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Ontogenetic Differences in the Effects of EEDQ on Dopamine-Mediated Behaviors

MONJA MESTLIN AND SANDERS A. McDOUGALL[1]

Department of Psychology, California State University, San Bernardino, CA 92407

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MESTLIN, M. AND S. A. McDOUGALL. *Ontogenetic differences in the effects of EEDQ on dopamine-mediated behaviors.* PHARMACOL BIOCHEM BEHAV **45**(4) 797–802, 1993.—Previous results suggest that 17-day-old rat pups may have substantial reserves of *both* D_1 and D_2 receptors. To assess this possibility, the behavioral effects of a nonselective dopamine (DA) agonist, R-propylnorapomorphine (NPA), were measured in 11- and 17-day-old rat pups previously treated with the irreversible DA receptor antagonist *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ). Rat pups were treated with EEDQ (7.5 mg/kg) either alone or in combination with the D_1 and D_2 antagonists, SCH 23390 (1.0 mg/kg) and sulpiride (100 mg/kg), respectively. (The SCH 23390 and sulpiride were used to protect dopamine receptors from EEDQ-induced inactivation.) NPA's effects on stereotyped sniffing and locomotor activity were then assessed 1, 2, and 4 days after EEDQ pretreatment. Results showed that NPA (0.01, 0.1, 1.0, or 5.0 mg/kg) produced a dose-dependent increase in the stereotyped sniffing of both aged rats. Unexpectedly, however, EEDQ did not disrupt the NPA-induced stereotyped sniffing of either the 11- or 17-day-old rat pups. Thus a behavior (i.e., stereotyped sniffing) that requires the activation of a large complement of DA receptors was not sensitive to the receptor-depleting actions of EEDQ. Moreover, the behaviors of 11-day-old rats, which have fewer DA receptors than older pups or adults, were also not susceptible to the effects of EEDQ. When taken together, these results suggest that EEDQ's inability to block the agonist-induced behaviors of preweanling rat pups cannot be explained by ontogenetic changes in DA receptor reserves.

EEDQ Dopamine D_1 and D_2 receptors NPA Sniffing Ontogeny

DOPAMINE (DA) D_1 and D_2 receptors mediate unlearned behaviors in both preweanling and adult rats. Selective activation of D_2 receptors by quinpirole or pergolide increases the locomotor activity, yawning, and rearing of adult rats; whereas, selective activation of D_1 receptors by SKF 38393 increases grooming and repetitive jaw movements (1,7,28). Simultaneous activation of both D_1 and D_2 receptors by the nonselective agonists apomorphine and R-propylnorapomorphine (NPA) induces stereotyped behaviors, including stereotyped sniffing and decreased grooming in adult rats (1,7). In general, DA agonist drugs have similar actions across ontogeny, as both young and adult rats show increased grooming after SKF 38393 and increased locomotor activity after quinpirole and NPA (18–20).

Recently, irreversible receptor antagonism has been used as a tool to further elucidate the role of DA receptors in behavior. For example, results indicate that some of the behaviors induced by DA agonists are maintained even after a substantial percentage of DA receptors are inactivated (3, 12,28,31). Although controversial, these results may best be explained by the presence of a DA receptor reserve, as reserve receptors could compensate for receptors inactivated by drug treatment (3,22,28,31). In adult rats, a receptor reserve has been postulated for D_1 receptors, but not D_2 receptors, because only the actions of D_1 agonists are unaffected by irreversible receptor antagonism (3,22,24,28,31). For example, the D_1 agonist SKF 38393 produces a full repetitive jaw movement response in adult rats, even after 70–80% of their D_1 receptors were inactivated by the irreversible DA antagonist, *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) (28). In contrast, behavioral effects normally induced by D_2 agonists are not apparent in adult rats pretreated with EEDQ—thus indicating the absence of a D_2 receptor reserve (3,17,19,23–25).

