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# Intracisternal Administration of SKF-38393 and SCH-23390: Behavioral Effects in the Rat Fetus

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VARLINSKAYA, E. I., E. S. PETROV, S. R. ROBINSON AND W. P. SMOTHERMAN. *Intracisternal administration of SKF-38393 and SCH-23390: Behavioral effects in the rat fetus.* PHARMACOL BIOCHEM BEHAV 48(3) 741-748, 1994. — The dopamine D<sub>1</sub> agonist SKF-38393 and the D<sub>1</sub> antagonist SCH-23390 were administered into the central nervous system of the E21 rat fetus via intracisternal (IC) injection. IC injection of SKF-38393 promoted a dose-dependent increase in fetal motor activity, principally including movements of the forelimbs, head, and body trunk. IC injection of SCH-23390 did not affect overall activity, but selectively suppressed forelimb, rearlimb, and head movements and promoted an increase in mouthing, licking, and facial wiping. Administration of SCH-23390 after IC injection of SKF-38393 was effective in completely reversing the behavioral effects of the D<sub>1</sub> agonist. These findings suggest that central manipulation of dopamine D<sub>1</sub> receptors can have direct and potent behavioral effects in the term rat fetus.

SKF-38393    SCH-23390    D<sub>1</sub>    Dopamine    Rat fetus

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PRENATAL exposure to drugs of abuse is now recognized as an important cause of behavioral as well as morphological abnormalities (1,2). To date, experimental approaches to understanding the underlying mechanisms of prenatal drug effects have involved application of drugs to fetal tissues in vitro or administration of drugs to the female during pregnancy and inference of cause from postnatal consequences (13). These approaches have provided important information about the potential for drugs of abuse to exert effects on functional systems in utero. However, little direct evidence is available regarding the effects of pharmacological agents on the central nervous system of the fetus in vivo.

Recent studies have documented that drugs of abuse, such as cocaine (6), and synthetic agents that act on neurotransmitter or neuropeptide systems, such as the dopamine (3) and endogenous opioid systems (10,11), are effective in producing behavioral changes when administered peripherally to the rat fetus. Stimulation of activity at D<sub>1</sub> receptors with the selective

agonist SKF-38393 results in higher levels of fetal motor activity when the drug is administered to individual fetal subjects via intraperitoneal injection. A relatively high dose of the D<sub>1</sub> antagonist SCH-23390 also alters fetal activity by promoting mouthing and facial wiping (a fetal action pattern involving paw-to-face contact) (3,11). These findings suggest that peripheral manipulations of dopamine activity can engage the motor system of the E21 rat fetus.

It is possible to administer pharmacological agents into the central nervous system of the fetal rat via injection into the cisterna magna (12,14). In the present study, changes in motor behavior were used as a bioassay to assess effects of a dopaminergic agonist and antagonist on the central nervous system of the rat fetus on E21 of gestation. The aims of these experiments were a) to provide information about the behavioral effects of SKF-38393 and SCH-23390 administered into the cisterna magna of the rat fetus, b) to establish dose-response relationships for the D<sub>1</sub> agonist and antagonist, and c) to se-

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quentially administer SKF-38393 and SCH-23390 to confirm that behavioral effects of the dopamine agonist are mediated through its interaction with the  $D_1$  receptor.

#### GENERAL METHOD

##### *Subjects*

Subjects were fetuses produced in timed matings of pregnant Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA). Breeding female rats were housed in groups of three in plastic breeding cages (36 × 47 × 20 cm) with one male rat during a 4-day breeding period. Daily vaginal smears were collected to date conception, with embryonic day 0 (E0) defined as the day sperm were detected (birth occurs on E21.5). Pregnant female rats were maintained under conditions of constant room temperature (22°C), on a 12 L : 12 D cycle (lights on at 0700) until the date of fetal testing (E21). Food and water were available ad lib. At all times, rats were maintained and treated in accordance with guidelines for animal care established by the National Institutes of Health (PHS publication 86-23).

##### *Prenatal Preparation*

Each pregnant rat was surgically prepared on E21 of gestation for pharmacological manipulation and testing of fetuses. With the rat under ether anesthesia, 100% ethanol (70  $\mu$ l) was injected into the spinal canal between the first and second lumbar vertebrae. This chemomyelotomy procedure results in irreversible blockade of neural transmission within the spinal cord at the low thoracic level, eliminating sensation in the lower part of the body. The prepared rat then was placed in a holding device and immersed to chest depth in a buffered isotonic saline bath at 37.5°C. The activity of the pregnant rat was visually monitored throughout the period of fetal testing to ensure completeness of the spinal preparation. Direct access to fetal subjects was accomplished by exteriorizing the uterus into the bath through a midline laparotomy. Individual fetuses selected as experimental subjects were delivered from the uterus and the embryonic membranes were removed. The fetal subject, thus, was suspended within the saline bath with its umbilical attachment to the placenta intact. The condition of the fetus was visually monitored during the experiment; only fetuses that remained pink, indicating good oxygenation, were used as subjects. To avoid confounding litter effects with treatment effects, only one subject from each pregnancy was assigned to each treatment group, and all treatments were represented within the pregnancy whenever feasible. The order of testing subjects was randomized among the treatments in each pregnancy. After surgical preparation, a 20-min period elapsed before behavioral testing to provide time for the pregnant rat and subject fetuses to accommodate to the bath environment (9).

##### *Central Administration of $D_1$ Agonist and Antagonist*

Solutions containing various dosages of the selective  $D_1$  agonist SKF-38393 (SKF, a selective  $D_1$  agonist), SCH-23390 (SCH, a selective  $D_1$  antagonist), or isotonic saline (SAL) were administered into the central nervous system of individual fetal subjects (drugs obtained from Research Biochemicals Inc., Natick, MA). All drug or vehicle solutions were administered to individual fetal subjects by intracisternal (IC) injection. Drug solutions were loaded into the terminal section of transparent polyethylene tubing (PE-10). A 30 ga hypodermic nee-

dle at the end of this tubing was inserted under visual guidance into the foramen magnum between the occipital bone and the first cervical vertebra, with the tip placed in the cisterna magna. Successful placement of the needle into the cisterna magna was confirmed by the immediate appearance of a small volume of cerebrospinal fluid in the tubing. Drug and vehicle solutions were injected slowly (8–10 s pulse) in a volume of 1.0  $\mu$ l. Additional details about this injection procedure have been described in an earlier report (14). Solutions were prepared in advance in an isotonic saline vehicle (with dosages calculated from the weight of the salt), refrigerated, and warmed to fetal body temperature before injection. The volumetric equivalent of isotonic saline was used as the vehicle in preparing these solutions.

