number of receptors remain to mediate agonist-induced effects (see McDougall, Crawford, & Nonneman, 1992). In contrast to locomotor activity, drug-induced sniffing requires that a large percentage of D1 and D2 receptors must be activated for the behavior to occur (Arnt et al., 1988; Longoni et al., 1987; McDougall et al., 1990). This feature suggests that sniffing should be much more sensitive to EEDQ's effects. Therefore, in Experiment 2, the NPA-induced sniffing of 11- and 17-day-old pups was assessed after EEDQ treatment. In addition, by using both a high and low dose of NPA, it will be determined behaviorally if there are significant ontogenetic differences in recovery of dopamine D1 and D2 receptors for 11- and 17-day-old rat pups.

Method

Subjects and Apparatus. Subjects were 144 male and female rat pups of Harlan Sprague-Dawley descent, born and

reared at California State University, San Bernardino. Rearing conditions and apparatus were the same as in Experiment 1.

Procedure and Drugs. Using 0.01 and 5.0 mg/kg NPA, 11- and 17-day-old rats were tested for receptor recovery after EEDQ treatment. Rats from both age groups were divided into two conditions - protected or nonprotected condition. Protected subjects received two injections of dopamine antagonists to block receptors from EEDQ inactivation (this allows assessment of nondopaminergic effects of EEDQ). Rats in the protected condition were given an initial injection of sulpiride (100.0 mg/kg) followed, 30 min later, by an injection of SCH 23390 (1.0 mg/kg). The nonprotected rats received two injections of vehicle. Thirty minutes after the second injection, rats were injected with either EEDQ (7.5 mg/kg) or its vehicle. (A protected/vehicle group was not included, since protection has not been shown to significantly affect behavior.) For each rat, behavioral testing occurred 1, 2, and 4 days after initial drug treatments. On each of these test days, rats were injected with either saline or NPA (0.01 and 5.0 mg/kg). Each rat was given the identical treatment three times across a four day span. Five minutes after agonist treatment, locomotor activity and sniffing were recorded in the same manner as in Experiment 1.

All drugs were injected i.p. and were given at a volume of 5.0 ml/kg. Both sulpiride and SCH 23390 were dissolved in distilled water, with the former drug requiring a small volume of glacial acetic acid. EEDQ was dissolved in 95% ethanol:distilled water (1:4). EEDQ (N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline) was acquired from Sigma (USA). Sulpiride and SCH 23390 were obtained from Research Biochemicals Inc. (USA).

Statistics. Repeated measures 3 X 3 X 3 ANOVAs were used to examine locomotor activity and sniffing. The three test days were treated as a repeated variable. NPA and EEDQ were separated into three levels: saline, 0.01 mg/kg, and 5.0 mg/kg NPA; protected/EEDQ, nonprotected/EEDQ, and nonprotected/vehicle. Two- and one-way ANOVA's were used to further examine significant interactions. Newman-Keuls' tests supplemented ANOVAs when appropriate ($p < 0.05$).

Results

Locomotor Activity. No three-way interaction was found for 11-day-old pups ($F(8,126) = 1.07$, $p > 0.05$). The only interaction for 11-day-olds was between NPA and day ($F(4,126) = 7.32$, $p < 0.001$) (See Figure 3). A significant main-effect on Day 1 was found for NPA ($F(2,69) = 7.935$, $p < 0.001$). Eleven-day-old pups were significantly more active with the lower dose of NPA (0.01 mg/kg) than with 5.0 mg/kg or saline ($p < 0.05$). On Day 2 ($F(2,69) = 7.745$, $p <$