Unexpectedly, evidence consistent with the presence of both a D_1 and D_2 receptor reserve has been found using the preweanling rat. For example, 17-day-old rat pups treated with moderate (7.5 mg/kg) or high (15 mg/kg) doses of EEDQ showed normal increases in locomotor activity after challenge with NPA or quinpirole, and normal increases in grooming after challenge with SKF 38393 (19,20). Thus both D_1 and D_2 agonists produced normal behavioral effects in spite of EEDQ treatment: results consistent with the presence of a D_1 and a D_2 receptor reserve. This receptor reserve explanation is not consistent with the majority of receptor binding studies, however, because D_1 and D_2 receptors increase linearly across development and do not reach maturity until approximately 60 days of age [(27,32); but see (13,16)]. Thus when considered

[1] To whom requests for reprints should be addressed.

in terms of receptor numbers, a D_2 receptor reserve should be less, rather than more, likely in the preweanling rat.

The present study, therefore, will attempt to determine whether a receptor reserve hypothesis can account for EEDQ's inability to affect the DA agonist-induced behaviors of preweanling rat pups. To that end, the stereotyped sniffing and locomotor activity of NPA-treated 17-day-old rat pups was assessed after EEDQ pretreatment. Full expression of the sniffing response requires greater activation of both D_1 and D_2 receptors than does locomotor activity (3,4,12). Thus stereotyped sniffing should be more sensitive than locomotor activity to the behavior disrupting effects of EEDQ. In addition, the sniffing and locomotor activity of 11-day-old rat pups was also determined. Rats at this age have significantly fewer D_1 and D_2 receptors than older pups or adults (16,27,32), making it unlikely that the 11-day-old has an extensive D_2 receptor reserve. If this hypothesis is correct, 11-day-olds should be more sensitive than 17-day-olds to the behavioral effects of EEDQ. In the first experiment, the effects of various doses of NPA (0.01–5.0 mg/kg) on the locomotor activity and sniffing of 11- and 17-day-old rat pups was assessed across a 4 day span.

METHOD

Animals

Subjects were 224 male and female rat pups of Sprague-Dawley descent (Harlan). Litters were culled to a maximum of 10 pups or a minimum of 8 pups at 3 days of age. Pups were kept with the dam throughout behavioral testing. Assignment of subjects was random according to gender and within each litter. The colony room was maintained at 23–25°C and kept under a 14L : 10D cycle. Behavioral testing was conducted during the light phase of the cycle.

Drugs

All drugs were injected IP 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. NPA was dissolved in saline and EEDQ was dissolved in 95% ethanol : distilled water (1 : 4). Sulpiride, SCH 23390, and NPA (R-propylnorapomorphine) were acquired from Research Biochemicals Inc. (Natick, MA). EEDQ (*N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline) was acquired from Sigma (St. Louis, MO).

Apparatus

Behavioral testing was done in grey plywood chambers (25 × 25 × 18 cm) with the floors divided by lines into four equal quadrants. The testing chambers were housed in a large glass-topped incubator maintained at 31 ± 1°C, which is approximate thermoneutrality for 10- to 20-day-old rat pups (8).

Procedure

Experiment 1. In the first experiment (n = 8), behaviors of 11- and 17-day-old rat pups (age at initial testing) were assessed across a 4-day span. On each test day, pups were habituated to the testing apparatus for 20 min. After habituation, rat pups were injected with either saline or NPA (0.01, 0.1, 1.0, or 5.0 mg/kg) and placed immediately back into the testing apparatus. After 5 min, the locomotor activity (line crossings) and stereotyped (head down) sniffing of the pups was assessed during a 20-min testing session. Line crossings were measured for the entire 20 min; whereas, the occurrence of stereotyped sniffing was counted every 20 s using a time-sampling procedure. Both sniffing and line crossings were assessed by a single observer blind to treatment conditions. The drug-induced behaviors of the same pups were assessed again both 1 and 3 days after initial agonist treatment. For example, an 11-day-old rat pup given 5.0 mg/kg NPA would receive the same dose of NPA when 12 and 14 days old.

