



Asymmetrical Development of the Dopamine System in the Fetal Rat as Indicated by Lateralized Administration of SKF-38393 and SCH-23390

ELENA I. VARLINSKAYA,* EVGENIY S. PETROV,* SCOTT R. ROBINSON†
AND WILLIAM P. SMOTHERMAN†¹

*Pavlovian Physiological Department, Institute for Experimental Medicine,
Russian Academy of Medical Sciences, St. Petersburg, Russia

†Laboratory of Perinatal Neuroethology, Center for Developmental Psychobiology,
Department of Psychology, State University of New York at Binghamton, Binghamton, NY 13902-6000

Received 28 January 1994

VARLINSKAYA, E. I., E. S. PETROV, S. R. ROBINSON AND W. P. SMOTHERMAN. *Asymmetrical development of the dopamine system in the fetal rat as indicated by lateralized administration of SKF-38393 and SCH-23390*. PHARMACOL BIOCHEM BEHAV 50(3) 359-367, 1995.—The dopamine D₁ agonist SKF-38393 and the D₁ antagonist SCH-23390 were administered into the left or right cerebral hemisphere of the rat fetus on E21 of gestation. Intrahemispheric (IH) injection of the agonist promoted a large-magnitude increase in fetal motor behavior, which involved movements of the head, limbs, and body trunk. Although no lateral asymmetries were evident in left or right injections of the agonist, IH injection of the antagonist into the fetus's left hemisphere produced more pronounced effects on oral behavior, including mouth, lick, and facial wipe movements. Administration of SCH-23390 into the same hemisphere as SKF-38393 was effective in reversing the behavioral effects of the agonist, with left IH injections showing more immediate and complete blockade of agonist-induced behavioral activation. These data provide evidence for functional asymmetries in D₁ receptors of the dopamine system in the term rat fetus.

SKF-38393 SCH-23390 D₁ Dopamine Rat fetus Brain asymmetry

TECHNIQUES that permit delivery of drugs directly into the central nervous system of the rat fetus have recently provided new information about the prenatal development of different neurochemical systems. Selective agonists of dopamine (18), opioid (10,16), or vasopressin systems (17) have been shown to produce large-magnitude changes in motor behavior of fetal subjects on the last 2 days of gestation (E20-E21). For example, SKF-38393, a selective agonist of the D₁ dopamine receptor, when administered into the cisterna magna of the rat fetus, results in dosage-dependent increases in motor activity. In contrast, intracisternal injection of the D₁ antagonist SCH-23390 has little effect on overall motor activity, but promotes distinctive patterns of oral behavior, including mouthing, lick-

ing, and wiping the forelimbs over the surface of the head (facial wiping). SCH-23390 also is effective in reversing the behavioral effects promoted by intracisternal injection of SKF-38393 (18). These findings confirm that D₁ receptors of the fetal dopamine system are functional and capable of engaging the motor system during the late prenatal period.

A body of evidence obtained has suggested that many neurochemical systems exhibit distributions or functional organizations that differ between left and right sides of the brain. Lateral asymmetry in the dopamine system has been reported to vary among individual rats (12), and to contribute to individual differences in lateralized behavior (e.g., rotational behavior, tail posture, paw preference), stress reactivity, and

¹ Requests for reprints should be addressed to William P. Smotherman, Ph.D., Laboratory of Perinatal Neuroethology, Department of Psychology, P.O. Box 6000, Binghamton University, Binghamton, NY 13902-6000.

sensitivity to drugs. Most of the available information on asymmetry in the dopamine system derives from studies of adult rodents (3,4,6); relatively little work has been done to assess functional asymmetries in developing animals. One noteworthy exception is the tendency for neonatal rats to exhibit postural asymmetry while in a prone position. Postural preference typically is measured by placing the pup on a substrate in a bilaterally symmetrical starting position and noting whether the pup's tail is shifted to the left or right of the midline. Lateral bias in tail posture has been shown to be predictive of asymmetric spatial behavior in adulthood (11,13). Further, postural lateralization in neonates has been shown to correlate with asymmetry in dopamine activity in adult rats (1,11). Although lateral asymmetry *per se* was not assessed in fetal subjects, prenatal stress induced by manipulation of pregnant rats can enhance the expression of lateral asymmetries in the dopamine system of adults (5). Collectively, these studies suggest that functional asymmetries in the dopamine system may have their ontogenetic origin in the prenatal period.