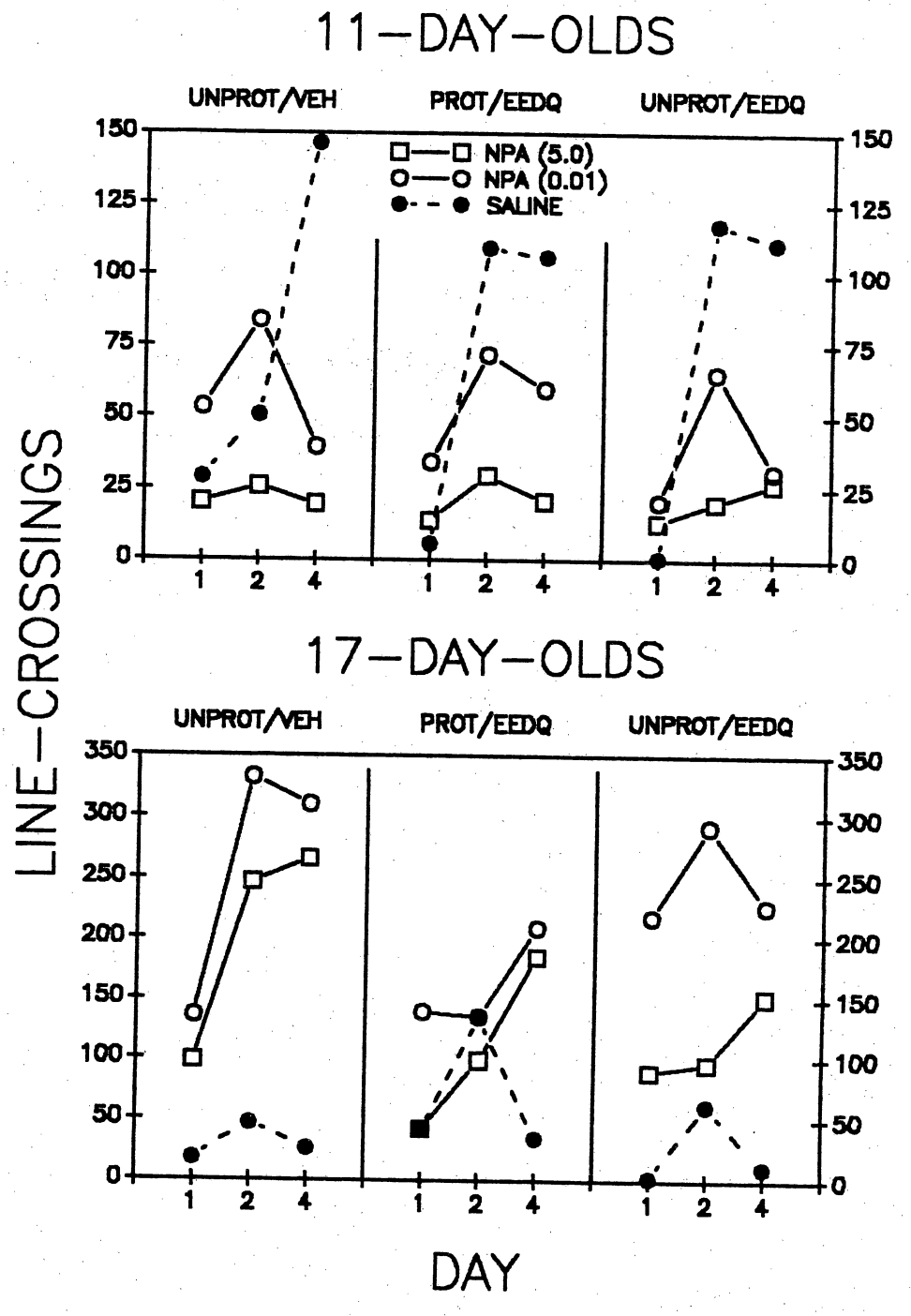

FIGURE 3 Mean number of line-crossings for 11- and 17-day-old rat pups in the three EEDQ pretreatment conditions of Experiment 2 by NPA dosage on each test day.