Experiment 2. In a second experiment (n = 8), 10- and 16-day-old rat pups were randomly placed into a DA receptor protected or nonprotected condition. In the protected condition, pups were given an initial injection of the D_2 antagonist sulpiride (100 mg/kg) followed, 30 min later, by an injection of the D_1 antagonist SCH 23390 (1.0 mg/kg). The nonprotected pups received two injections of vehicle. Thirty minutes after the second injection, rat pups were injected with EEDQ (7.5 mg/kg) or its vehicle. The 7.5 mg/kg dose of EEDQ was appropriate, because a greater dose (15.0 mg/kg) of EEDQ did not produce enhanced behavioral or receptor binding effects in 17-day-old rat pups (9,19). Only nonprotected rat pups were given vehicle, because we have previously shown that DA receptor protection (i.e., SCH 23390 and sulpiride) does not significantly affect the behavior of non-EEDQ-treated animals (19). Pretreatment with D_1 and D_2 antagonists was used to control for EEDQ's effects on nondopaminergic systems (2,4,14,19–21,28).

For each rat pup, behavioral testing occurred 1, 2, and 4 days after initial drug treatments. On each of these test days, pups were habituated to the testing apparatus for 20 min. After habituation, pups were injected with either saline or NPA (0.1 or 5.0 mg/kg) and behaviors were assessed as in Experiment 1. Each rat pup received only one drug sequence (e.g., sulpiride/SCH 23390 and EEDQ followed by three daily injections of 5.0 mg/kg NPA).

Statistics

Analyses of variance (ANOVAs) with repeated measures were used for statistical analysis of line crossing and stereotyped sniffing data. ANOVAs were performed across the 3 test days and were supplemented, when appropriate, by Newman-Keuls tests ($p < 0.05$).

RESULTS

Experiment 1

Line crossings. The mean number of line crossings for the 11- and 17-day-old rat pups are presented in Fig. 1. Overall, 11-day-old pups given NPA had significantly fewer line crossings than pups given saline, $F(4, 35) = 5.38$, $p < 0.01$. This effect varied across test days, as there was no difference between the various groups of 11-day-olds when tested on days 1 and 2. On day 4, the NPA-treated 11-day-olds had significantly fewer line crossings than their saline controls, $F(8, 70) = 7.87$, $p < 0.001$. This difference was due to a substantial day-dependent increase in the line crossings of the saline-treated pups and a complete lack of any day-dependent changes in the NPA-treated 11-day-olds.

As can be seen in Fig. 1, the 17-day-old rat pups showed a completely different pattern of responding after NPA treatment. For example, 17-day-olds given the lowest dose of NPA (0.01 mg/kg) had significantly more line crossings than all other groups, $F(4, 35) = 12.07$, $p < 0.001$. Pups given the three higher doses of NPA (0.1, 1.0, and 5.0 mg/kg) were

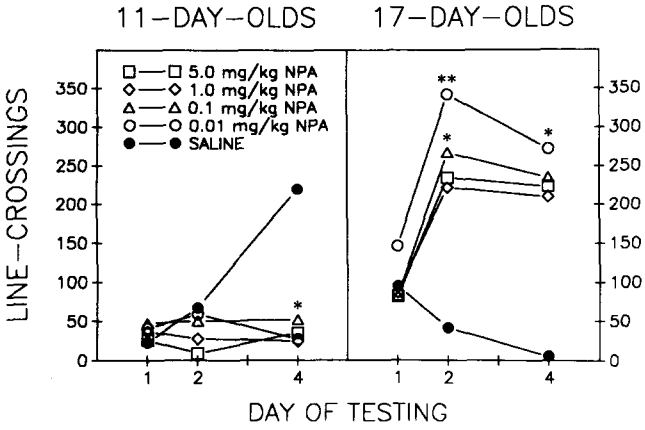

FIG. 1. Mean line crossings of 11- and 17-day-old rat pups (age at initial testing) injected IP with saline or NPA (0.01, 0.1, 1.0, or 5.0 mg/kg) immediately after a 25-min habituation period. The testing sessions lasted 20 min and occurred 5 min after drug injections. Agonist-induced responding of the same pups was assessed across a 4-day span. **Indicates a significant difference from the saline and low-dose (0.01 and 0.1 mg/kg) NPA groups ($p < 0.05$). *Indicates a significant difference from the saline group ($p < 0.05$).

