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Paradoxical Effects of Kappa-Opioid Stimulation on the Locomotor Activity and Fos Immunoreactivity of the Preweanling Rat: Role of Dopamine Receptors

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The kappa-opioid agonist U-50,488 increases the locomotor activity of preweanling rats. The authors attempted to determine whether this effect was modulated by dopamine (DA) system functioning. Surprisingly, U-50,488's locomotor activating effects were attenuated by both the DA receptor antagonist flupenthixol and the DA receptor agonist *R*(–)-propylnorapomorphine (NPA). In order to determine those brain areas stimulated by U-50,488, Fos immunoreactivity was assessed in 17- and 80-day-old rats. U-50,488 not only enhanced the locomotor activity of the younger rats, but it also enhanced Fos expression in various brain areas, including the nucleus accumbens and medial striatum. NPA blocked U-50,488-induced Fos expression in the latter region. When considered together, these results indicate that U-50,488 does not increase locomotion by stimulating a DA mechanism. Instead, either agonizing or antagonizing DA receptors is sufficient to disrupt U-50,488's locomotor activating effects in the preweanling rat.

In general, preweanling rats exhibit adultlike behavior patterns when treated with dopamine (DA) receptor agonists and antagonists. For example, nonselective D_1–D_2 receptor agonists (e.g., apomorphine or *R*(–)-propylnorapomorphine, NPA) increase the locomotor activity, rearing, and sniffing of both preweanling and adult rats (Arnt, 1987; McDougall, Crawford, & Nonneman, 1993; Mestlin & McDougall, 1993; Shalaby & Spear, 1980). Selective DA receptor agonists also have adultlike behavioral effects in young rats. More specifically, SKF 38393 (a selective D_1 agonist) increases the locomotor activity and grooming of 10-, 17-, and 21-day-old rats, whereas quinpirole (a selective D_2 agonist) enhances locomotor activity and wall climbing (McDougall, Arnold, & Nonneman, 1990; McDougall et al., 1993; Moody & Spear, 1992). Reversible DA antagonists also affect rats similarly across ontogeny, as the quinpirole-induced locomotor activity of preweanling and adult rats is blocked by either a D_1 or D_2 receptor antagonist, whereas SKF 38393-induced grooming is blocked by a D_1 receptor antagonist (Arnt, 1987; Clark & White, 1987; McDougall et al., 1990). DA agonists and antagonists occasionally cause age-dependent behavioral differences; however, these differences usually are quantitative or involve the emergence of an age-specific response (Moody & Spear, 1992; Shalaby & Spear, 1980).

In contrast to DA-acting drugs, agonists at kappa-opioid receptors induce pronounced age-dependent behavioral differences. In adult rats and mice, kappa-opioid agonists (e.g., U-50,488, tifluadom, and bremazocine) attenuate locomotor activity, rearing, and grooming while producing both place and taste aversions (Di Chiara & Imperato, 1988; A. Jackson & Cooper, 1988; Mucha & Herz, 1985; Ukai & Kameyama, 1985). Conversely, these same kappa-opioid agonists markedly increase various unlearned behaviors of developing rats. More specifically, U-50,488 stimulates limb movements in fetal rats (Day 21 of gestation) and produces a dose-dependent increase in the locomotor activity and wall climbing of 5- to 20-day-old rats (Bolanos, Garmsen, Clair, & McDougall, 1996; Carden, Barr, & Hofer, 1991; Carden, Davachi, & Hofer, 1994; H. C. Jackson & Kitchen, 1989; Kehoe & Boylan, 1994; Smotherman, Moody, Spear, & Robinson, 1993). It is curious that the age-dependent paradoxical effects of U-50,488 appear to be confined to locomotor activity and wall climbing, because the analgesic and aversive properties of kappa-opioid agonists are similar in preweanling and adult rats (Bals-Kubic, Herz, & Shippenberg, 1989; Barr, Paredes, Erickson, & Zukin, 1986; Barr, Wang, & Carden, 1994; Mucha & Herz, 1985; Ohno, Yamamoto, & Ueki, 1992).

At present it is uncertain why kappa-opioid agonists cause behavioral activation in the preweanling rat, but it is possible that U-50,488 enhances locomotor activity by stimulating a

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dopaminergic mechanism. In adult rats, the kappa-opioid and DA systems have a generally antagonistic relationship, because U-50,488 inhibits DA release from the nucleus accumbens (Di Chiara & Imperato, 1988), reduces the firing rate of DA cells in the substantia nigra pars compacta (Walker, Thompson, Frascella, & Friederich, 1987), and attenuates cocaine-induced place preference conditioning and Fos immunoreactivity (Crawford, McDougall, Bolanos, Hall, & Berger, 1995). It is not known whether a similar antagonistic relationship exists during the preweanling period, but the fact that U-50,488 and NPA have similar behavioral profiles in the developing rat suggests that the relationship may be facilitatory rather than antagonistic. To examine this idea, preweanling rats were coadministered U-50,488 and either the nonselective D_1–D_2 receptor antagonist flupenthixol (Experiment 1) or the nonselective D_1–D_2 receptor agonist NPA (Experiment 2) prior to behavioral testing. We predicted that flupenthixol would attenuate, and NPA would potentiate, the U-50,488-induced locomotor activity of 17-day-old rats. In addition, we determined whether U-50,488 or NPA would stimulate Fos immunoreactivity in various brain regions of preweanling and adult rats (Experiment 3). Fos, the product of the early-response gene *c-fos,* is regionally expressed after acute or chronic treatment with a variety of drugs, and it has been used both as a marker of neuronal activity and as a tool for assessing the effects of drugs on neuronal mechanisms (Brown, Robertson, & Fibiger, 1992; Dragunow & Faull, 1989; G. S. Robertson & Fibiger, 1992; Sagar, Sharp, & Curran, 1988; Wang, Smith, & McGinty, 1995).