0.001), saline-treated pups showed significantly higher levels of activity than 11-day-olds treated with 5.0 mg/kg NPA ($p < 0.05$). Higher activity counts were also produced by 0.01 mg/kg NPA on Day 2, when compared with 5.0 mg/kg ($p < 0.05$). Saline-treated rats displayed significantly higher activity counts on Day 4 ($F(2,69) = 12.121$, $p < 0.001$), than either 0.01 or 5.0 mg/kg NPA ($p < 0.05$).

For 11-day-olds treated with saline, significant increases in activity were seen from Day 1 to Day 2 ($p < 0.05$); this increase leveled off on Day 4. Significant increases in locomotor activity were also seen from Day 1 to Day 2 for 11-day-olds treated with 0.01 mg/kg NPA.

Significant three-way interactions between NPA, EEDQ, and day were found for 17-day-old pups ($F(8,126) = 2.01$, $p < 0.050$) (See Figure 3). Two-way interactions between EEDQ and day, and NPA and day were also significant for 17-day-olds ($F(4,126) = 2.59$, $p < 0.040$, and $F(4,126) = 4.14$, $p < 0.003$, respectively). Significant two-way interactions between NPA and EEDQ were found on Day 2 ($F(4,63) = 4.642$, $p < 0.002$) for 17-day-old pups. Activity counts of pups treated with 0.01 mg/kg of NPA were significantly lower on Day 2 while when in the protected/EEDQ group rather than the unprotected/EEDQ or unprotected/vehicle group ($p < 0.05$). When given 5.0 mg/kg NPA, both unprotected/EEDQ and protected/EEDQ groups showed significantly lower levels of

activity than the unprotected/vehicle group ($p < 0.05$). The protected/EEDQ group treated with saline had significantly higher counts of activity for Day 2 than the unprotected/vehicle group ($p < 0.05$).

On Day 1, unprotected/EEDQ pups treated with 0.01 mg/kg NPA had significantly higher locomotor activity counts than saline-treated pups in the unprotected/EEDQ, unprotected/vehicle, or protected/EEDQ groups; 5.0 mg/kg NPA treated pups in the protected/EEDQ group also had significantly lower activity counts than 0.01 mg/kg treated unprotected/EEDQ pups ($p < 0.05$). For 17-day-old pups in the unprotected/vehicle group, activity increased significantly from Day 1 to Day 2 for pups in the 0.01 and 5.0 groups, and then leveled off ($p < 0.05$). Saline-treated pups showed significantly lower levels of activity on Day 4 in all three conditions than either 0.01 or 5.0 mg/kg NPA treated pups ($p < 0.05$). Unprotected/vehicle pups given 0.01 mg/kg NPA were significantly more active than unprotected/EEDQ pups given 5.0 mg/kg on Day 4 ($p < 0/05$).

Sniffing. As with the locomotor activity results, 11-day-olds showed no significant three-way interaction ($F(8,126) = 0.80$, $p > 0.05$) (See Figure 4). A significant two-way interaction was found for NPA by day for 11-day-olds ($F\ (4,126) = 8.60$, $p < 0.001$). Saline-treated 11-day-old pups had significantly lower levels of sniffing than either