also significantly more active than the saline controls, but not to the same extent as the 0.01 NPA group. The effects of drug treatment varied according to test day, $F(8, 70) = 6.39, p < 0.001$. For example, on day 1, the various groups of 17-day-olds did not differ; however, on day 2, all of the NPA groups were more active than the saline group, with pups given 0.01 mg/kg NPA being the most active. By day 4, none of the NPA groups differed among themselves, but all had more line crossings than the saline-treated pups. When assessed across test days, the saline-treated 17-day-olds showed a progressive

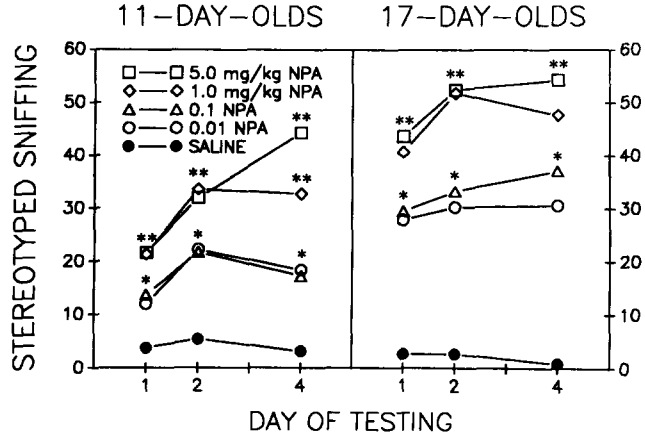

FIG. 2. Mean stereotyped sniffing of 11- and 17-day-old rat pups. The occurrence of stereotyped sniffing was measured every 20 s using a time-sampling procedure. Drugs and testing conditions were the same as described for Fig. 1. **Indicates a significant difference from the saline and low-dose (0.01 and 0.1 mg/kg) NPA groups ($p < 0.05$). *Indicates a significant difference from the saline group ($p < 0.05$).