##### *Behavioral Observation*

The motor behavior of fetal subjects was visually monitored and recorded to assess the effects of drug treatments. An observer noted each instance of fetal movement and called the appropriate behavioral category to an assistant, who recorded the data using a microcomputer-based event recorder (the assistant conducted the drug administrations, permitting the observer to remain blind to specific treatments in each fetal subject). Six basic categories of fetal movement were distinguished by reference to the region of the fetal body that was involved in the movement: forelimb (movement involving one or both forelimbs), rearlimb (movement involving one or both rearlimbs), head (change in head position relative to the body trunk), trunk (ventriflextion or lateral flexion of the body trunk), mouth (opening and closing of the mouth), and lick (protrusion of the tongue outside the oral cavity). A seventh behavioral category was facial wiping, an action pattern involving placement of one or both forepaws against the side of the head and movement of the forepaw(s) in a rostral direction, with contact sliding across the face. Facial wiping is uncommonly expressed by fetal rats in the absence of explicit sensory stimulation (5). The sum of movement events in all seven categories was used as a measure of overall fetal activity. These techniques for recording and quantifying fetal motor behavior preserve information about the frequency and timing of fetal movements and are highly consistent between observation sessions (reliability > 0.90). A more detailed description about the observation and scoring techniques used in our laboratory has been reported previously (7,9).

##### *Data Analysis*

In each experiment, a series of statistical analyses was conducted to assess the effects of drug treatment. An overall analysis comprising all treatment groups involved a multifactor analysis of variance (ANOVA), with scores during successive minutes of observation treated as a repeated measure. Significant main or interaction effects involving the drug treatment factor were followed by planned ANOVAs to determine which experimental groups differed from saline-injected controls. In these planned comparisons, a significant main or interaction effect involving the drug treatment factor indicated that the drug treatment differed from the saline-injected control group. A similar series of analyses was conducted to characterize drug effects on each of the seven categories of fetal movement. Owing to the large number of analyses involved, discussion of statistical significance will be limited to differences indicated by an alpha level of  $p < 0.01$ .

EXPERIMENT 1.  
IC ADMINISTRATION OF SKF-38393

Peripheral administration of SKF-38393 has been shown to influence motor behavior in the E21 rat fetus (3). Intraperitoneal injection of 1.0 mg/kg SKF resulted in significantly elevated movements of the forelimbs, rearlimbs, and head of fetal subjects. The objective of Experiment 1 was to determine the behavioral effects of SKF-38393 administered into the central nervous system of the E21 rat fetus. In addition, this experiment was designed to provide information about the effects of varying dosage of the D<sub>1</sub> agonist on fetal motor behavior.

A total of 101 fetuses served as subjects in Experiment 1. Fetuses received a control injection of the isotonic saline vehicle (SAL;  $n = 26$ ), or a drug injection consisting of one of five doses of SKF-38393 (0.05  $\mu\text{g}$  per fetus,  $n = 10$ ; 0.2  $\mu\text{g}$ ,  $n = 15$ , 1.0  $\mu\text{g}$ ,  $n = 15$ ; 5.5  $\mu\text{g}$ ,  $n = 20$ ; 10.0  $\mu\text{g}$ ,  $N = 15$ ). Immediately following IC injection of the SKF solution or saline vehicle, the behavior of fetal subjects was recorded in a 10-min observation session.

### Results

Overall fetal activity was compared in a two-factor repeated measures ANOVA (five doses of SKF or saline  $\times$  10 min), which indicated the significant interaction of drug treatment and time,  $F(45, 855) = 10.3$ ,  $p < .001$ . Pair-wise ANOVAs comparing each SKF dose with saline-injected controls indicated no differences between 0.05  $\mu\text{g}$  SKF and SAL ( $p$  values  $> 0.10$ ), but significant increases in overall activity in the 0.2  $\mu\text{g}$  [drug  $\times$  time interaction:  $F(9, 351) = 4.6$ ,  $p < 0.001$ ], 1.0  $\mu\text{g}$  [drug  $\times$  time interaction:  $F(9, 351) = 16.9$ ,  $p < 0.001$ ], 5.5  $\mu\text{g}$  [drug  $\times$  time interaction:  $F(9, 396) = 6.3$ ,  $p < 0.001$ ], and 10.0  $\mu\text{g}$  SKF groups [drug  $\times$  time interaction:  $F(9, 351) = 33.2$ ,  $p < 0.001$ ]. The increase in motor activity produced by IC injection of SKF during min 10 amounted to 247% (0.2  $\mu\text{g}$ ), 424% (1.0  $\mu\text{g}$ ), 460% (5.5  $\mu\text{g}$ ), and 783% (10.0  $\mu\text{g}$ ) of SAL controls (Fig. 1). At the three higher dosages, which produced the most pronounced effects on fetal activity, movements increased to approximately the

same stable level in the 1.0 and 5.5  $\mu\text{g}$  groups and showed a monotonic increase throughout the 10-min session in the 10.0  $\mu\text{g}$  group.

The two-factor repeated measures ANOVAs that examined changes in the individual categories of fetal movement indicated that IC administration of SKF resulted in significant increases of forelimb, rearlimb, head, trunk, mouth, and facial wiping movements, but no change in licking ( $p > 0.05$ ) (Fig. 2). A significant interaction of drug treatment  $\times$  time was evident for movements of forelimb,  $F(45, 855) = 9.2$ ,  $p < 0.001$ ; head,  $F(45, 855) = 11.1$ ,  $p < 0.001$ ; trunk,  $F(45, 855) = 1.9$ ,  $p < 0.001$ ; wipe,  $F(45, 855) = 2.5$ ,  $p < 0.001$ . Main effects of drug treatment were evident for rearlimb,  $F(5, 95) = 3.8$ ,  $p < 0.01$ , and mouth,  $F(5, 95) = 6.0$ ,  $p < 0.001$ .