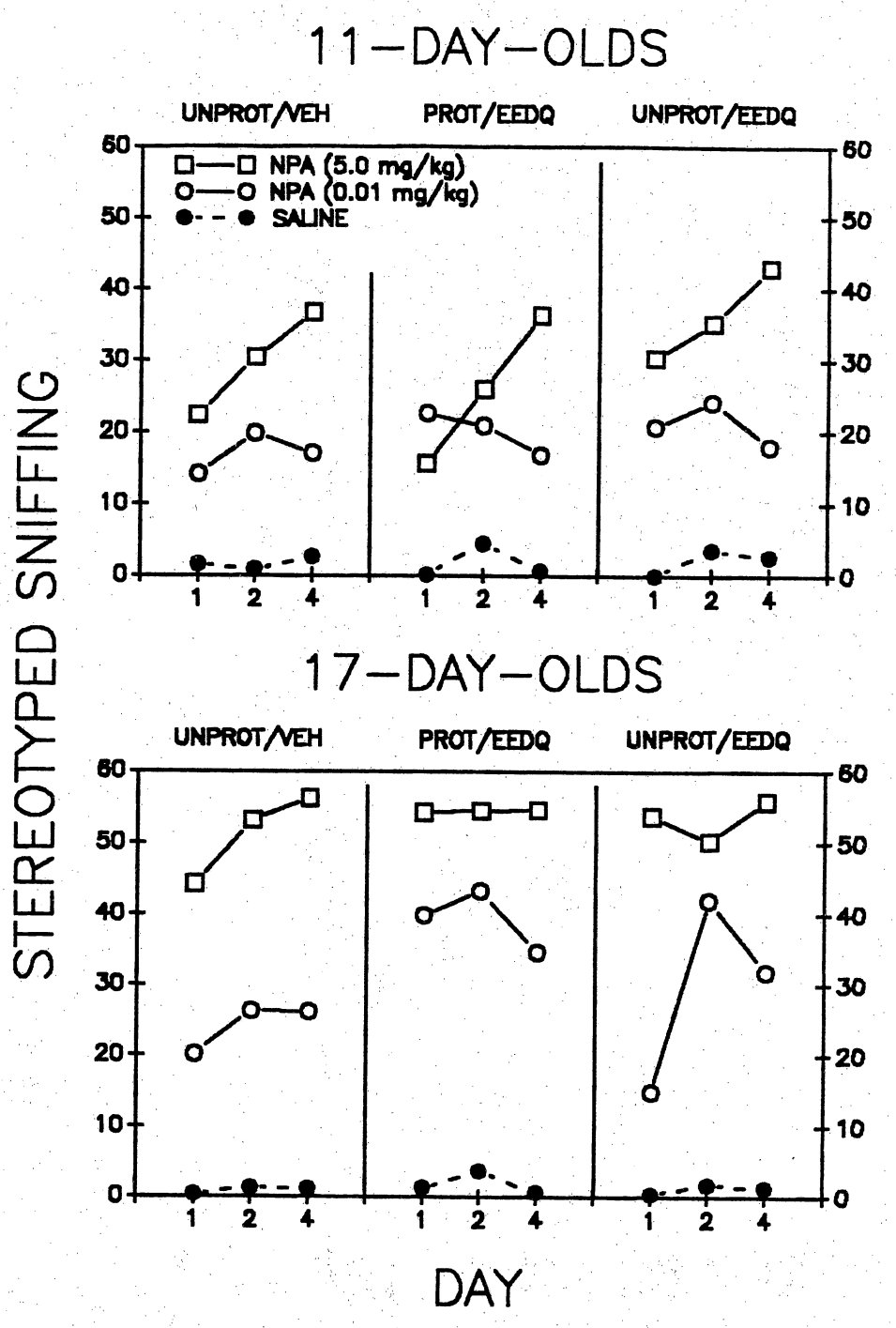

FIGURE 4 Mean number of sniffing counts for 11- and 17-day-old rat pups in the three EEDQ pretreatment conditions of Experiment 2 by NPA dosage on each test day.

0.01 or 5.0 mg/kg NPA ($p < 0.05$). On Days 2 and 4, ($F(2,69) = 43.795$, $p < 0.001$ and $F(2,69) = 123.240$, $p < 0.001$, respectively) 5.0 mg/kg produced significant increases in activity compared with 0.01 mg/kg NPA ($p < 0.05$).

Over the four testing days, there was a significant increase in sniffing for 5.0 mg/kg NPA treated 11-day-olds ($p < 0.05$); all other doses remained stable with EEDQ having no significant effect.