General Method

Subjects

Subjects were male and female rats of Sprague-Dawley descent (Harlan Sprague Dawley, Inc., Indianapolis, IN). Litters were culled to 10 rat pups at 3 days of age. Rats were tested at either 17 or 80 days of age, with no more than 1 rat from each litter being placed into a particular group. Preweanling rats were housed with their littermates and dam until testing, whereas postweanling rats were housed individually in standard wire mesh cages and given food and water ad lib. The colony room was maintained at 21–23 °C and kept under a 12-hr light–dark cycle. Behavioral testing was conducted during the light phase of the cycle.

Apparatus

Two gray plywood chambers (30 × 30 × 42 cm) were used for testing. The floors of the test chambers were divided by lines into four equal quadrants.

Drugs

R(–)-propylnorapomorphine hydrochloride (Research Biochemicals International), (±)-*trans*-U-50,488 methanesulfonate, and *cis*-(Z)-flupenthixol dihydrochloride were mixed in saline at a volume of 1 ml/kg for adult rats and 5 ml/kg for preweanling rats. U-50,488 was injected subcutaneously (sc), and flupenthixol was injected intraperitoneally (ip).

Behavioral Assessment

In all experiments, drug-induced behaviors were assessed during a single 60-min testing session. We assessed line crosses (a measure of forward locomotion) continuously across the testing session, whereas we assessed the occurrence of stereotyped (head down) sniffing every 20 s using a time-sampling procedure.

Statistics

Analyses of variance (ANOVAs) were used for the statistical analysis of the behavioral data. For the locomotor activity data, repeated measures ANOVAs were performed across six 10-min time blocks. The stereotyped sniffing data were collapsed across the testing session because no interactions involving time as a variable were apparent. In addition, the behavioral scores of male and female rats did not differ significantly, so analyses were collapsed across gender. When appropriate, Tukey tests ($p < .05$) were used for making post hoc comparisons.

Experiment 1

Unlike adults, preweanling rats show increased locomotor activity after U-50,488 treatment. To determine whether this U-50,488-induced locomotion is ultimately mediated through a dopaminergic mechanism, we treated 17-day-old rats with flupenthixol (a nonselective D_1–D_2 receptor antagonist) prior to U-50,488 treatment. Flupenthixol was expected to attenuate U-50,488-induced locomotor activity.

Method

Five groups of 17-day-old rats ($n = 8$ per group) were injected with saline or flupenthixol (0.025, 0.1, 0.4, or 0.8 mg/kg ip). Rats were returned to their home cages for 30 min and then injected (sc) with 5.0 mg/kg U-50,488 (a kappa-opioid agonist). An additional control group was given two injections of saline. We immediately placed rats in the testing apparatus and assessed line crosses and stereotyped sniffing for 60 min.

Results

Line crosses. Overall, U-50,488 increased the line crossing of saline-pretreated rats (see Figure 1): condition main effect, $F(5, 42) = 50.50$, $p < .001$, and Tukey tests, $p < .05$. Pretreatment with 0.8 mg/kg flupenthixol significantly attenuated U-50,488-induced locomotor activity on all six of the time blocks, whereas 0.4 mg/kg flupenthixol reduced the locomotor activity of the U-50,488-treated rats on only the last four time blocks: Condition × Time interaction, $F(25, 210) = 4.72$, $p < .001$, and Tukey tests, $p < .05$. Neither 0.025 nor 0.1 mg/kg flupenthixol affected U-50,488-induced locomotor activity.

Stereotyped sniffing. The number of stereotyped sniffing counts varied only slightly according to treatment condition, $F(25, 210) = 0.60$, $p > .05$, with the group means ranging from 0 to 1.63 sniffing counts.

Experiment 2

Flupenthixol attenuated U-50,488-induced locomotor activity in the 17-day-old rat. This finding suggests that

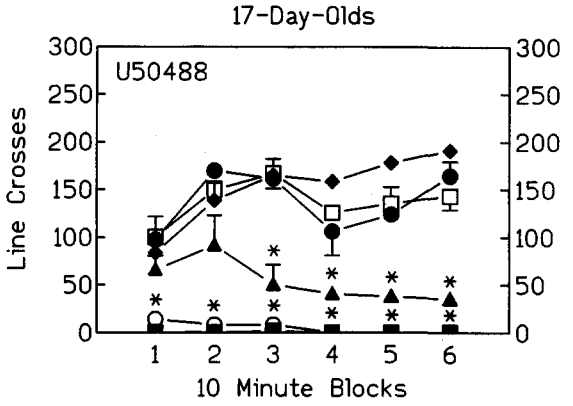

Figure 1. Mean number of line crosses during the 60-min behavioral testing session. The 17-day-old rats (n = 8 per group) were injected with saline or flupenthixol (0.025, 0.1, 0.4, or 0.8 mg/kg) 30 min prior to behavioral testing. Rats were then given saline or U-50,488 (5.0 mg/kg) immediately prior to testing (○ = saline/saline; □ = saline/U-50,488; ● = 0.025 mg/kg flupenthixol/U-50,488; ◆ = 0.1 mg/kg flupenthixol/U-50,488; ▲ = 0.4 mg/kg flupenthixol/U-50,488; ■ = 0.8 mg/kg flupenthixol/U-50,488). *Significantly different from the saline/U-50,488 group ($p < .05$).