The purpose of the present study was to investigate the early ontogeny of lateralized differences in the dopamine system of the rat fetus. Individual fetal subjects received injections of selective D₁ dopamine agonist (SKF-38393) or antagonist (SCH-23390) into the left or right cerebral hemisphere in the vicinity of the lateral ventricle. Behavioral effects of these prenatal manipulations were assessed by observation of the motor behavior of fetuses *in vivo*. Specifically, these experiments examined whether (a) lateralized administration of SKF-38393 or SCH-23390 is effective in promoting changes in fetal motor behavior, (b) whether left or right injections of the D₁ agonist or antagonist differ in their effects on fetal behavior, and (c) whether ipsilateral injection of SCH-23390 is effective in reversing the behavioral effects promoted by intrahemispheric injection of SKF-38393.

GENERAL METHOD

Subjects

Fetuses produced in timed matings of pregnant Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) served as experimental subjects. A total of 191 fetal subjects derived from 61 pregnancies were used in the three experiments of this study. Breeding female rats were housed in groups of three in plastic breeding cages (36 × 47 × 20 cm) and were time mated by introducing one male rat into the cage. Vaginal smears were collected daily during a 4-day breeding period to date conception; embryonic day 0 (E0) was defined as the first day sperm were detected (birth occurs on E21.5). Pregnant rats were maintained on a 12L : 12D cycle (lights on at 0700 h) under conditions of constant room temperature (22°C) 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.

Prenatal Preparation

Pregnant rats were surgically prepared on E21 of gestation for testing of fetal subjects. The rat was placed under ether anesthesia and 100% ethanol (70 µl) was injected into the spinal canal between the first and second lumbar vertebrae to produce a chemical transection of the spinal cord. 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. After chemomyelotomy, the prepared rat 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 monitored visually throughout the experiment to ensure completeness of the spinal transection. Access to fetal subjects was provided by exteriorizing the uterus through a low midline incision in the rat's abdomen. Individual fetuses selected as experimental subjects were externalized through a small incision in the uterus and the embryonic membranes were removed. Approximately the same proportion of male and female fetuses were used as subjects in each treatment group within the three experiments. [The sex of each fetus was determined by examination of anogenital distance, which is a reliable indicator of gender on E21 (2).] The fetal subject was attached to the placenta, which remained inside the uterus. Subjects remained under the water surface throughout experimentation and were monitored to ensure that they remained pink and showed no behavioral indications of hypoxia, including lateral trunk curls (14). 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 (7). The order of testing subjects and their location within the uterus were randomized among the treatments in each pregnancy. After surgical preparation, a 20-min period elapsed before behavioral testing (15).

Central Administration of D₁ Agonist and Antagonist

Solutions containing various dosages of the selective D₁ agonist SKF-38393 (SKF, a selective D₁ agonist), SCH-23390 (SCH, a selective D₁ antagonist), or isotonic saline (SAL) were administered into the central nervous system of individual fetal subjects (drugs obtained from Research Biochemicals Inc., Natick, MA). The dosages of SKF (5.5 µg/subject) and SCH (5.5 µg) used in this study were determined by dose-response studies (18). All drug or vehicle solutions were administered to individual fetal subjects by intrahemispheric (IH) injection. To deliver IH injections, drug solutions were loaded into the terminal section of transparent polyethylene tubing (PE-10). A 30-ga hypodermic needle at the end of this tubing was inserted under visual guidance into the subject's left or right cerebral hemisphere to a depth of 1.5 mm at a point located 1.0 mm anterior and 1.0 mm lateral from bregma. These coordinates correspond to the location of the lateral ventricle in the brain of the term rat fetus (E21-P0) (9). A fixed volume (1.0 µl) of the drug or vehicle solution was injected in a pulse lasting 8–10 s. Additional details about this procedure for central administration of drugs to fetal subjects have been described previously (17). Drug 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

Effects of drug treatment were assessed by scoring the motor behavior of fetuses over an experimental session lasting 5–12 min. The observer noted each instance of fetal movement and called the appropriate behavioral category to an assistant, who entered the category of behavior into a microcomputer-based event recorder. Because the assistant conducted the drug administrations, the observer remained blind to drug treat-

ments received by each fetal subject. Seven categories of fetal behavior were distinguished: 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 (ventriflexion or lateral flexion of the body trunk), mouth (opening and closing of the mouth), lick (protrusion of the tongue outside the oral cavity), and wipe [placement of one or both forepaws against the side of the head and movement of the forepaw(s) in a rostral direction, in contact with the face]. No attempt was made to distinguish movements directed toward or involving body parts on the left or right side of the body. The sum of movement events in all seven categories was used as a measure of overall fetal activity. The event recording system also recorded the time of each data entry, thereby providing information about the frequency and timing of fetal movements. We have reported that this observation and recording protocol is highly consistent between observation sessions (reliability > 0.90) (15).