Planned ANOVAs indicated that forelimb movements were elevated relative to the SAL group in the 0.2  $\mu\text{g}$  [drug  $\times$  time interaction:  $F(9, 351) = 6.5$ ,  $p < 0.001$ ], 1.0  $\mu\text{g}$  [drug  $\times$  time interaction:  $F(9, 351) = 15.3$ ,  $p < 0.001$ ], 5.5  $\mu\text{g}$  [drug  $\times$  time interaction:  $F(9, 396) = 5.9$ ,  $p < 0.001$ ], and 10.0  $\mu\text{g}$  groups [drug  $\times$  time interaction:  $F(9, 351) = 29.2$ ,  $p < 0.001$ ], but were not elevated in the 0.05  $\mu\text{g}$  group. Changes in forelimb activity resulting from IC injection of SKF followed the same general pattern as overall motor activity, with the 1.0 and 5.5  $\mu\text{g}$  groups increasing to a plateau and the 10.0  $\mu\text{g}$  group showing a steady increase through the session. Rearlimb movements did not differ from SAL controls in the 0.05, 0.2, and 1.0  $\mu\text{g}$  SKF groups, but were significantly elevated in the 5.5  $\mu\text{g}$  [drug main effect:  $F(1, 44) = 7.2$ ,  $p < 0.01$ ], and 10.0  $\mu\text{g}$  groups [drug main effect:  $F(1, 39) = 10.6$ ,  $p < 0.01$ ]. Although rearlimb movements were affected by SKF injection, the increase at the highest dosage only amounted to 192% of SAL levels. Movements of the head increased relative to SAL controls at dosages of 0.2  $\mu\text{g}$  [drug  $\times$  time interaction:  $F(9, 351) = 8.4$ ,  $p < 0.001$ ], 1.0  $\mu\text{g}$  [drug  $\times$  time interaction:  $F(9, 351) = 24.3$ ,  $p < 0.001$ ], 5.5  $\mu\text{g}$  [drug  $\times$  time interaction:  $F(9, 396) = 13.3$ ,  $p < 0.001$ ], and 10.0  $\mu\text{g}$  [drug  $\times$  time interaction:  $F(9, 351) = 32.3$ ,  $p < 0.001$ ], but were not affected by the 0.05  $\mu\text{g}$  dose. As with forelimb activity, head movements exhibited a pattern of increasing to a plateau in the 1.0 and 5.5  $\mu\text{g}$  groups, and showed a much more pronounced increase in the 10.0  $\mu\text{g}$  group. Trunk movements were elevated after SKF injection in the 1.0  $\mu\text{g}$  [drug main effect:  $F(1, 39) = 17.7$ ,  $p < 0.001$ ], 5.5  $\mu\text{g}$  [drug main effect:  $F(1, 44) = 133.9$ ,  $p < 0.001$ ], and 10.0  $\mu\text{g}$  [drug  $\times$  time interaction:  $F(9, 351) = 6.2$ ,  $p < 0.001$ ] groups. Fetuses in the 0.05 and 0.2  $\mu\text{g}$  groups showed trunk activity that did not differ from SAL controls. The two highest dosages of SKF appeared to produce comparable increases in trunk movements. Mouthing movements were significantly elevated relative to the SAL group only at the 5.5  $\mu\text{g}$  dosage of SKF [drug main effect:  $F(1, 44) = 12.3$ ,  $p < 0.001$ ]. Facial wiping behavior increased following SKF injection at dosages of 0.2  $\mu\text{g}$  [drug  $\times$  time interaction:  $F(9, 351) = 3.3$ ,  $p < 0.001$ ], 1.0  $\mu\text{g}$  [drug  $\times$  time interaction:  $F(9, 351) = 7.6$ ,  $p < 0.001$ ], 5.5  $\mu\text{g}$  [drug  $\times$  time interaction:  $F(9, 396) = 2.8$ ,  $p < 0.01$ ], and 10.0  $\mu\text{g}$  [drug  $\times$  time interaction:  $F(9, 351) = 5.8$ ,  $p < 0.001$ ]. Wiping was virtually absent in SAL controls and in the 0.05  $\mu\text{g}$  SKF group.

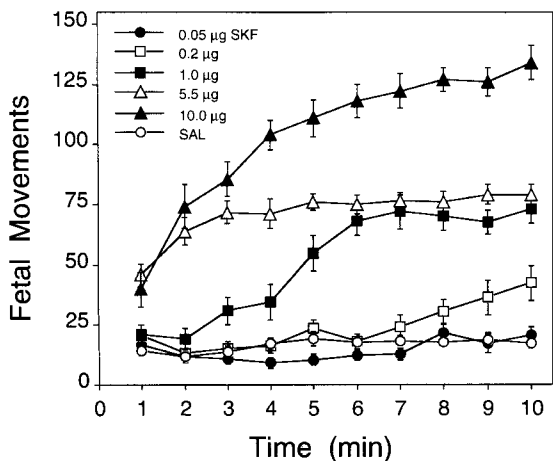


FIG. 1. Overall motor activity of E21 rat fetuses following intracisternal (IC) injection of SKF-38393 (SKF) in dosages of 0.05, 0.2, 1.0, 5.5, or 10.0  $\mu\text{g}$ , or the isotonic saline vehicle (SAL) in Experiment 1. Points depict the mean number of fetal movements per minute; vertical lines show SEM.

### Discussion

The results of this experiment demonstrate that injection of SKF-38393 into the cisterna magna of the E21 rat fetus results in dramatic changes in fetal motor behavior. The three highest dosages of SKF-38393 produce the most pronounced