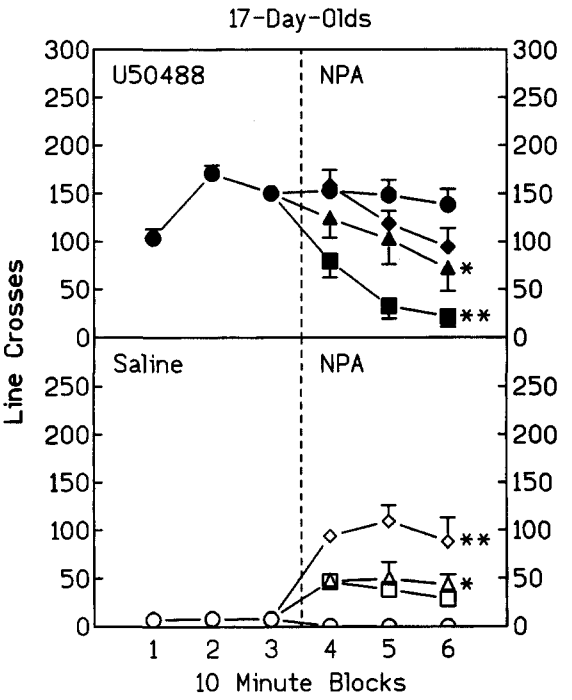

Figure 2. Mean number of line crosses during the 60-min behavioral testing session. The 17-day-old rats (n = 8 per group) were injected with saline or U-50,488 (5.0 mg/kg) immediately prior to behavioral testing. Rats were then injected (indicated by the dashed line) with saline or R(–)-propylnorapomorphine (NPA; 0.01, 0.1, or 1.0 mg/kg) 30 min into the testing session (circles = saline; diamonds = 0.01 mg/kg NPA; triangles = 0.1 mg/kg NPA; squares = 1.0 mg/kg NPA). *Significantly different from the saline-treated group (i.e., the circles) receiving the identical pretreatment ($p < .05$). **Significantly different from all other groups receiving the identical pretreatment ($p < .05$).

activation of kappa-opioid receptors increases locomotor activity by modulating a dopaminergic mechanism. Alternatively, it is possible that flupenthixol decreased U-50,488-induced locomotor activity by producing a general depression in motoric functioning. Therefore, to further examine the interaction between the kappa-opioid and DA systems, 17-day-old rats were treated with U-50,488 prior to being injected with the nonselective D_1–D_2 receptor agonist NPA. If kappa-opioid agonists enhance the locomotor activity of preweanling rats by modulating a dopaminergic mechanism, then U-50,488 pretreatment should potentiate NPA-induced locomotor activity.

Method

Eight groups of 17-day-old rats (n = 8 per group) received an acute injection of saline or U-50,488 (5.0 mg/kg sc) immediately prior to a 60-min behavioral testing session. These same rats then received an acute injection of saline or NPA (0.01, 0.1, or 1.0 mg/kg ip) 30 min into the testing session. Behavioral assessment was the same as described in the General Method section.

Results

Line crosses. On the initial three time blocks, U-50,488-pretreated rats had significantly more line crosses than rats pretreated with saline (see left panels, Figure 2): Pre × Time interaction, $F(2, 124) = 32.23$, $p < .001$. Collapsed over the final three time blocks, NPA attenuated U-50,488-induced line crosses in a dose-related manner (see top graph, right panel, Figure 2): Pre × Post interaction, $F(3, 56) = 11.45$, $p < .001$, and Tukey tests, $p < .05$. More specifically, 1.0 mg/kg NPA produced the greatest decline in U-50,488-induced locomotor activity, whereas 0.1 mg/kg NPA produced a lesser, but still significant, decline (see top graph, right panel, Figure 2, Tukey tests, $p < .05$).

When given alone, NPA enhanced the locomotor activity of 17-day-old rats (see bottom graph, right panel, Figure 2): Pre × Post interaction, $F(3, 56) = 11.45$, $p < .001$, and Tukey tests, $p < .05$. The 0.01-mg/kg dose was the most effective at increasing locomotor activity; however, rats receiving 0.1 mg/kg NPA also had more line crosses than saline controls (Tukey tests, $p < .05$). Interactions involving time as a variable were not significant.

Stereotyped sniffing. During the first 30-min testing period (prior to NPA treatment), sniffing was not affected by U-50,488 pretreatment (data not shown). Overall, during the second 30-min testing period, 17-day-old rats receiving NPA (0.01, 0.1, or 1.0 mg/kg) had significantly more stereotyped sniffing counts than saline-treated rats (see Table 1): post main effect, $F(3, 56) = 28.18$, $p < .001$. This effect varied according to pretreatment condition, as saline-pretreated rats given 0.01, 0.1, or 1.0 mg/kg NPA sniffed significantly more than rats in the saline–saline control group: Pre × Post interaction, $F(3, 56) = 10.68$, $p < .001$, and Tukey tests, $p < .05$. U-50,488 attenuated NPA-induced sniffing, because U-50,488-pretreated rats given 0.01, 0.1, or 1.0 mg/kg NPA

Table 1
Mean Number of Stereotyped Sniffing Counts (±SEM) of 17-Day-Old Rats

Drug treatment	Stereotyped sniffing counts
With saline	
Saline	0.50 ± 0.5
0.01 mg/kg NPA	30.50 ± 6.4[a]
0.1 mg/kg NPA	48.75 ± 4.8[a]
1.0 mg/kg NPA	61.37 ± 5.7[a]
With U-50, 488	
Saline	0.37 ± 0.4
0.01 mg/kg NPA	3.12 ± 1.2[b]
0.1 mg/kg NPA	8.62 ± 5.5[b]
1.0 mg/kg NPA	16.12 ± 5.3[b]