Data Analysis

In each experiment, a series of statistical analyses was conducted to assess the effects of drug treatment and site of injection. 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 tests for simple main effects to further characterize treatment effects. 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, an alpha level of $p < 0.01$ was used to judge statistical significance.

EXPERIMENT 1. INTRAHEMISPHERIC ADMINISTRATION OF SKF-38393

Peripheral administration or injection of SKF-38393 into the cisterna magna of the rat fetus results in dose-dependent increases in overall fetal motor activity. Peripheral administration of the D_1 agonist resulted in elevated movements of the forelimbs, rearlimbs, and head (8), whereas central injection promoted head and forelimb activity but no change in rearlimb movement (18). These findings confirm that peripheral or intracisternal injection of dopaminergic drugs can engage D_1 receptors to bring about changes in fetal motor behavior. Both methods of drug administration are likely to have resulted in equivalent stimulation of receptors located in left and right cerebral hemispheres. The objective of Experiment 1 was to administer the D_1 agonist SKF-38393 into the left or right hemisphere to provide information about possible asymmetries in the developing dopamine system of the fetal rat.

Method

A total of 71 fetuses from 22 pregnancies served as subjects in Experiment 1. Each subject received a single IH injection at the beginning of the test session consisting of the isotonic saline vehicle (SAL) or SKF-38393 (SKF) administered into the left or right hemisphere. The combination of drug and site of injection thus yielded four treatment groups: SAL-Left ($N = 10$ subjects), SAL-Right ($N = 10$), SKF-Left ($N = 26$), and SKF-Right ($N = 25$). Immediately following IH injection, the behavior of fetal subjects was recorded in a 10-min observation session.

Results

Overall fetal activity was compared in a three-factor repeated-measures ANOVA (SKF or SAL \times left or right injection \times 10 min). This analysis indicated the significant interaction of drug treatment and time, $F(9, 603) = 7.2, p < 0.001$. Post hoc tests for the simple main effect of drug treatment for each minute of the session indicated significant differences between SKF and SAL, regardless of side of injection, during minutes 2–10 ($p < 0.01$). Tests for the simple main effect of time indicated that overall activity increased in subjects treated with SKF ($p < 0.001$), but not in SAL-treated subjects. Intrahemispheric injection of SKF resulted in three- to fourfold increases in motor activity that were evident within 1–2 min after injection and persisted without decline for the remainder of the 10-min session (Fig. 1). The increase in activity was evident following both left and right IH injection of SKF.

Three-factor repeated-measures ANOVAs also were conducted for each of the seven categories of fetal movement (Fig. 2). A significant interaction of drug treatment \times time was evident for movements of head, $F(9, 603) = 13.7, p < 0.001$, and forelimb, $F(9, 603) = 5.4, p < 0.001$. Main effects of drug treatment were evident for rearlimb, $F(1, 67) = 17.7, p < 0.001$, trunk, $F(1, 67) = 17.6, p < 0.001$, mouth, $F(1, 67) = 8.8, p < 0.01$, and wipe, $F(1, 67) = 27.8, p < 0.001$. Post hoc tests for simple main effects indicated that head movements of SKF-treated fetuses were elevated relative to the SAL group during minutes 2–10 ($p < 0.01$). Head movements also changed with time in SKF-treated subjects ($p < 0.01$), but not in SAL-treated subjects. SKF also resulted in an increase in forelimb movements relative to SAL during minutes 4–10 ($p < 0.01$). Forelimb movements increased with time only in subjects treated with SKF ($p < 0.01$). Trunk, mouth, and wiping movements were elevated after IH injection of SKF relative to SAL controls ($p < 0.01$). However, rearlimb movements decreased following IH injection of SKF compared to SAL-treated subjects ($p < 0.01$). These analyses indicated that IH administration of SKF resulted in significant increases of head, forelimb, rearlimb, trunk, mouth, and wiping, but no change in licking. There was no evidence for signif-