Significant three-way interactions between NPA, EEDQ, and day were present for the 17-day-old pups ($F(8,126) = 4.76$, $p < 0.001$) (See Figure 4). Significant two-way interactions for the 17-day-olds included EEDQ by day ($F(4,126) = 3.73$, $p < 0.001$), and NPA by day ($F(4,126) = 6.52$, $p < 0.007$). Significant interactions between EEDQ and NPA were found for 17-day-old rats on Days 1 and 2 ($F(4,63) = 5.462$, $p < 0.001$ and $F(4,63) = 7.253$, $p < 0.001$). On Day 1, the protected/EEDQ group had significantly more sniffing counts than the unprotected/EEDQ and unprotected/vehicle groups when treated with 0.01 mg/kg NPA ($p < 0.05$). Moreover, on Day 1, treatment with 5.0 mg/kg NPA produced significantly higher counts of sniffing for protected/EEDQ and unprotected/EEDQ groups, compared with the unprotected/vehicle group ($p < 0.05$). On Day 2, 0.01 mg/kg produced the least amount of sniffing in unprotected/vehicle pups, rather than pups in the other two conditions ($p <$

0.05).

On all three test days, saline-treated pups had significantly lower levels of sniffing in all three conditions; whereas 5.0 mg/kg NPA produced the highest counts, significantly higher than 0.01 mg/kg treated pups on Days 1 and 4 in all three conditions ($p < 0.05$). On Day 2, 5.0 mg/kg produced significantly more sniffing counts than 0.01 mg/kg in the unprotected/vehicle and protected/EEDQ conditions ($p < 0.05$). A significant increase from Day 1 to Day 2 was noted for pups given 0.01 mg/kg NPA in the unprotected/EEDQ condition ($p < 0.05$).

Summary. For 11-day-old pups, EEDQ did not appear to affect locomotor activity, as the protected/EEDQ and unprotected/EEDQ conditions reflected similar patterns of activity as the unprotected/vehicle condition. As in Experiment 1, on Days 2 and 4 saline-treated pups showed higher levels of activity than pups given either 0.01 or 5.0 mg/kg NPA. Sniffing of 11-day-olds was also unaffected by EEDQ treatment. As in Experiment 1, 5.0 mg/kg NPA-treated pups had higher levels of sniffing than 0.01 mg/kg NPA- or saline-treated pups.

Seventeen-day-old pups' locomotor activity and sniffing were affected by EEDQ treatment. When EEDQ inactivated D1 and D2 receptors in the unprotected/EEDQ condition, locomotor activity of NPA (0.01 mg/kg) treated pups was

enhanced on Day 2, as compared with the pups in protected/EEDQ condition. Both the unprotected/EEDQ and protected/EEDQ conditions had lower levels of activity on Day 4 after 0.01 mg/kg and 5.0 mg/kg NPA than did pups in the unprotected/vehicle condition. Sniffing data showed response patterns similar to Experiment 1, with 5.0 mg/kg NPA enhancing sniffing behavior significantly more than 0.01 mg/kg NPA. On Day 1, 5.0 mg/kg NPA produced higher sniffing counts in the two EEDQ conditions (protected and unprotected) than in the unprotected/vehicle condition; also on Day 2, pups given 0.01 mg/kg NPA showed higher sniffing counts when previously given EEDQ (protected and unprotected) than if given vehicle.

Discussion

In the present study, dose-dependent effects of NPA were established for sniffing and locomotor activity in both 11- and 17-day-old pups. In Experiment 1, locomotor activity of 17-day-olds was typically increased by NPA, with 0.01 mg/kg NPA producing the highest levels of activity. For 11-day-old pups, however, NPA generally decreased locomotor activity in comparison to saline-treated pups. Sniffing of 11- and 17-day-olds was enhanced in a dose-dependent fashion by NPA; 5.0 and 1.0 mg/kg produced higher counts of sniffing than lower doses of NPA or saline.