Note. Rats were injected with saline or U-50,488 (5.0 mg/kg) followed, 30 min later, by an injection of saline or *R*(–)-propylnorapomorphine (NPA; 0.01–1.0 mg/kg). Behavioral testing occurred immediately after U-50,488 pretreatment and lasted for 60 min. Data presented are from the second 30-min testing period (i.e., after rats had received saline or NPA injections).
[a]Significantly different from the saline–saline group, $p < .05$. [b]Significantly different from saline-pretreated rats receiving the identical dose of NPA, $p < .05$.

had significantly fewer sniffing counts than saline-pretreated rats given identical doses of NPA (Tukey tests, $p < .05$).

Experiment 3

Unexpectedly, NPA attenuated (not potentiated) U-50,488-induced locomotor activity in preweanling rats. To further assess this finding, we treated 17- and 80-day-old rats as in Experiment 2, with the exception that regional Fos immunoreactivity was assessed after pharmacological manipulation. Because U-50,488 enhances the locomotor activity of only preweanling rats, it was hypothesized that U-50,488 would induce Fos in the nucleus accumbens and striatum of 17- but not 80-day-old rats. Further, based on the behavioral results of Experiment 2, NPA was predicted to attenuate U-50,488-induced Fos immunoreactivity in the younger animals. The nucleus accumbens and striatum were of special interest because these brain regions are important for locomotor activity (Arnt, 1987) and contain a substantial number of kappa-opioid receptors (Mansour, Fox, Akil, & Watson, 1995; Mansour, Khachaturian, Lewis, Akil, & Watson, 1987). Fos immunoreactivity was apparent in brain regions other than those about which we formed hypotheses, so these regional effects are also presented in the Results section.

Method

Behavioral procedures. Eight groups of 17- and 80-day-old rats ($n = 8$ per group) received an acute injection of saline or U-50,488 (5.0 mg/kg sc) immediately prior to being placed in the testing apparatus. Rats were given an acute injection of saline or NPA (0.1 mg/kg ip) 30 min into the 60-min behavioral testing session. After testing, rats were returned to their home cages for 60 min, were given an overdose of phenobarbital, and were rapidly perfused with 4% paraformaldehyde. Thus, rats were euthanized 120 min after receiving saline or U-50,488 and 90 min after receiving saline or NPA. Fos immunoreactivity was later assessed with this tissue.

Supplies. The primary antibody was a monoclonal antibody made to the N-terminal end of the Fos peptide in mouse myeloma cells.[1] This primary antibody recognizes Fos and not Fos-related antigens. The secondary antibody was a biotinylated mouse–anti-rat secondary antibody (Vector Laboratories, Inc., Burlingame, CA). An avidin–biotin–horseradish peroxidase conjugate from an ABC horse kit was also used (Vector Laboratories, Inc.).

Immunohistochemistry procedures. Following a postfixation period, 100-μm sections were cut from each brain using a Vibratome 1000 (Ted Pella, Inc., Redding, CA). The sections were washed three times with 0.1 M phosphate buffer (PB) before being incubated with the Fos primary antibody at a 1:50,000 dilution in horse serum solution (HSS) (0.1 M PB containing 2% horse serum, 0.1% Triton-X100, and 0.1% bovine serum albumin). Sections were incubated in the primary antibody for 48–72 hr. Control sections from each rat were run in the absence of the primary antibody. All sections were then washed three times in PB and incubated with a biotinylated mouse–anti-rat secondary antibody for 2 hr (1:10,000 dilution in HSS). Sections were washed three times in PB and incubated for 2 hr in avidin–biotin–horseradish peroxidase conjugate from ABC horse kits. Sections were then washed three more times, and the Fos protein was visualized using 3,3′-diaminobenzidine tetrahydrochloride and hydrogen peroxide. Sections were then rinsed in PB and mounted on chrom–alum slides. The slides were then air dried, dehydrated, and coverslipped with Permount. All washes lasted 5 min. This general procedure was based on the method of Sharp, Gonzalez, Sharp, and Sagar (1989).

A total of 5–6 rats from each treatment group were randomly chosen for analysis of Fos immunoreactivity (an additional 8 rats served as uninjected controls). Similar coronal sections from each rat were selected for quantitative analysis. These coronal sections were then examined to determine those brain areas containing Fos-positive nuclei. On the basis of this initial examination, the number of distinguishable immunoreactive nuclei present within the core and shell of the nucleus accumbens, medial and lateral striatum, amygdala, habenula, mammillary bodies, olfactory tubercles, piriform cortex, preoptic area, septal area, and Areas 1 and 3 of the temporal cortex were manually counted using a magnification of 40×. The rat brain atlas of Paxinos and Watson (1986) was used to identify brain areas in adult rats, and the developing rat brain atlas of Sherwood and Timiras (1970) was used for the preweanling rats. For each region, one to four sample areas were counted, with the size of each sample area being 0.16 mm^2.

Behavioral Results

Line crosses. On each of the first three time blocks, 17-day-old rats pretreated with 5.0 mg/kg U-50,488 had significantly more line crosses than saline-pretreated rats (see left panels, Figure 3): Pre × Time interaction, $F(2, 60) = 20.42$, $p < .001$, and Tukey tests, $p < .05$. U-50,488 continued to induce high levels of locomotor activity on the last three time blocks (see top graph, right panel, Figure 3). As in Experiment 2, rats given both U-50,488 and NPA (0.1 mg/kg) had significantly fewer line crosses than rats given U-50,488 alone—an effect that became more pronounced as the testing session progressed—Pre × Post × Time interac-

[1] The Fos primary antibody was generously supplied by the laboratories of Frank Sharp and Stephen Sagar.