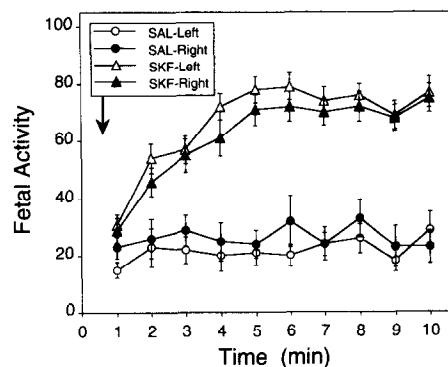


FIG. 1. Overall motor activity of E21 rat fetuses following left or right intrahemispheric (IH) injection of SKF-38393 (SKF; 5.5 μ g per subject) or the isotonic saline vehicle (SAL) in Experiment 1. Points depict the mean number of fetal movements per minute; vertical lines show SEM. Injection of SKF-38393 into either the left or right hemisphere resulted in significant, threefold increases in fetal activity that were evident 2 min after injection and persisted through the end of the 10-min test session.

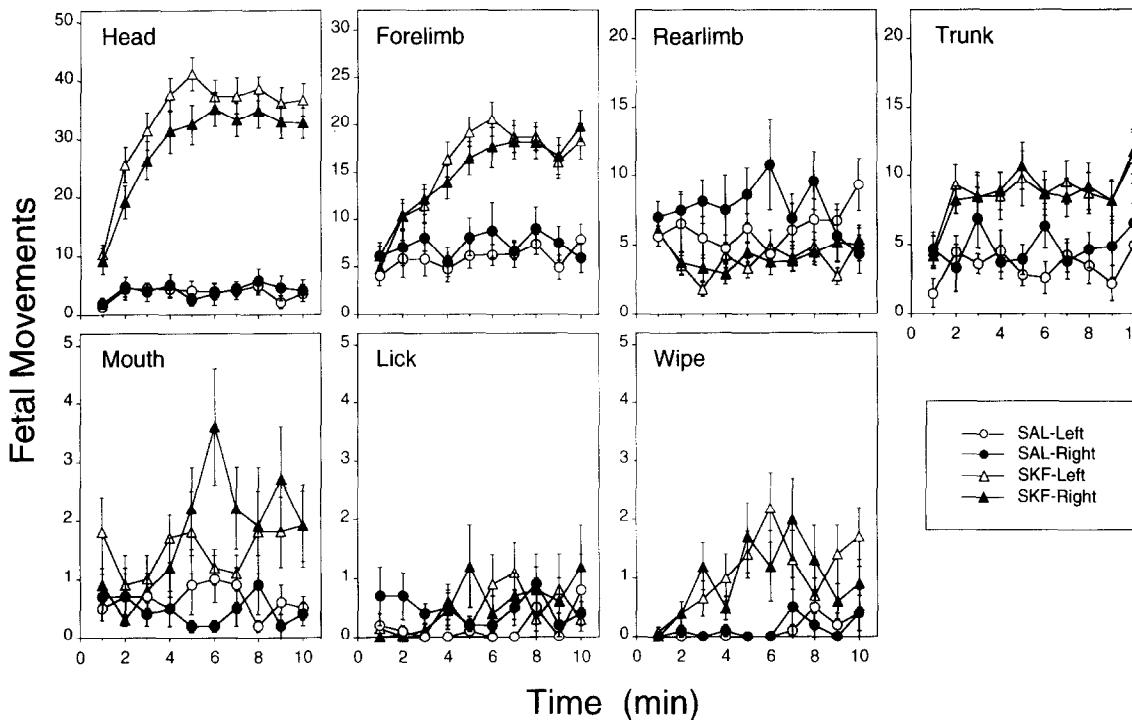


FIG. 2. Changes in seven categories of fetal behavior following left or right IH injection of different dosages of SKF or SAL in Experiment 1. Data for head, forelimb, rearlimb, trunk, mouth, lick, and wipe are shown in separate graphs. Left or right IH injections of SKF-38393 promoted significant increases in head, forelimb, trunk, mouth, and wipe movements, and a reduction of rearlimb movements.

ificant main or interaction effects involving the side of IH injection, suggesting no differences in the behavioral effects of SKF administered into the left or right hemispheres.