In general, results for 17-day-old pups are consistent with previous research, as NPA and quinpirole have been shown to increase locomotor activity (McDougall, Crawford, & Nonneman, 1992; McDougall et al., 1990). Moody and Spear (1992) have shown that quinpirole increases forward locomotor activity in all ages including 10- and 21-day-old pups; whereas, SKF 38393-induced increases in locomotor activity which were most pronounced for 3- and 21-day-old pups and sniffing was first observed at 10-days-old. Combinations of quinpirole and SKF 38393 maximally increased locomotor activity in 10-day-olds. Researchers have also shown locomotor activity and sniffing to increase in 11-day-olds in response to quinpirole and the nonselective dopamine agonist, apomorphine (McDougall et al., 1990; Moody & Spear,

1992; Shalaby & Spear, 1980). Importantly, these results are in contrast with the results of Experiment 1, as NPA was shown to have no effect on the locomotor activity of 11-day-old pups (See Figure 1). It is likely that this discrepancy is due to potency considerations, as NPA is much more potent than apomorphine or quinpirole. Thus, it is possible that a lower dosage is necessary to produce an increase in locomotor activity in 11-day-old pups.

The present results indicate that lower levels of NPA, compared to higher levels, increase locomotor activity in 17-day-olds. In general, previous findings conflict with this result. McDougall, Crawford, and Nonneman (1992) found increased locomotor activity with higher doses of NPA (1.0 compared to 0.1 mg/kg), but locomotor activity was defined differently in that study and probably more reflects general stereotyped behavior. Other studies have also shown that higher doses of D1 and D2 agonists increase locomotor activity in younger animals. For example, larger doses of SKF-38393 and quinpirole typically increase forward locomotion for 3-, 10-, and 21-day-old pups (Moody & Spear, 1992), and for 11- and 17-day-olds (McDougall et al., 1990). Of course, these selective agonists are not as potent as NPA. Consistent with the present results, increasing doses of apomorphine were shown to decrease locomotor movements in younger animals (Shalaby & Spear, 1980).

The dose-dependent increase in NPA-induced stereotyped sniffing reported here is consistent with past research, as Moody and Spear (1992) found increased sniffing with higher doses of SKF-38393 in 10-day-olds. Shalaby and Spear (1980) also reported increased sniffing for 7- and 14-day-old pups with increased apomorphine doses. Sniffing also increases in 11-day-olds in response to quinpirole (McDougall, Crawford, & Nonneman, 1992). Stereotyped sniffing requires both D1 and D2 receptor activation, suggesting that both D1 and D2 receptors were fully activated for 11- and 17-day-old pups.

Adult studies have shown that low levels of dopamine agonists decrease locomotor activity (Arnt, 1987), possibly due to autoreceptor activation; whereas, activation of postsynaptic D1 and D2 receptors with higher doses of dopamine agonists increase locomotor activity and sniffing in rats (Arnt, 1987; Clark & White, 1987). It has been suggested that autoreceptors do not develop in the rat until after PD 35 (Shalaby & Spear, 1980). This would indicate that low doses of dopamine agonists should increase locomotor activity in younger rats. The present results are consistent with the hypothesis that autoreceptors are not present in 11- or 17-day-old rat pups. Similar to adults, both 11- and 17-day-olds showed enhanced sniffing behavior with higher doses of NPA, indicating that dopamine agonist-

induced stereotyped behaviors are similar for younger rats as well as adults.

In Experiment 2, EEDQ inactivation of D1 and D2 receptors was behaviorally assessed for 11- and 17-day-old rat pups using NPA. In general, 11- and 17-day-old pups did not display adult typical responses to EEDQ, as NPA-induced behavior was not inactivated by EEDQ. McDougall, Crawford, and Nonneman (1992) found similar results for 17-day-old pups. Although 11-day-olds do not show full receptor recovery after 8 days as do 17-day-olds, no evidence showed that EEDQ affected behaviors. Both locomotor activity and sniffing patterns of the pups were similar in both Experiments.