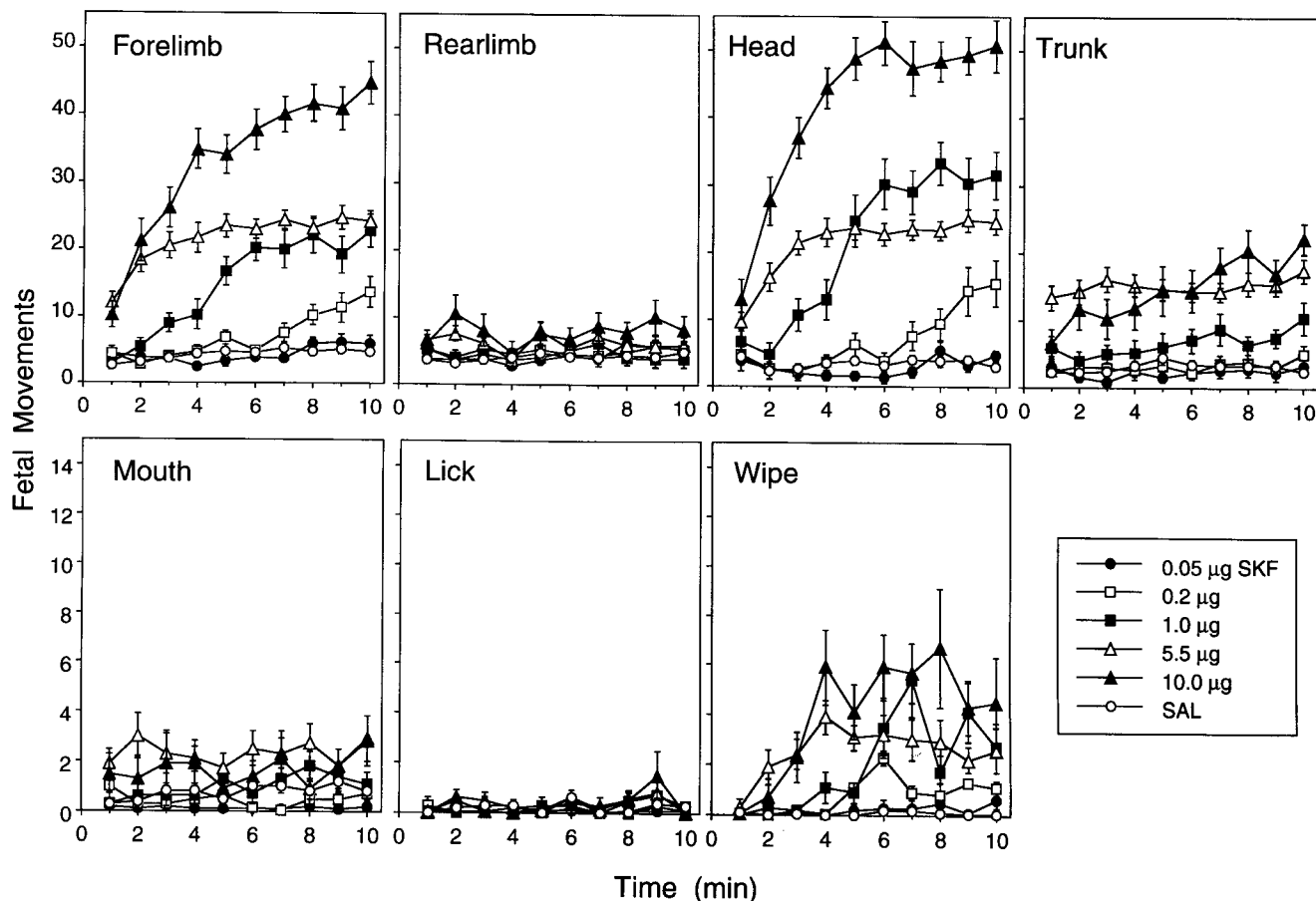


FIG. 2. Changes in seven categories of fetal behavior following IC injection of different dosages of SKF or SAL in Experiment 1. Data for forelimb, rearlimb, head, trunk, mouth, lick, and facial wipe are shown in separate graphs.

effects on fetal behavior, with distinctive changes in movements principally involving rostral parts of the body (head, forelimbs, mouth, facial wiping). The effects of the 1.0 and 5.5  $\mu\text{g}$  groups were very similar, reaching stable levels of activity in forelimb and head movements, which constituted the greatest increase in activity. This contrasted with the 10.0  $\mu\text{g}$  dose, which produced a steady increase in activity over the 10-min observation session. The increase in facial wiping following IC administration of SKF-38393 was particularly noteworthy, because wiping behavior occurs rarely among unmanipulated fetal subjects without explicit sensory stimulation. Peak levels of wiping were evident 3–8 min after injection of SKF-38393.

#### EXPERIMENT 2. IC ADMINISTRATION OF SCH-23390

Experiment 1 confirmed that central administration of a  $D_1$  agonist results in a significant increase in common patterns of fetal behavior, principally including forelimb and head movements. Previous experiments have shown that blockade of  $D_1$  receptors by peripheral administration of the antagonist SCH-23390 also produces behavioral effects in the fetal rat. Unlike agonist effects, IP injection of SCH promoted movements in the perioral region, including mouthing and spontaneous facial wiping (3). The aim of Experiment 2 was to deter-

mine the behavioral effects of different dosages of SCH-23390 when administered into the fetal CNS.

A total of 73 fetuses served as subjects in Experiment 2. Fetuses received a control injection of the isotonic saline vehicle (SAL;  $n = 11$ ), or a drug injection consisting of one of four doses of SCH-23390 (1.0  $\mu\text{g}$  per fetus,  $n = 16$ ; 5.0  $\mu\text{g}$ ,  $n = 16$ ; 10.0  $\mu\text{g}$ ,  $n = 16$ ; 20.0  $\mu\text{g}$ ,  $n = 14$ ). Immediately following IC injection of the SCH solution or saline vehicle, the behavior of fetal subjects was recorded in an 8-min observation session.

#### Results

Overall fetal activity was compared in a two-factor repeated measures ANOVA (four doses of SCH or saline  $\times$  8 min). This analysis indicated no significant main or interaction effects involving the drug factor on overall motor activity ( $p > 0.05$ ), but a significant main effect of time,  $F(7, 476) = 3.6$ ,  $p < 0.001$  (Fig. 3). However, closer examination of individual behavioral categories revealed that SCH did have effects on specific patterns of fetal movement. The two-factor repeated measures ANOVAs that examined changes in the individual categories of fetal movement indicated that IC administration of SCH resulted in modest but significant decreases of forelimb, rearlimb, and head movements, and significant increases in mouthing, licking and facial wiping

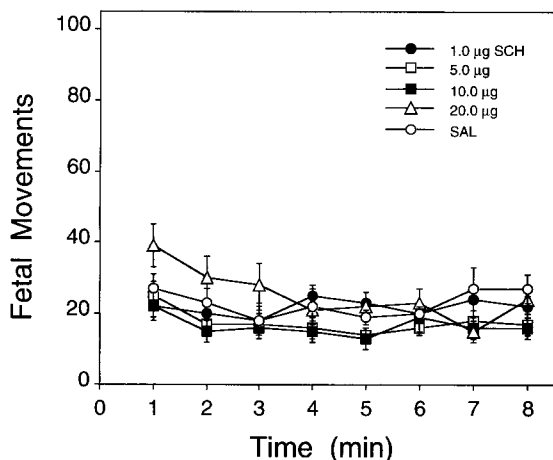


FIG. 3. Overall motor activity following IC injection of SCH-23390 (SCH) in dosages of 1.0, 5.0, 10.0, or 20.0  $\mu$ g, or the isotonic saline vehicle (SAL) in Experiment 2.