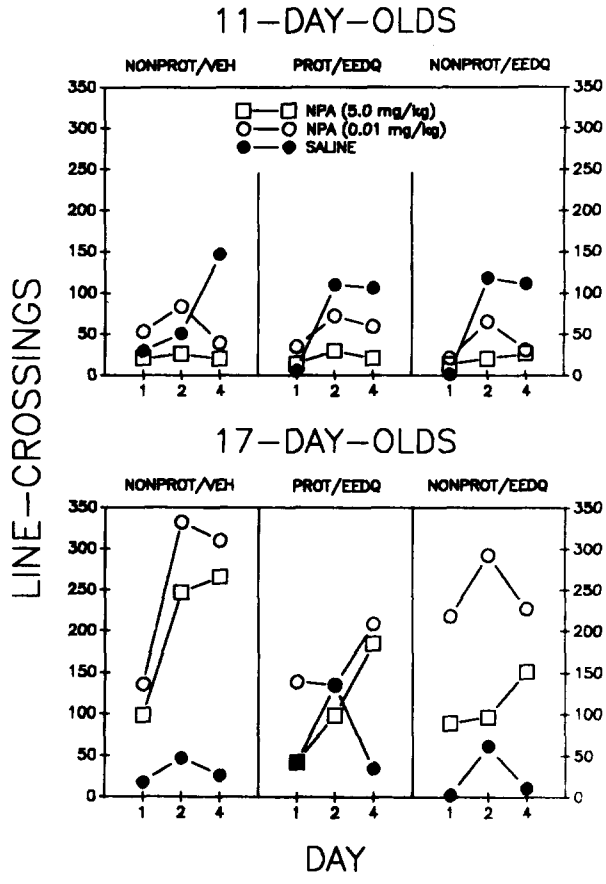

FIG. 3. Mean line crossings of 11- and 17-day-old rat pups injected IP with saline or NPA (0.01 or 5.0 mg/kg) 5 min prior to testing. The testing sessions lasted 20 min and occurred 1, 2, and 4 days after pretreatment with EEDQ (7.5 mg/kg) or its vehicle (VEH). Prior to EEDQ or vehicle pretreatment rats were given injections of both 100 mg/kg sulpiride and 1.0 mg/kg SCH 23390 (the protected condition; PROT) or two vehicle injections (the nonprotected condition; NONPROT). The sulpiride and SCH 23390 were used to protect D_1 and D_2 receptors from EEDQ-induced receptor inactivation. Significant main effects and interactions are presented in the Results section.

decrease in line crossings. In contrast, all of the NPA-treated pups showed a substantial increase in line crossings from day 1 to day 2, $F(8, 70) = 6.39, p < 0.001$.

Stereotyped sniffing. Mean occurrences of stereotyped sniffing for the 11- and 17-day-old rat pups are presented in Fig. 2. Overall, NPA-treated 11-day-olds sniffed significantly more than their saline controls, $F(4, 35) = 53.37, p < 0.001$. This effect varied according to dose, as 11-day-olds injected with the two greater doses of NPA (1.0 and 5.0 mg/kg) sniffed significantly more than pups given 0.01 or 0.1 mg/kg NPA. This dose-dependent effect was apparent on all test days; moreover, on day 4, 11-day-olds given 5.0 mg/kg NPA sniffed significantly more than pups given 1.0 mg/kg NPA, $F(8, 70) = 5.30, p < 0.001$. There was a general increase in sniffing from days 1 to 2, $F(2, 70) = 26.92, p < 0.001$; but only the 11-day-olds treated with 5.0 mg/kg NPA showed a progressive increase in sniffing across all test days, $F(8, 70) = 5.30, p < 0.001$.

A similar pattern of responding was shown by the 17-day-olds (Fig. 2), as pups given the greater doses of NPA (1.0 and

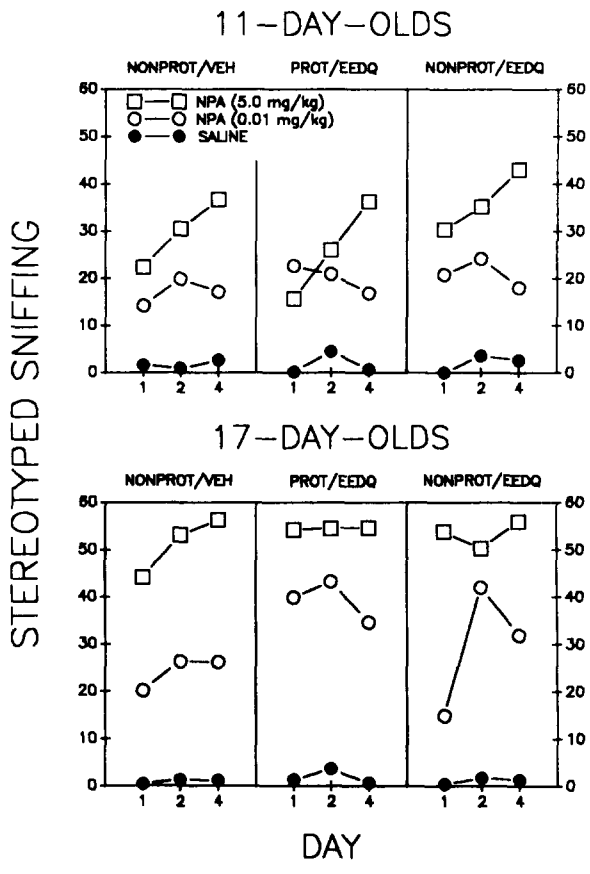

FIG. 4. Mean stereotyped sniffing of 11- and 17-day-old rat pups. Stereotyped sniffing was assessed every 20 s using a time-sampling procedure. Drugs and testing conditions were the same as described in Fig. 3. Significant main effects and interactions are presented in the Results section.