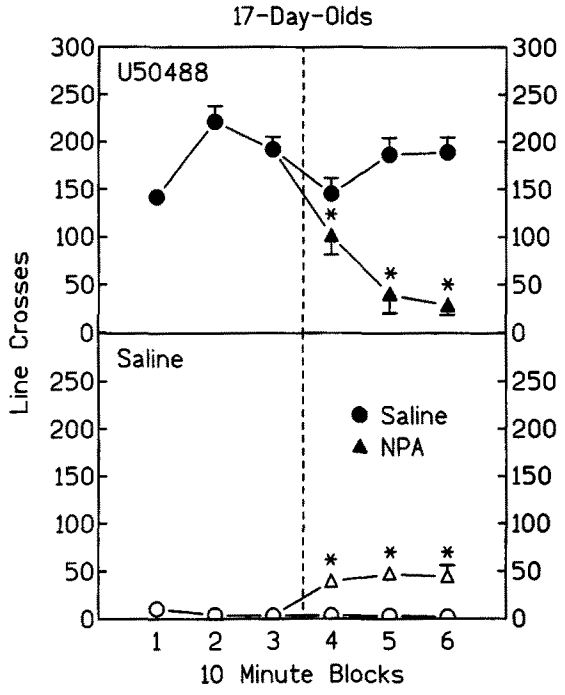

Figure 3. Mean number of line crosses during the 60-min behavioral testing session. The 17-day-old rats ($n = 8$ per group) were injected with saline or U-50,488 (5.0 mg/kg) immediately prior to behavioral testing. Rats were then injected (indicated by the dashed line) with saline or *R*(−)-propylnorapomorphine (NPA; 0.1 mg/kg) 30 min into the testing session. *Significantly different from the saline-treated group (i.e., the circles) receiving the identical pretreatment ($p < .05$).

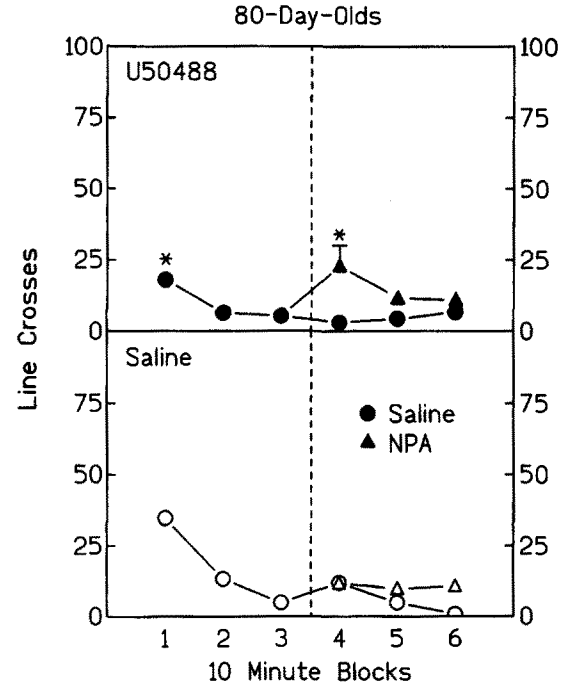

Figure 4. Mean number of line crosses during the 60-min behavioral testing session. The 80-day-old rats ($n = 8$ per group) were injected with saline or U-50,488 (5.0 mg/kg) immediately prior to behavioral testing. Rats were then injected (indicated by the dashed line) with saline or *R*(−)-propylnorapomorphine (NPA; 0.1 mg/kg) 30 min into the testing session. *Significantly different from the saline-treated group (i.e., the circles) receiving the identical pretreatment ($p < .05$).

tion, $F(2, 56) = 20.68$, $p < .001$, and Tukey tests, $p < .05$. NPA, when given alone, significantly increased the line crosses of saline-pretreated rats on all three time blocks (bottom graph, right panel, Figure 3); however, NPA was not as effective as U-50,488 at increasing locomotor activity (Pre × Post × Time interaction and Tukey tests).

On the first time block, 80-day-old rats pretreated with U-50,488 had significantly fewer line crosses than saline-pretreated rats (see left panel, Figure 4): Pre × Time interaction, $F(2, 60) = 6.27$, $p < .001$, and Tukey tests, $p < .05$. There were no differences between groups on Time Blocks 2 and 3. On the fourth time block, 80-day-old rats treated with both U-50,488 and NPA had significantly more line crosses than all other groups (see right panel, Figure 4): Pre × Post × Time interaction, $F(2, 56) = 6.67$, $p < .001$, and Tukey tests, $p < .05$. There were no differences between groups on any of the subsequent time blocks (Tukey tests, $p < .05$).

Stereotyped sniffing. There were no significant differences between saline- and U-50,488-pretreated 17-day-old rats during the initial 30-min testing period (data not shown). During the final testing period, 17-day-old rats given NPA had significantly more stereotyped sniffing counts than saline-treated rats (see Table 2): post main effect, $F(1, 28) = 80.49$, $p < .001$. In contrast to the results of Experiment 2, U-50,488 did not significantly attenuate the NPA-induced sniffing of preweanling rats. The reasons for this difference are not known.