movements (Fig. 4). A significant main effect of drug treatment was evident for movements of forelimb,  $F(4, 68) = 4.3$ ,  $p < 0.01$ ; rearlimb,  $F(4, 68) = 9.7$ ,  $p < 0.001$ , head,  $F(4, 68) = 5.5$ ,  $p < 0.001$ , mouthing,  $F(4, 68) = 5.3$ ,  $p < 0.001$ , and facial wiping,  $F(4, 68) = 10.5$ ,  $p < 0.001$ . The significant interaction of drug treatment  $\times$  time was found for licking movements,  $F(7, 456) = 2.5$ ,  $p < 0.001$ . Trunk movements were not affected by IC injection of SCH.

Planned ANOVAs indicated that forelimb movements decreased relative to the SAL group in the 5.0  $\mu$ g [drug main effect:  $F(1, 25) = 11.1$ ,  $p < 0.01$ ], and 10.0  $\mu$ g groups [drug main effect:  $F(1, 25) = 14.8$ ,  $p < 0.001$ ], but did not differ from SAL in the 1.0 and 20.0  $\mu$ g groups. The modest decrease in forelimb movements amounted to 65% and 53% of SAL controls in the 5.0 and 10.0  $\mu$ g groups, respectively. Rearlimb movements were reduced in the 5.0  $\mu$ g [drug main effect:  $F(1, 25) = 37.2$ ,  $p < 0.001$ ], 10.0  $\mu$ g [drug main effect:  $F(1, 25) = 26.6$ ,  $p < 0.001$ ], and 20.0  $\mu$ g groups [drug main effect:  $F(1, 23) = 14.6$ ,  $p < 0.001$ ] relative to the SAL group. The decrease in rearlimb movements amounted to 51% (5.0  $\mu$ g), 43% (10.0  $\mu$ g), and 51% (20.0  $\mu$ g) of the SAL group. Head movements were reduced relative to SAL controls in the 5.0  $\mu$ g [drug main effect:  $F(1, 25) = 10.5$ ,  $p < 0.01$ ], 10.0  $\mu$ g [drug main effect:  $F(1, 25) = 14.9$ ,  $p < 0.001$ ], and 20.0  $\mu$ g groups [drug main effect:  $F(1, 23) = 8.7$ ,  $p < 0.01$ ]. The decrease in head movements amounted to 58% (5.0  $\mu$ g), 47% (10.0  $\mu$ g), and 55% (20.0  $\mu$ g) of the SAL group. Mouthing was elevated relative to SAL at all SCH dosages: 1.0  $\mu$ g [drug main effect:  $F(1, 25) = 9.4$ ,  $p < 0.01$ ], 5.0  $\mu$ g [drug main effect:  $F(1, 25) = 10.5$ ,  $p < 0.01$ ], 10.0  $\mu$ g [drug main effect:  $F(1, 25) = 16.6$ ,  $p < 0.001$ ], 20.0  $\mu$ g [drug main effect:  $F(1, 23) = 17.4$ ,  $p < 0.001$ ]. Mouthing in the SAL group was infrequent, and increased by 400% (1.0  $\mu$ g), 575% (5.0  $\mu$ g), 475% (10.0  $\mu$ g), and 775% (20.0  $\mu$ g) after IC administration of SCH. Licking was elevated only at the 20.0  $\mu$ g dose of SCH [drug treatment  $\times$  time interaction:  $F(7, 161) = 2.8$ ,  $p < 0.01$ ]. However, the increase in Licking in the highest dosage of SCH was pronounced; SAL-treated subjects showed no licking during the first min after injection, whereas fetuses in the 20.0  $\mu$ g group exhibited an average of 7.4 licking movements in the first min. Facial wiping movements were elevated

relative to SAL controls in the 5.0  $\mu$ g [drug main effect:  $F(1, 25) = 24.8$ ,  $p < 0.001$ ], 10.0  $\mu$ g [drug main effect:  $F(1, 25) = 22.6$ ,  $p < 0.001$ ], and 20.0  $\mu$ g groups [drug main effect:  $F(1, 23) = 27.0$ ,  $p < 0.001$ ]. The increase in Wiping amounted to 735% (5.0  $\mu$ g), 875% (10.0  $\mu$ g), and 1005% (20.0  $\mu$ g) of the SAL group.

### Discussion

The absence of drug effects on overall motor activity in Experiment 2 appears to be the result of SCH selectively decreasing movements of forelimbs, rearlimbs, and head while increasing mouthing, licking, and wiping activity. Because increases in perioral movements were most pronounced during the first minutes after IC injection of SCH, the net effect of the drug treatment on overall activity was a modest decrease over time. Although SCH-23390 did produce effects on fetal behavior, they were distinctively different from the effects of the D<sub>1</sub> agonist. In particular, the D<sub>1</sub> antagonist promoted increases only in behavioral patterns in the perioral area: mouthing, licking, and facial wiping. These increases were apparent immediately after IC injection, which further contrasts with the time-dependent increases in facial wiping promoted by SKF-38393.

### EXPERIMENT 3.

#### REVERSAL OF SKF-38393 EFFECTS WITH SCH-23390

Central administration of a D<sub>1</sub> agonist results in distinctive changes in fetal motor behavior. Although central administration of a D<sub>1</sub> antagonist also can influence fetal behavior, SCH-23390 does not result in a pronounced change in overall motor activity and produces behavioral effects that are distinctively different from those seen following administration of SKF-38393. The purpose of Experiment 3 was to confirm that the behavioral effects produced by central administration of SKF-38393 are mediated by the D<sub>1</sub> receptor. The design of the experiment was first to administer SKF-38393 to promote behavioral effects reported in Experiment 1. Five minutes after injection, when the effects of SKF-38393 were equally evident in all three treatment groups, a second IC injection involving SCH-23390 was administered in an attempt to reverse changes in fetal motor behavior induced by the D<sub>1</sub> agonist.