5.0 mg/kg) sniffed more than the saline controls, with the 0.01 and 0.1 mg/kg NPA groups being intermediate, $F(4, 35) = 81.58$, $p < 0.001$. The differences between the high-dose (1.0 and 5.0 mg/kg) NPA groups, the low-dose (0.01 and 0.1 mg/kg) NPA groups, and the saline controls was maintained on all test days, $F(8, 70) = 2.77$, $p < 0.05$. Overall, sniffing of the 17-day-olds increased across the first 2 test days, $F(2, 70) = 13.97$, $p < 0.001$; however, this day-dependent effect was apparent for only the pups given 1.0 or 5.0 mg/kg NPA, $F(8, 70) = 2.77$, $p < 0.05$.

Experiment 2

Line crossings. Mean line crossings of EEDQ- and vehicle-pretreated 11- and 17-day-old rat pups are presented in Fig. 3. Neither EEDQ or protection pretreatments affected the line crossings of the 11-day-old pups. Overall, 11-day-olds given NPA had significantly fewer line crossings than pups given saline, with pups given 5.0 mg/kg NPA being the least active, $F(2, 63) = 14.37$, $p < 0.001$. This pattern of responding varied across test days, because on day 1 the 11-day-olds given the lower dose of NPA (0.01 mg/kg) had significantly more line crossings than the other two groups, $F(4, 126) = 7.32$, $p < 0.001$. On day 2, groups given saline or 0.01 mg/kg NPA did not differ, but both had significantly more line crossings than the 5.0 mg/kg NPA group. By day 4, the saline-treated 11-day-olds were more active than either of the NPA groups. This pattern of responding was due to the progressive day-dependent increase in line crossings shown by the saline-treated 11-day-olds and the day-dependent increase and subsequent decline in responding shown by pups given 0.01 mg/kg NPA. Line crossings by the 5.0 mg/kg NPA group were stable across test days.

As can be seen in Fig. 3, 17-day-olds given 0.01 mg/kg NPA had significantly more line crossings than pups given 5.0 mg/kg NPA, with the saline controls being the least active, $F(2, 63) = 35.53$, $p < 0.001$. The effects of EEDQ and protection pretreatment on the line crossings of 17-day-olds varied according to both agonist treatment and test day, condition × agonist interaction, $F(4, 63) = 3.11$, $p < 0.05$; condition × day interaction, $F(4, 126) = 2.59$, $p < 0.05$; agonist × day interaction, $F(4, 126) = 4.14$, $p < 0.01$; condition × agonist × day interaction, $F(8, 126) = 2.01$, $p < 0.05$. For example, on day 1, 17-day-olds given 0.01 mg/kg NPA had significantly more line crossings than their saline controls in all pretreatment conditions. EEDQ did not affect the responding of the 17-day-olds on this test day, because NPA- and saline-treated pups in the nonprotected/EEDQ (NONPROT/EEDQ) and nonprotected/vehicle (NONPROT/VEH) conditions did not differ. In contrast, protection pretreatment did affect responding slightly on Day 1, as protected pups given 5.0 mg/kg NPA were not more active than pups given saline. On day 2, the line crossings of 17-day-olds given 0.01 mg/kg NPA were again not affected by EEDQ pretreatment; however, pups given the greater dose of NPA (5.0 mg/kg) were less active if previously treated with EEDQ. The various groups of pups in the protected/EEDQ (PROT/EEDQ) condition did not vary amongst themselves on day 2. On day 4, all NPA-treated pups had significantly more line crossings than their saline controls regardless of pretreatment condition. When assessed across test days it was apparent that NPA-treated pups in the NONPROT/VEH condition showed a substantial increase in line crossings from days 1 to 2. Pups given EEDQ did not show any significant day-dependent changes in activity.