The stereotyped sniffing of U-50,488- and saline-pretreated adult rats did not differ during the initial 30-min testing period (data not shown). During the final testing period, 80-day-old rats given NPA had significantly more

Table 2
Mean Number of Stereotyped Sniffing Counts (±SEM) of 17- and 80-Day-Old Rats

	Stereotyped sniffing counts	
Drug treatment	17-day-olds	80-day-olds
With saline		
Saline	0.62 ± 0.5[b]	5.38 ± 2.3[b]
NPA	42.62 ± 5.3	66.13 ± 2.8
With U-50,488		
Saline	1.25 ± 0.9[b]	3.38 ± 1.8[a,b]
NPA	20.72 ± 5.9	50.00 ± 6.8[a]

Note. Rats were injected with saline or U-50,488 (5.0 mg/kg) followed, 30 min later, by an injection of saline or *R*(−)-propylnorapomorphine (NPA; 0.1 mg/kg). Behavioral testing occurred immediately after U-50,488 pretreatment and lasted for 60 min. Data presented are from the second 30-min testing period (i.e., after rats had received saline or NPA injections).
[a]Main effect significantly different from saline-pretreated rats of the same age, $p < .05$. [b]Main effect significantly different from NPA-treated rats of the same age, $p < .05$.

stereotyped sniffing counts than their saline controls (see Table 2): post main effect, $F(1, 28) = 83.49$, $p < .001$. U-50,488-pretreated rats had significantly fewer sniffing counts than saline-pretreated rats, an effect most clearly observed in the two NPA groups: pre main effect, $F(1, 28) = 5.23$, $p < .05$.

Immunohistochemistry Results

Preweanling rats. U-50,488 enhanced Fos immunoreactivity in a number of different brain regions of the preweanling rat (see Table 3). More specifically, compared with rats pretreated with saline, U-50,488-pretreated rats had significantly more Fos-positive nuclei in the nucleus accumbens (data from the core and shell were combined because they provided similar results), $F(1, 20) = 5.56$, $p < .05$; medial striatum (the lateral portions of the striatum had very few Fos-positive nuclei), $F(1, 20) = 18.31$, $p < .001$; amygdala, $F(1, 20) = 8.14$, $p < .01$; habenula, $F(1, 20) = 36.52$, $p < .001$; olfactory tubercles, $F(1, 20) = 20.83$, $p < .001$; piriform cortex, $F(1, 20) = 8.57$, $p < .01$; preoptic area, $F(1, 20) = 28.00$, $p < .001$; septal area, $F(1, 20) = 34.66$, $p < .001$; and Areas 1 and 3 of the temporal cortex, $F(1, 20) = 8.03$, $p < .01$. NPA (0.1 mg/kg) attenuated U-50,488-induced Fos expression in only the medial striatum: Pre × Post interaction, $F(1, 20) = 5.58$, $p < .05$, and Tukey tests, $p < .05$. NPA-treated rats (when compared with saline controls) had significantly more Fos-positive nuclei in the mammillary bodies, $F(1, 20) = 7.28$, $p < .05$; piriform cortex, $F(1, 20) = 15.46$, $p < .001$; and Areas 1 and 3 of the temporal cortex, Pre × Post interaction, $F(1, 20) = 9.45$, $p < .01$.

Adult rats. U-50,488 did not enhance Fos expression in any brain region of the 80-day-old rat (see Table 4). Instead, U-50,488-pretreated rats had significantly fewer Fos-positive nuclei in the mammillary bodies than saline-pretreated rats, $F(1, 20) = 4.53$, $p < .05$. Drug-induced Fos expression was evident, however, because NPA (0.1 mg/kg) increased the number of Fos-positive nuclei in the olfactory tubercles, $F(1, 16) = 10.82$, $p < .001$; piriform cortex, Pre × Post interaction, $F(1, 16) = 7.15$, $p < .05$; and septal area, Pre × Post interaction, $F(1, 16) = 7.41$, $p < .05$. U-50,488 attenuated NPA-induced Fos expression in the latter two brain regions (Tukey tests, $p < .05$).

General Discussion

The purpose of our study was to examine U-50,488's paradoxical ability to enhance the locomotor activity of preweanling rats. Because DA systems are so intimately involved in locomotor activity, it was hypothesized that kappa-opioid agonists might enhance locomotion by stimulating a dopaminergic mechanism. Contrary to this hypothesis, the nonselective D_1–D_2 receptor agonist NPA, which increases locomotor activity when given alone, attenuated U-50,488-induced activity in a dose-dependent manner. Curiously, flupenthixol (a nonselective D_1–D_2 receptor antagonist) also depressed U-50,488's locomotor activating effects, thus showing that stimulating or blocking DA receptors will disrupt U-50,488-induced locomotor activity. The antagonistic relationship between the kappa-opioid and DA receptor systems appears to be reciprocal because U-50,488 attenuated the NPA-induced stereotyped sniffing of preweanling rats (see Experiment 2). Thus, when considered together, these results clearly show that kappa-opioid agonists do not increase the locomotor activity of preweanling rats by activating a dopaminergic mechanism.