Discussion

The results of this experiment demonstrate that intrahemispheric injection of SKF-38393 produces pronounced increases in overall motor activity in the E21 rat fetus. The increase in activity is primarily attributable to elevated head and forelimb movements, with smaller increases occurring in trunk, mouth, and wiping behavior. The cerebrum of the rat fetus is still undergoing rapid growth and differentiation on E21 of gestation, yet administration of a selective D_1 dopamine agonist into the hemisphere is effective in producing almost immediate effects on fetal motor behavior. Moreover, SKF-38393 exerted effects on fetal motor activity after administration into either hemisphere, providing no evidence for lateralization in the ability of D_1 receptors to produce changes in fetal behavior.

EXPERIMENT 2. INTRAHEMISPHERIC ADMINISTRATION OF SCH-23390

Intraperitoneal or intracisternal administration of SCH-23390 can promote modest effects on fetal motor behavior. Both peripheral and central injections of the D_1 antagonist increase categories of movement in the perioral region of the fetus, including elevated mouth, licking, and facial wiping behavior (8,18). These findings demonstrate that the term rat fetus exhibits endogenous activity in the dopamine system and that blockade of endogenous activity at D_1 receptors promotes the expression of perioral behavior. The objective of Experi-

ment 2 of this study was to administer the D_1 antagonist SCH-23390 into the left or right hemisphere to provide information about asymmetries in endogenous activity at D_1 receptors in the fetal rat. This experiment provided necessary information preliminary to using the D_1 antagonist to reverse behavioral effects of SKF-38393, reported below (Experiment 3).

Method

A total of 80 fetuses from 22 pregnancies served as subjects in Experiment 2. Each subject received a single IH injection at the beginning of the test session consisting of the isotonic saline vehicle (SAL) or SCH-23390 (SCH) administered into the left or right hemisphere. The combination of drug and site of injection thus yielded four treatment groups: SAL-Left ($N = 20$ subjects), SAL-Right ($N = 20$), SCH-Left ($N = 20$), and SCH-Right ($N = 20$). Immediately following IH injection, the behavior of fetal subjects was recorded in a 5-min observation session.

Results

Overall fetal activity was compared in a three-factor repeated-measures ANOVA (SCH or SAL \times left or right injection \times 5 min). This analysis indicated the significant interaction of drug treatment and site of injection, $F(1, 76) = 7.3, p < 0.01$, and the main effect of time, $F(4, 304) = 5.1, p < 0.001$. Post hoc tests for the simple main effect of drug treatment separately for left and right IH injections indicated a significant difference between SCH and SAL following injection into the subject's left hemisphere ($p < 0.001$), but no difference between SCH and SAL injected into the right hemisphere. Tests for the simple main effect of site of injection

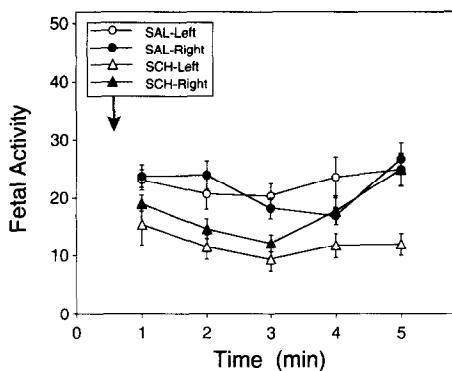


FIG. 3. Overall motor activity following left or right IH injection of SCH-23390 (SCH; 5.5 μ g per subject) or the isotonic saline vehicle (SAL) in Experiment 2. A small but significant decrease in fetal activity was evident following injection of SCH-23390 into the left, but not the right, hemisphere. A main effect of time was also indicated, with the lowest levels of fetal activity expressed during minute 3.

indicated that overall activity differed between left and right injections only in subjects that received an IH injection of SCH ($p < 0.001$), but not in SAL-treated subjects. These post hoc analyses indicate that SCH resulted in an overall decrease in fetal motor activity only when administered into the left hemisphere (Fig. 3). Post hoc comparisons of fetal activity at each minute after injection (collapsed across groups) indicated a small but significant reduction in fetal activity at minute 3 relative to minute 5 ($p < 0.01$).