EEDQ pretreatment paradoxically enhanced some of the behaviors of 17-day-old pups. When D1 and D2 receptors were inactivated by EEDQ (the unprotected/EEDQ condition) locomotor activity was enhanced on Day 2 by 0.01 mg/kg NPA, compared with pups whose D1 and D2 receptors were left intact (protected/EEDQ). Sniffing behavior was also enhanced by both EEDQ conditions (protected and unprotected), as 5.0 mg/kg NPA produced higher sniffing counts on Day 1, compared with the unprotected/vehicle condition; 0.01 mg/kg NPA also produced increased activity on Day 2 in both EEDQ pretreatment conditions. A similar enhancement of locomotor activity after EEDQ induced

receptor inactivation has previously been reported (McDougall, Crawford, & Nonneman, 1992). One possible explanation is that D1 and D2 receptors recover faster in young rats than adults. This possibility appears plausible, since locomotor activity enhancement did not occur until day 2. Although the absence of this effect on Day 4 is difficult to explain. This possibility also cannot explain the EEDQ-induced enhancement of sniffing on Day 1. Another possibility is that the D1 and D2 receptors still functioning after EEDQ inactivation are sensitized by EEDQ and become hyper-responsive to NPA. Thus, the receptors remaining may still be able to fully mediate behavior. Although EEDQ inactivation of dopamine receptors is greater for adult rats than pups (Crawford et al., 1992; Crawford et al., 1991), increases in sniffing behavior two days after EEDQ treatment have been reported in adult rats (Meller et al., 1989). Meller et al. suggest that the enhanced sniffing may be the result of a sufficient number of dopamine receptors being available for sniffing behavior without competing behaviors interfering.

A number of adult studies indicate that EEDQ treatment results in a loss of responses to dopamine D2 agonists (Arnt & Hyttel, 1989; Arnt et al., 1988; Hamblin & Creese, 1983; Meller et al., 1989). The D2 agonist, quinpirole had no effect on stereotyped behaviors after EEDQ treatment,

indicating a sufficient depletion of D2 receptors in the adults (Arnt et al., 1988). McDougall, Crawford, and Nonneman (1992) also found EEDQ treatment to inhibit NPA-induced increases in locomotor activity and decreases in grooming behavior for at least two days in 90-day-old rats.

A number of possibilities exist for the age-dependent behavioral differences in response to EEDQ treatment. One possibility is that dopamine receptors may be unaffected by EEDQ in the young rat. However, Crawford et al. (1992) found binding sites for 17-day-old-rats to be approximately 69% when compared with adult rats with an 86% depletion rate. This amount of inactivation should be sufficient to disrupt behavior in the younger animals (McDougall, Crawford, & Nonneman, 1992). Similar depletion rates are found for 11-day-old pups in comparison to 17-day-olds, and in contrast full recovery did not occur for the 11-day-olds even after eight days (Crawford et al., 1991). Even after 24 hours, receptor inactivation is still apparent in the younger rats, although they do show faster recovery rates than older rats (Crawford et al., 1992; Crawford et al., 1991; Leff et al., 1984).

Another possibility is that the young rat may have a receptor reserve sufficiently large enough to compensate for the loss of D1 and D2 receptors. Arnt et al. (1988) suggests that a receptor reserve may exist for D1 mediated

behaviors, thus explaining the lack of effect of EEDQ treatment on those behaviors. However, it is unlikely that 11- and 17-day-old pups have an overabundance of D1 and D2 receptors, since these receptors are still below adult levels (Murrin & Zeng, 1986; Rao et al., 1991; Rowlett et al., 1989; Zeng et al., 1988). A receptor reserve hypothesis also does not account for the 0.01 mg/kg NPA-induced increases in locomotor (in the unprotected/EEDQ condition) and sniffing (in both EEDQ conditions) in 17-day-olds on Day 2; or the 5.0 mg/kg NPA-induced increase in sniffing on Day 1 for the protected and unprotected/EEDQ conditions, since these findings were not seen across all days.