Instead, these results indicate that the kappa–DA interaction undergoes profound changes across ontogeny. During the late fetal period, DA receptor stimulation causes a

Table 3
Mean Number of Fos-Positive Nuclei (±SEM) in Various Brain Regions of the 17-Day-Old Rat

	Treatment condition				
	With saline		With U-50,488		
Brain region	Saline	NPA	Saline	NPA	Uninjected control
Amygdala	39.83 ± 4	46.08 ± 4	55.75 ± 6[a]	71.92 ± 12[a]	0.70 ± 0.5
Habenula	26.50 ± 3	28.33 ± 5	58.33 ± 7[a]	55.67 ± 3[a]	8.40 ± 2.0
Mammillary bodies	7.08 ± 1[b]	14.58 ± 3	8.83 ± 1[b]	13.75 ± 3	1.00 ± 0.8
Nucleus accumbens	0.83 ± 1	0.50 ± 1	4.61 ± 2[a]	2.25 ± 1[a]	0.23 ± 0.1
Olfactory tubercles	4.42 ± 2	0.92 ± 1	30.92 ± 8[a]	19.67 ± 6[a]	0.00 ± 0.0
Piriform cortex	14.14 ± 2[b]	22.00 ± 4	19.33 ± 2[a,b]	32.33 ± 2[a]	0.40 ± 0.1
Preoptic area	5.29 ± 1	7.96 ± 3	22.88 ± 3[a]	20.62 ± 1[a]	0.65 ± 0.3
Septal area	2.62 ± 1	8.88 ± 3	23.33 ± 2[a]	18.88 ± 2[a]	0.40 ± 0.3
Medial striatum	0.04 ± 0	0.04 ± 0	20.54 ± 6[a,c,d]	5.95 ± 3[a]	0.30 ± 0.3
Temporal cortex	6.75 ± 1	16.39 ± 3[c]	21.11 ± 3[a,c]	15.80 ± 2[a]	1.50 ± 0.2

Note. Rats were euthanized 120 min after receiving saline or U-50,488 (5.0 mg/kg) and 90 min after receiving saline or *R*(–)-propylnorapomorphine (NPA; 0.1 mg/kg). The behavioral scores of these rats are shown in Figure 3 and Table 2. The uninjected controls were not included in statistical analyses but were presented for comparative purposes. Numbers represent means for 0.16-mm^2 sample areas.
[a]Main effect significantly different from saline-pretreated rats, $p < .05$. [b]Main effect significantly different from NPA-treated rats, $p < .05$. [c]Significantly different from the Sal–Sal group, $p < .05$. [d]Significantly different from the U50–NPA group, $p < .05$.

Table 4
Mean Number of Fos-Positive Nuclei (±SEM) in Various Brain Regions of the 80-Day-Old Rat

Brain region	Treatment condition				
	With saline		With U-50,488		
	Saline	NPA	Saline	NPA	Uninjected control
Amygdala	40.33 ± 8	66.58 ± 12	36.92 ± 9	35.58 ± 7	1.50 ± 1.0
Habenula	24.67 ± 6	31.67 ± 4	33.50 ± 4	29.58 ± 5	1.00 ± 0.8
Mammillary bodies	19.33 ± 4	29.83 ± 6	16.75 ± 7[a]	11.33 ± 3[a]	0.00 ± 0.0
Nucleus accumbens	6.57 ± 1	3.33 ± 1	7.90 ± 4	4.73 ± 2	0.00 ± 0.0
Olfactory tubercles	8.00 ± 2[b]	2.40 ± 1	4.00 ± 2[b]	0.90 ± 1	0.33 ± 0.3
Piriform cortex	20.20 ± 2	38.33 ± 2[c]	23.13 ± 4	22.20 ± 5	0.06 ± 0.1
Preoptic area	7.71 ± 2	18.04 ± 7	14.83 ± 3	10.42 ± 3	0.25 ± 0.2
Septal area	9.05 ± 1	18.65 ± 3[c]	11.80 ± 2	9.40 ± 3	0.00 ± 0.0
Medial striatum	5.65 ± 2	5.05 ± 2	7.30 ± 3	5.40 ± 5	0.08 ± 0.1
Temporal cortex	18.28 ± 5	22.36 ± 3	13.44 ± 3	22.80 ± 5	0.00 ± 0.0

Note. Rats were euthanized 120 min after receiving saline or U-50,488 (5.0 mg/kg) and 90 min after receiving saline or *R*(−)-propylnorapomorphine (NPA; 0.1 mg/kg). The behavioral scores of these rats are shown in Figure 3 and Table 2. The uninjected controls were not included in statistical analyses but were presented for comparative purposes. Numbers represent means for 0.16-mm^2 sample areas.
[a]Main effect significantly different from saline-pretreated rats, $p < .05$. [b]Main effect significantly different from NPA-treated rats, $p < .05$. [c]Significantly different from all other groups, $p < .05$.

unidirectional activation of the kappa-opioid system, whereas kappa receptor stimulation does not seem to modulate DA functioning (Robinson, Moody, Spear, & Smotherman, 1993; Smotherman et al., 1993). This conclusion is based on studies showing that the SKF 38393-induced limb movements of fetal rats are blocked by a kappa-opioid antagonist, whereas U-50,488-induced movements are not blocked by a D_1 receptor antagonist (Smotherman et al., 1993). Our study suggests a different relationship in the preweanling rat, because both a nonselective D_1–D_2 receptor agonist and antagonist attenuated U-50,488-induced locomotor activity, whereas a kappa-opioid agonist attenuated NPA-induced sniffing. Thus, there appears to be a complex reciprocal interaction between the kappa-opioid and DA systems during the preweanling period. The kappa–DA interaction does not become truly adultlike until after the preweanling period, because kappa-opioid receptor stimulation causes a unidirectional inhibition of DA functioning in the adult rat (the reverse is seen in fetal rats). For example, U-50,488 attenuates cocaine-induced locomotor activity and place preference conditioning in adult rats (Crawford et al., 1995; Suzuki, Shiozaki, Masukawa, Misawa, & Nagase, 1992), whereas DA-acting drugs have not been reported to modulate kappa-opioid–mediated behaviors. Therefore, it appears that the pattern of kappa–DA interaction differs during each of these three ontogenetic periods.