A total of 31 E21 fetuses were used as subjects in Experiment 3. All subject fetuses received an initial IC injection consisting of 5.5  $\mu$ g of SKF-38393. Fetal behavior was observed for 5 min, after which a second IC injection was delivered consisting of the saline vehicle (SAL;  $n = 10$ ), 10.0  $\mu$ g ( $n = 11$ ) or 20.0  $\mu$ g ( $n = 10$ ) of SCH-23390. After the second injection, fetal behavior was recorded for an additional 5 min.

### Results

An initial analysis examined overall fetal activity during the first 5 min of the observation session to confirm the effect of the D<sub>1</sub> agonist on fetal behavior. This two-factor repeated measures ANOVA indicated the significant main effect of time,  $F(4, 112) = 50.3$ ,  $p < 0.001$ . Administration of SKF promoted a pronounced increase in fetal activity (Fig. 5). The increase in fetal activity was comparable to that observed in the 5.5  $\mu$ g SKF group in Experiment 1. Further, the absence of main or interaction effects involving the drug treatment factor confirmed that no differences were evident among the three groups during the first 5 min of the session.

To assess effects of IC administration of the D<sub>1</sub> antagonist, overall fetal activity during the last 5 min of the session was compared in a two-factor repeated measures ANOVA (three

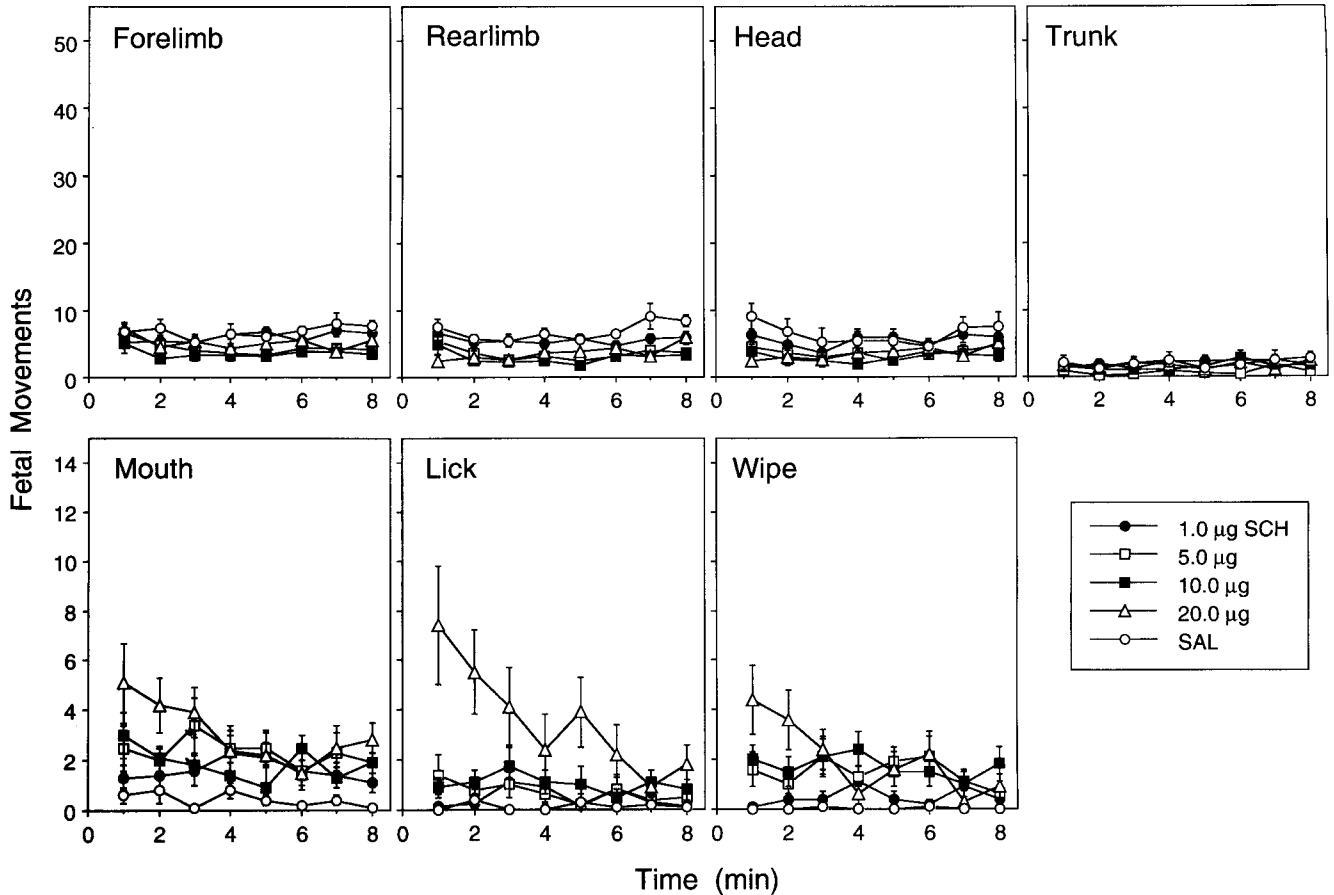


FIG. 4. Changes in seven categories of fetal behavior following IC injection of different dosages of SCH or SAL in Experiment 2. Data for forelimb, rearlimb, head, trunk, mouth, lick, and facial wipe are shown in separate graphs.

drug treatment groups  $\times$  5 min), which indicated the significant interaction of drug treatment and time,  $F(8, 112) = 2.8$ ,  $p < 0.01$ . Subjects in the SAL group continued to exhibit high levels of motor activity through the last 5 min of the session, but decreases in overall activity were evident in both the 10.0  $\mu\text{g}$  and 20.0  $\mu\text{g}$  SCH groups (Fig. 5). To confirm this pattern of difference, a planned ANOVA compared overall activity among the three groups during min 10 of the session (Experiment 1 demonstrated that SKF continues to promote high levels of fetal activity 10 min after IC injection). This one-way ANOVA indicated significant differences in fetal activity during min 10,  $F(2, 28) = 58.8$ ,  $p < 0.001$ . Post hoc comparison of group means by the method of Scheffe revealed that overall activity was reduced in both the 10.0  $\mu\text{g}$  and 20.0  $\mu\text{g}$  SCH groups relative to SAL controls. Further, no difference between the two SCH groups was evident. Qualitative comparison of the level of fetal activity in these two SCH groups with control subjects from Experiments 1 and 2 suggested that fetal movements remained modestly elevated at min 10, but that the effects of the  $D_1$  agonist were completely reversed by IC injection of SCH by the end of the observation session (min 12).