Stereotyped sniffing. Mean occurrences of stereotyped sniffing for the EEDQ- and vehicle-pretreated 11- and 17-day-old rat pups are presented in Fig. 4. Overall, NPA produced a dose-dependent increase in the sniffing of 11-day-old rat pups, $F(2, 63) = 116.58$, $p < 0.001$. This effect varied according to test day, as 11-day-olds given 5.0 mg/kg NPA showed a day-dependent increase in sniffing; whereas, pups treated with 0.01 mg/kg NPA or saline maintained a stable level of responding across test days, $F(4, 126) = 8.60$, $p < 0.001$. Neither EEDQ or protection pretreatment affected the stereotyped sniffing of the 11-day-old rat pups.

As with the younger pups, 17-day-olds showed a dose-dependent increase in stereotyped sniffing, $F(2, 63) = 595.81$, $p < 0.001$ (Fig. 4). Pretreatment condition affected the sniffing of 17-day-olds given the greater dose of NPA (5.0 mg/kg) on day 1 only, as pups in the PROT/EEDQ or NONPROT/EEDQ conditions sniffed significantly more than similarly treated pups in the NONPROT/VEH condition, $F(8, 126) = 4.76$, $p < 0.001$. The sniffing of 17-day-olds given the lower dose of NPA (0.01 mg/kg) varied according to test day. For example, on day 1, the sniffing induced by 0.01 mg/kg NPA was greatest in pups from the PROT/EEDQ condition. On days 2 and 4, NPA (0.01 mg/kg) treated pups from the PROT/

EEDQ and NONPROT/EEDQ conditions sniffed significantly more than pups from the NONPROT/VEH condition.

GENERAL DISCUSSION

Previous results suggest that preweanling rat pups may have substantial reserves of both D_1 and D_2 receptors (19,20). In the present study this possibility was assessed by measuring the NPA-induced stereotyped sniffing and locomotor activity of EEDQ-pretreated 11- and 17-day-old rat pups. The results showed that NPA (0.01, 0.1, 1.0, and 5.0 mg/kg) induced a dose-dependent increase in the stereotyped sniffing of the 11- and 17-day-olds (Fig. 2). In contrast, NPA did not increase the locomotor activity of the 11-day-olds, but did increase the activity of the older pups (Fig. 1). Importantly, EEDQ pretreatment did not disrupt NPA's actions on either the stereotyped sniffing or the locomotor activity of the 11- or 17-day-old rats (Figs. 3 and 4). The one exception was that the 17-day-olds pretreated with EEDQ did not show the usual progressive day-dependent increases in locomotor activity after high dose (5.0 mg/kg) NPA treatment (Fig. 3, bottom right panel). The importance of this finding is unclear, because a lower dose (0.01 mg/kg) of NPA did induce a day-dependent increase in activity. In general, protection pretreatment had few effects on behavior, although the locomotor activity, but not the stereotyped sniffing, of the 17-day-old pups was depressed by SCH 23390 and sulpiride.

Previous studies have shown that the NPA- and quinpirole-induced locomotor activity of 17-day-old rat pups is unaffected by EEDQ pretreatment (19,20). One possibility is that a D_1 and a D_2 receptor reserve was responsible for the maintenance of these behaviors after receptor inactivation. Contrary to this hypothesis, the present results showed that the stereotyped sniffing of the 17-day-old rat pups was not affected by EEDQ. This was unexpected because stereotyped sniffing requires substantial activation of D_1 and D_2 receptors (3,4,12) and, thus, should have been very sensitive to the receptor inactivating effects of EEDQ. For a receptor reserve explanation to account for these results the existence of very large D_1 and D_2 receptor reserves would have to be postulated: a possibility not consistent with the adult rat literature (2, 19,23,25). Moreover, EEDQ did not diminish the NPA-induced stereotyped sniffing of 11-day-old rat pups. This finding is also contrary to a receptor reserve explanation, because a substantial D_1 and D_2 reserve is unlikely at an age that has relatively fewer D_1 and D_2 receptors (16,27,32). Therefore, when taken together, these results suggest that EEDQ's inability to block the agonist-induced behaviors of preweanling rat pups cannot be fully accounted for by ontogenetic changes in DA receptor reserves.