At present, the neuroanatomical mechanisms responsible for these kappa–DA interactions are not fully understood. Smotherman et al. (1993) have speculated that the kappa–DA interaction mediating fetal limb movements may occur in the spinal cord. In the adult rat, systemically administered U-50,488 does not increase locomotor activity; however, U-50,488's ability to inhibit DA-mediated behaviors is most commonly ascribed to actions in the nucleus accumbens or striatum. In these brain regions, kappa-opioid receptors are located predominantly on DA terminal fibers, and their stimulation directly inhibits DA release (Di Chiara & Imperato, 1988; Maisonneuve, Archer, & Glick, 1994; Spanagel, Herz, & Shippenberg, 1990, 1992). Additional kappa-opioid receptors are located on cell bodies of striatal neurons projecting to the substantia nigra pars compacta. Stimulation of these receptors indirectly reduces striatal DA levels by inhibiting DA neurons of the nigrostriatal pathway (Matsumoto, Brinsfield, Patrick, & Walker, 1988; Walker et al., 1987). During the preweanling period, both flupenthixol and NPA attenuate U-50,488-induced locomotor activity. Although speculative, one possibility is that flupenthixol nonspecifically attenuates U-50,488-induced locomotor activity by depressing neuronal activity in the mesolimbic pathway, nigrostriatal pathway, or both. Furthermore, it is possible that DA receptor stimulation may attenuate U-50,488-induced locomotor activity by inhibiting nondopaminergic pathways that modulate locomotor activity. More specifically, stimulation of kappa-opioid receptors in the substantia nigra pars reticulata induces locomotor excitation in the adult rat, probably by inhibiting GABAergic output projections (Matsumoto et al., 1988; Thompson & Walker, 1990, 1992). Thus, it is possible that NPA attenuates U-50,488's locomotor activating effects by inhibiting these nondopaminergic output projections.

In addition to behavioral testing, the neuronal response to U-50,488 and NPA was examined by measuring the induction of Fos. In the preweanling rat, U-50,488 significantly increased Fos expression in a number of brain areas, including the nucleus accumbens, medial striatum, amygdala, habenula, olfactory tubercle, piriform cortex, preoptic area, septal area, and temporal cortex. In contrast, U-50,488 did not increase Fos expression in any brain region of the adult rat. This pronounced age-dependent difference in Fos immunoreactivity is consistent with the relative amounts of

behavioral activation produced by U-50,488 in the two age groups. The medial striatum was the only brain region in the preweanling rat where NPA attenuated U-50,488-induced Fos expression. This pattern of neuronal activation correlates with NPA's ability to block U-50,488-induced locomotor activity, thus providing some evidence that the medial striatum may be the locus for NPA's locomotor inhibiting effects.

NPA was able to stimulate Fos expression in adult and preweanling rats, with both age groups showing a similar pattern of Fos immunoreactivity. More specifically, NPA increased the number of Fos-positive nuclei in the mammillary bodies, piriform cortex, and septal area of adult rats, whereas 17-day-old rats showed enhanced Fos expression in the mammillary bodies and piriform cortex. In general, this pattern of Fos expression is similar to the pattern observed after acute treatment with the nonselective DA agonist apomorphine—with the important exception that NPA did not increase Fos immunoreactivity in the nucleus accumbens or striatum (Dilts, Helton, & McGinty, 1993). The lack of Fos expression in these two structures may have been due to the dose (0.1 mg/kg) of NPA used, because low doses of apomorphine are incapable of stimulating Fos immunoreactivity in the striatum (Cenci, Kalen, Mandel, Wictorian, & Bjorklund, 1992; Dilts et al., 1993). Alternatively, NPA is relatively selective for the D_2 receptor, and activation of the D_2 receptor is not sufficient for inducing striatal Fos expression (LaHoste, Yu, & Marshall, 1993; Wirtshafter & Asin, 1994).

The pattern of our results provides further evidence that the relationship between Fos immunoreactivity and behavior is very complex (see Numan & Numan, 1995; Sandstrom, Sarter, & Bruno, 1996). This complexity is best realized by comparing the results of other studies that have shown that (a) the absence of Fos immunoreactivity in a particular brain area does not preclude the involvement of that brain area in the mediation of a drug-induced behavior (G. S. Robertson, Vincent, & Fibiger, 1992), (b) 6-OHDA lesioned rats exhibit a dissociation between Fos expression and rotational behavior (H. A. Robertson, Peterson, Murphy, & Robertson, 1989), (c) Fos induction is reflective of those neurons that underlie the expression of behavior (Numan & Numan, 1995), and (d) Fos expression may in itself be sufficient for the occurrence of some motor behaviors (Heilig, Engel, & Soderpalm, 1993; Hooper, Chiasson, & Robertson, 1994). On the basis of these sometimes seemingly inconsistent findings, it is apparent that the relationship between Fos and behavior is not yet well understood.

In summary, the kappa-opioid agonist U-50,488 dramatically increased the locomotor activity of preweanling rats while attenuating NPA-induced sniffing. The increased locomotor activity does not appear to be mediated through a dopaminergic mechanism; instead, our results show that either agonizing or antagonizing DA receptors will disrupt U-50,488-induced locomotion. Although speculative, we believe that the medial striatum may be the locus for NPA's locomotor inhibiting effects, because NPA was capable of blocking U-50,488-induced Fos expression in only this brain region. Future research involving the microinjection of kappa-opioid and DA agonists will be necessary to definitively determine the neuroanatomical location of U-50,488's and NPA's actions.

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