Additional two-factor repeated measures ANOVAs were conducted to assess the effects of SCH on individual movement categories during the last 5 min of the observation session (Fig. 6). Forelimb activity gradually decreased following administration of SCH [drug  $\times$  time interaction:  $F(8, 112) =$

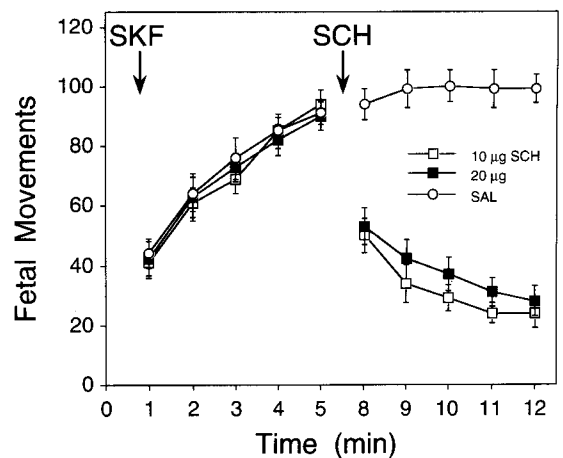


FIG. 5. Overall motor activity in Experiment 3. Fetuses received an IC injection of SKF (5.5  $\mu\text{g}$ ) at the beginning of the session. Five minutes later, subjects received an IC injection of one of two doses of SCH (10.0 or 20.0  $\mu\text{g}$ ) or SAL.

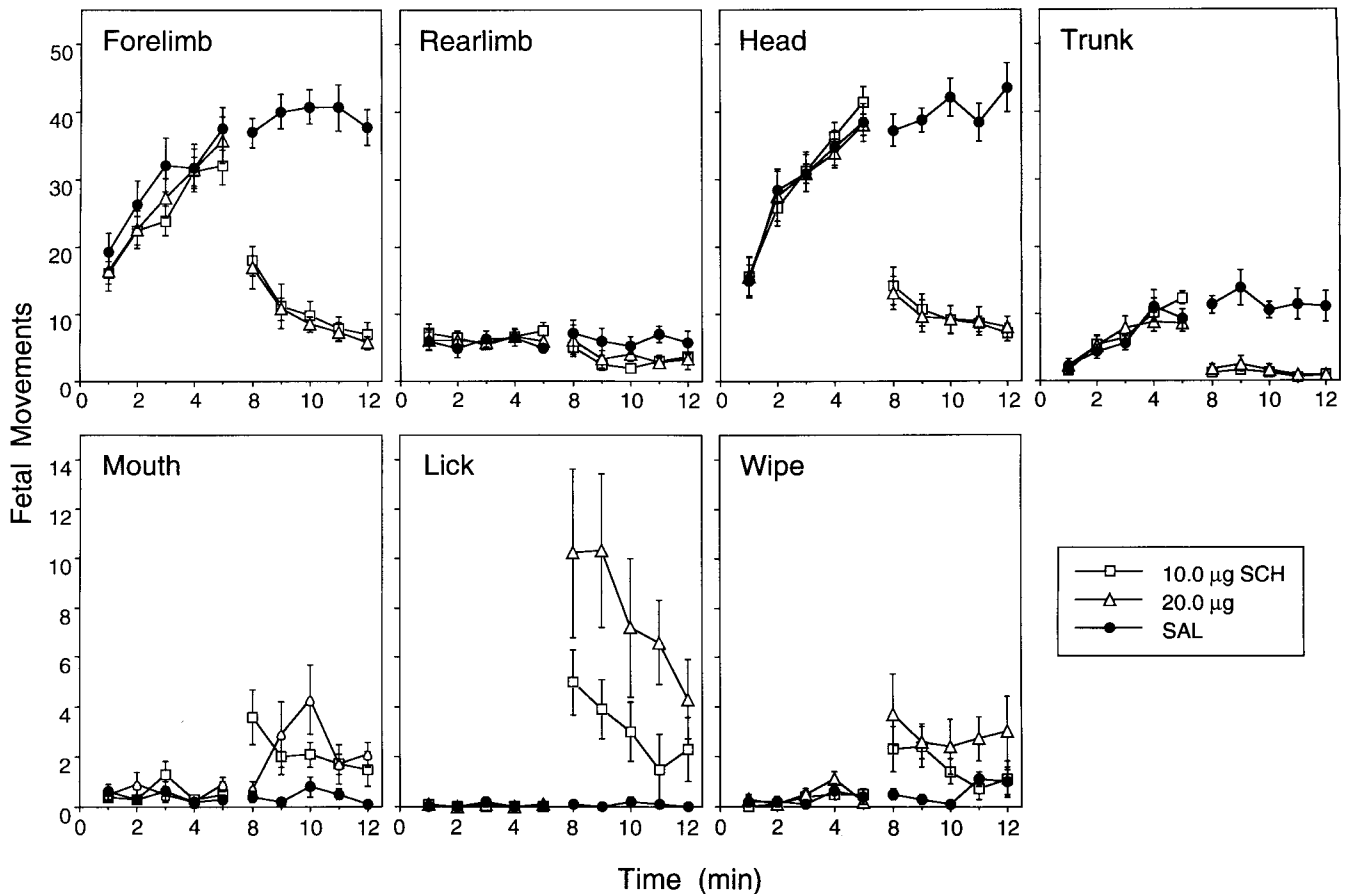


FIG. 6. Changes in seven categories of fetal behavior after IC injection of SKF followed by IC injection of SCH or SAL in Experiment 3. Data for forelimb, rearlimb, head, trunk, mouth, lick, and facial wipe are shown in separate graphs.