Three-factor repeated-measures ANOVAs were conducted

for each of the seven categories of fetal movement (Fig. 4). A significant interaction of drug treatment \times site of injection was evident for movements of Forelimb, $F(1, 76) = 12.5$, $p < .001$. Post hoc tests for simple main effects indicated that forelimb movements were depressed relative to the SAL group in subjects that received an IH injection of SCH into either the left or right hemisphere ($p < 0.001$). Differences in forelimb activity after IH injection were evident only after administration of SCH ($p < 0.001$), not SAL. Main effects of drug treatment were indicated for head, $F(1, 76) = 35.4$, $p < 0.001$, trunk, $F(1, 76) = 10.3$, $p < 0.01$, mouth, $F(1, 76) = 43.9$, $p < 0.001$, and lick, $F(1, 76) = 46.7$, $p < 0.001$, with SCH resulting in significant decreases in head, rearlimb, and trunk movements, and significant increases in mouth and lick movements. Main effects of time after injection were found for head, $F(4, 304) = 5.1$, $p < 0.001$, forelimb, $F(4, 304) = 3.5$, $p < 0.01$, and rearlimb, $F(4, 304) = 8.8$, $p < 0.001$. Post hoc comparisons of different times after injection (collapsed across groups) revealed that head, forelimb, and rearlimb movements were suppressed at minutes 3 and 4 relative to minute 1 ($p < 0.01$). The significant interaction of drug treatment \times time was found for wipe, $F(4, 304) = 3.6$, $p < 0.01$. Tests for simple main effects on wiping movements indicated that wiping was elevated following IH administration of SCH during minutes 2, 4, and 5 relative to SAL-injected subjects ($p < 0.001$). The simple effect of time was indicated only for subjects treated with SCH ($p < 0.001$).

Discussion

The results of this experiment demonstrate that IH injection of SCH-23390 results in significant changes in motor behavior in the E21 rat fetus. The IH administration of the D₁

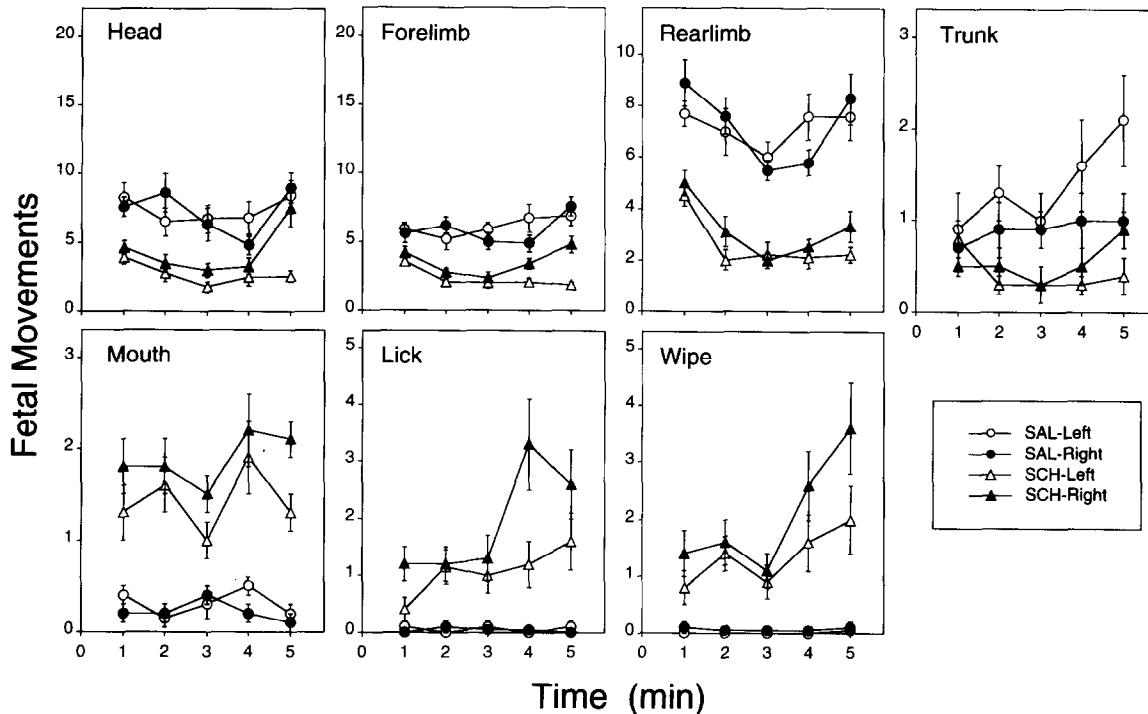


FIG. 4. Changes in seven categories of fetal behavior following left or right IH injection of different dosages of SCH or SAL in Experiment 2. Data for head, forelimb, rearlimb, trunk, mouth, lick, and wipe are shown in separate graphs. Administration of SCH-23390 into the left or right hemisphere resulted in significant decreases in head, forelimb, rearlimb, and trunk movements, and significant increases in mouth, lick, and wipe.