A last possibility is that dopamine receptors in the young rat may be sensitized by EEDQ treatment, thus compensating for those destroyed. Some results from Experiment 2 can best be explained by this possibility, since EEDQ produced higher activity levels when D1 and D2 receptors were inactivated on Day 2 for 17-day-old pups. However, the general inability of EEDQ to inhibit NPA-induced behaviors is still unexplained. It may be that postsynaptic mechanisms are insufficient for explaining this phenomenon, and EEDQ is acting at presynaptic sites to produce these behaviors. Although it is known that DOPAC levels are elevated in adult rats, but not rat pups, treated

with EEDQ, few studies have assessed the possibility that presynaptic sites may be responsible for the ontogenetic differences in EEDQ-induced responses.

For both Experiments 1 and 2, day-dependent increases in locomotor activity were generally observed for 17-day-old pups treated with NPA. Only for the unprotected/EEDQ condition did activity significantly decrease with 0.01 mg/kg NPA from Day 2 to Day 4. Interestingly, 11-day-olds in Experiment 1 showed no increase in activity over days with NPA treatment, although in Experiment 2 a significant increase in activity from Day 1 to Day 2 was noted for 0.01 mg/kg NPA. Sniffing behavior showed day-dependent increases, as 11- and 17-day-olds in Experiment 1 exhibited general increases in sniffing over testing days with all doses of NPA. In Experiment 2, 11-day-olds in the 5.0 mg/kg NPA group showed day-dependent increases in sniffing; whereas, 17-day-olds showed this increase in the unprotected/vehicle condition only. As with locomotor activity, the unprotected/vehicle pups had increased levels of sniffing from Day 1 to Day 2 with 0.01 mg/kg NPA, which then decreased on Day 4; this was also seen in the protected/EEDQ condition.

In Experiment 1, a drug-sensitization effect appears to be occurring for the 17-day-old pups in both locomotor activity and sniffing, and for 11-day-old sniffing

responses. Although some researchers have argued that sensitization does not occur in rats under PD 21 to amphetamine (Kolta et al., 1990), others have observed sensitization-like effects with NPA treatment in preweanling rat pups (McDougall, Crawford, & Nonneman, 1992). Performance of the 17-day-olds in Experiment 2 is also consistent with drug-sensitization. The decrease in locomotor activity and sniffing on Day 4 for NPA (0.01 mg/kg) treated pups in the unprotected/vehicle condition, may suggest D1 and D2 receptors have been sensitized by EEDQ, making it impossible for NPA to induce its normal sensitizing effects.

EEDQ has been shown to inactivate receptor types other than dopamine D1 and D2, including: alpha-adrenergic, serotonin and GABA receptors (Meller, Bohmaker, Goldstein, & Friedhoff, 1985; Miller, Lumpkin, Galpern, Greenblatt, & Shader, 1991). By selectively protecting dopamine receptor subtypes with D1 and D2 antagonists, behavioral effects due to nondopaminergic receptors are assumed to be equal between protected and unprotected conditions (Hamblin & Creese, 1983; Henry et al., 1987; Leff et al., 1984; Meller et al., 1989). Dopamine receptor subtypes D1 and D2 were selectively protected by using sulpiride and SCH 23390 in the current study, thus allowing dopaminergic and nondopaminergic effects to be separated. As seen on Day 2,

17-day-old pups were significantly less active in the protected/EEDQ condition after 0.01 mg/kg NPA treatment, as compared with the unprotected conditions, suggesting nondopaminergic processes affected locomotor activity in the older pups.

In summary, low doses of NPA increased locomotor activity in 17-day-old pups; however, 11-day-old pups displayed lower activity levels with NPA. It is possible that 11-day-olds require a lower dose of NPA than those tested here to display increases in locomotor activity. Sniffing behavior was enhanced for both 11- and 17-day-olds, with 5.0 mg/kg producing the most pronounced effect. EEDQ pretreatment did not produce an adult-typical response in either 11- or 17-day-old pups; and in some cases produced an enhanced effect in 17-day-olds. These results suggest that either dopamine receptors recover faster in young animals or that a sufficient numbers of dopamine receptors were left intact after EEDQ treatment to mediate these behaviors.

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