An adequate explanation for the ontogeny of EEDQ's actions remains unavailable; however, it is unlikely that age-dependent changes in drug efficacy are responsible for EEDQ's inability to affect behavior. For example, [^{3}H]-SCH 23390 and [^{3}H]-spiperone receptor binding assays have shown that a 7.5 mg/kg dose of EEDQ inactivates a substantial percentage of striatal D_1 (69%) and D_2 (61%) receptors in 17-day-old rat pups (9). It is also unlikely that an insufficient dose of EEDQ was used in the present study (i.e., that behavioral disruption would have been observed at greater doses), as 7.5 and 15.0 mg/kg EEDQ produce similar amounts of striatal D_1 and D_2 receptor depletion in preweanling rat pups (11). Moreover, the behaviors of 17-day-old rat pups are not differentially affected by moderate (7.5 mg/kg) or large (15.0 mg/kg) doses of EEDQ (19). Therefore, it remains uncertain why EEDQ does not diminish the agonist-induced behaviors of preweanling rat pups; however, presynaptic processes may be involved, because EEDQ is known to produce age-dependent changes in DOPAC levels and DA metabolism (9,10,15).

An interesting feature of these data is that the locomotor activity of the 17-day-old pups was maximally stimulated by lower (0.01 and 0.1 mg/kg), rather than higher (1.0 and 5.0 mg/kg), doses of NPA (Fig. 1). In the case of the 11-day-olds, NPA did not increase locomotor activity, but actually depressed activity (compared to saline controls) when assessed across test days. In contrast, NPA induced a dose-dependent increase in the stereotyped sniffing of both aged pups (Fig. 2). These results are not entirely consistent with past research, as previous studies have shown that apomorphine and quinpirole do not affect the sniffing of preweanling rat pups, but do have locomotor activating properties in rats as young as 4 days of age (6,18,26,30). The discrepancy between the present study using NPA and past studies using apomorphine and quinpirole may be due to differences in drug efficacy. Alternately, unlike apomorphine and quinpirole, NPA is a full agonist at both D_1 and D_2 receptors (22,29). This may be significant, because a pronounced activation of both D_1 and D_2 receptors is necessary for maximal sniffing (3,4,5,12).

The present results are consistent with a general framework of stereotypy mentioned by Bordi et al. (3). These authors have suggested that DA-mediated behaviors are on a continuum: locomotor activity and rearing are induced after lower doses of a nonselective DA agonist (e.g., apomorphine or NPA); low-intensity stereotypies (e.g., stereotyped sniffing) predominate after moderate doses; and high-intensity stereotypies (e.g., vacuous oral movements) are observed after larger doses. Locomotor and rearing are inhibited after moderate and large doses, perhaps due to behavioral competition (3). This framework of stereotypy can be used to explain the present results, as the lack of locomotor activity in the 11-day-olds may have been due to NPA-inducing stereotyped sniffing at the expense of locomotor activity. Moreover, the finding that greater doses of NPA (1.0 and 5.0 mg/kg) induced maximal sniffing in the 17-day-olds, while the 0.01 mg/kg dose produced the most locomotor activity, is consistent with Bordi et al.'s (3) framework of stereotypy.

In conclusion, the present results show two interesting ontogenetic differences. First, the pattern of NPA's behavioral effects changed from 11 to 17 days of age. In the younger animal, NPA induced moderate levels of stereotyped sniffing and no locomotor activity; whereas, in the older pup, NPA preferentially induced locomotor activity at lower doses and stereotyped sniffing at higher doses. This is consistent with recent research which has shown that the behaviors induced by DA agonists change across development (26). Second, EEDQ did not affect the NPA-induced behaviors of 11- and 17-day-old rat pups, whereas it is well established that EEDQ blocks the D_2 agonist-induced behaviors of adult rats (2, 17,19,23-25). The present study suggests that differences between preweanling and adult rats are not due to large D_1 and D_2 receptor reserves in the younger animals. In the future, an accurate understanding of EEDQ's age-dependent effect may increase our knowledge of the ontogeny of DA receptor systems and how these systems mediate behavior.

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