3.4,  $p < 0.01$ ). The effect of drug treatment was apparent at min 10,  $F(2, 28) = 78.3$ ,  $p < 0.001$ , with forelimb activity remaining elevated in the SAL control group relative to both the 10.0  $\mu\text{g}$  and 20.0  $\mu\text{g}$  SCH groups ( $p$  values  $< 0.01$ ). Rearlimb activity was not affected by the SCH treatment. Movements of the head also exhibited a significant decrease after SCH injection [drug main effect:  $F(2, 28) = 106.0$ ,  $p < 0.001$ ]. The effect of SCH was evident at min 10,  $F(2, 28) = 66.2$ ,  $p < 0.001$ , with fewer head movements in the 10.0 and 20.0  $\mu\text{g}$  SCH groups than among SAL controls. Trunk movements, which were modestly elevated by SKF, were nearly eliminated by administration of SCH [drug main effect:  $F(2, 28) = 45.9$ ,  $p < 0.001$ ]. An effect of SCH on trunk movements was found at min 10,  $F(2, 28) = 33.6$ ,  $p < 0.001$ , with significantly fewer movements in the 10.0 and 20.0  $\mu\text{g}$  SCH groups compared to SAL ( $p$  values  $< 0.01$ ).

As in Experiment 2, IC administration of SCH appeared to promote increases in mouthing, licking, and facial wiping behavior. Mouthing movements increased in frequency after SCH injection [drug main effect:  $F(2, 28) = 6.5$ ,  $p < 0.01$ ]. This effect was confirmed by a planned one-way ANOVA at min 8, after IC injection of SCH (Experiment 2 demonstrated that SCH promotes increased mouthing, licking, and facial wiping immediately after IC administration). At min 8, a drug effect on mouthing activity was indicated,  $F(2, 28) = 6.9$ ,  $p < 0.01$ , with subjects in the 10.0  $\mu\text{g}$  group showing more

mouthing movements than SAL controls or the 20.0  $\mu\text{g}$  group ( $p$  values  $< 0.01$ ). Licking movements also increased after SCH treatment [drug main effect:  $F(2, 28) = 5.8$ ,  $p < 0.01$ ]. The effect on licking was apparent immediately after SCH injection; at min 8, a drug effect on licking activity was indicated,  $F(2, 28) = 6.9$ ,  $p < 0.01$ , with subjects in the 20.0  $\mu\text{g}$  group showing more licking movements than SAL controls ( $p < 0.01$ ). Facial wiping was elevated over the last 5 min of the session in the SCH groups [drug main effect:  $F(2, 28) = 8.0$ ,  $p < 0.01$ ].

#### Discussion

These findings are consistent with the interpretation that SCH-23390 was effective in reversing the behavioral effects produced by IC injection of SKF-38393 in the fetal rat. The most distinctive effects of SKF-38393, namely the pronounced increases in forelimb and head movements, were eliminated following administration of SCH-23390. Increases in mouthing and licking following injection of SCH-23390 may be attributed to the effects of the D<sub>1</sub> antagonist on fetal behavior, and not to residual effects of the agonist. The rapid increase in facial wiping, which persisted through the last 5 min of the observation session, probably was due to antagonist effects as well; control subjects that received an IC injection of SAL after administration of SKF-38393 showed low levels of facial wiping behavior.

## CONCLUSIONS

The series of experiments reported in this study provides information about the effects of dopaminergic agonist and antagonist drugs administered into the central nervous system of the E21 rat fetus. In Experiment 1, intracisternal injection of SKF-38393, a selective D<sub>1</sub> agonist, resulted in dose-dependent increases in fetal motor behavior. The highest dose stimulated a nearly eightfold increase in the frequency of fetal activity, which principally included movements of the forelimbs, head, and body trunk. Experiment 2 demonstrated that IC injection of SCH-23390, a selective D<sub>1</sub> antagonist, does not appreciably influence overall motor activity in the fetus over a range of doses. But blockade of D<sub>1</sub> receptors does result in behavioral effects, including a modest suppression of common movement patterns (forelimb, rearlimb, and head movements), and expression of other, less common patterns of fetal behavior (mouthing, licking, and facial wiping). Experiment 3 confirmed that the distinctive behavioral effects of SKF-38393 are, indeed, mediated at the D<sub>1</sub> receptor, because IC administration of the D<sub>1</sub> antagonist was effective in completely reversing agonist-induced increases in forelimb, head, and trunk movements. The findings of these three experiments corroborate inferences from peripheral administration of dopaminergic drugs to rat fetuses reported in earlier studies (3,4,11). Additionally, these data provide evidence that the magnitude but not the pattern of behavioral effects produced by pharmacological manipulation of D<sub>1</sub> receptors is influenced by dosage administered into the cisterna magna.

The pronounced increases in fetal movements cannot be attributed to some general increase in neural activity. Administration of SKF-38393 did not result in activation of all categories of fetal movement, but selectively increased the frequency of specific patterns of fetal behavior. In particular, movements of the head, forelimbs, and trunk were dramatically elevated after injection of the D<sub>1</sub> agonist. In contrast, central administration of SCH-23390 did not stimulate head or limb movements, but promoted increases in mouthing and

licking behavior. These unique behavioral effects of SCH-23390 have been described in an earlier report that employed peripheral administration of a single dosage of the drug (3); the present study confirms that similar effects are produced by IC injection of the D<sub>1</sub> antagonist across a range of dosages. Facial wiping behavior, which is commonly expressed in response to sensory stimulation but occurs rarely during non-evoked activity (5), also was observed in all three experiments. This pattern of paw-face contact occurred immediately after IC injection of SCH-23390 in Experiments 2 and 3, but appeared only after a delay of several minutes after injection of SKF-38393. These data imply that facial wiping was specifically activated by blockade of the D<sub>1</sub> receptor, but may have occurred through the adventitious cooccurrence of forelimb and head movements following stimulation of D<sub>1</sub> activity.

It is a widely held view that drugs of abuse can effect prenatal development through their action within the central nervous system (13). This view, however, is seldom subjected to direct empirical scrutiny and is based upon inference from *in vitro* experiments and postnatal evaluation of prenatal manipulations. The findings of the present study confirm that the central nervous system of the E21 rat fetus responds with pronounced and selective changes in motor behavior following central administration of dopaminergic agonist and antagonist drugs. The behavioral effects of SKF-38393 and SCH-23390 indicate that drugs of abuse that alter the dopamine activity, such as cocaine and amphetamine, indeed, can have access to a fetal neuromotor system that is intact and sufficiently mature to transduce changes at the level of receptors into modifications in motor behavior. Premature activation of an intact neuromotor system in the fetus is likely to be a source of lasting effects on behavioral development (8,10).

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