antagonist depressed movements of the head and appendages, but increased activity in the perioral region of the fetus (mouth, lick, and wipe). However, the effects of the D_1 antagonist on fetal motor activity, although significant, were modest in magnitude relative to the pronounced increases in activity that result from IH administration of the D_1 agonist (Experiment 1), and were evident only when SCH was injected into the left cerebral hemisphere. Experiment 2 thus provided evidence for asymmetric effects of SCH-23390 on fetal motor behavior. Overall activity was reduced when SCH was injected into the left hemisphere of the fetus, but not when administered into the right hemisphere. The pattern of reduced activity in fetuses receiving left IH injections was consistently expressed across head, forelimb, rearlimb, and trunk movements. Where SCH-23390 resulted in elevated fetal movements (mouth, lick, wipe), fetuses that received left IH injections showed smaller increases in activity. Across all seven categories, therefore, consistent but small differences in activity—with less activity following left IH injection—were cumulatively responsible for the asymmetrical pattern of effects on overall motor activity. Whereas intrahemispheric injection of SKF-38393 provided no evidence for differences in sensitivity of left and right hemispheres to exogenous stimulation of D_1 receptors (Experiment 1), differences in fetal behavior following IH injection of SCH-23390 suggests that endogenous activity at D_1 receptors differs between left and right cerebral hemispheres in the term rat fetus. Alternatively, asymmetrical effects of SCH-23390 may be due to differences in the action of the D_1 antagonist in left and right hemispheres and may not be reflective of endogenous dopamine activity.

EXPERIMENT 3. REVERSAL OF INTRAHEMISPHERIC SKF-38393 EFFECTS WITH IPSILATERAL ADMINISTRATION OF SCH-23390

Previous experiments with fetal subjects have confirmed that changes in behavior promoted by SKF-38393 are mediated by activity at the D_1 receptor. Peripheral administration of SCH-23390 can block behavioral effects by SKF-38393 (8). Similarly, elevated motor activity resulting from central administration of SKF-38393 can be reversed by injection of SCH-23390 into the cisterna magna (18). Data presented in Experiment 1 of this study confirmed that SKF-38393 also promotes large-magnitude increases in fetal activity when injected directly into the cerebral hemisphere of the rat fetus, and that administration of the D_1 agonist into the left or right hemisphere produces similar effects. However, the findings of Experiment 2, in which SCH-23390 was injected into the left or right hemisphere of the fetus, suggested asymmetric behavioral effects resulting from blockade of D_1 receptors. It is possible that these results are indicative of left-right differences in endogenous activity in the dopamine system, or that SCH-23390 exerts quantitatively or qualitatively different effects in the two hemispheres. The objective of Experiment 3 in this study was to provide further information about possible asymmetries in the functioning of the dopamine system in the term rat fetus by administering the D_1 agonist and D_1 antagonist into the same cerebral hemisphere.

Method

A total of 40 fetuses from 17 pregnancies served as subjects in Experiment 3. Each subject received a single IH injection at the beginning of the test session consisting of SKF-38393 (SKF) administered into the left or right hemisphere. Fetuses were observed for 5 min after SKF injection, then received a second IH injection consisting of SCH-23390 (SCH) into the

same hemisphere as the original SKF injection (ipsilateral hemisphere), yielding two experimental groups (SCH-Left, $N = 12$; SCH-Right, $N = 12$). Two additional control groups involved IH injection of SAL into the ipsilateral hemisphere (SAL-Left, $N = 8$; SAL-Right, $N = 8$). The purpose of these control groups was to confirm that SKF would continue to promote elevated motor activity after a second IH injection. IH injection of SCH or SAL occurred during a 2-min period (minutes 6–7 of the test session), which was followed by an additional 5-min period of observation.

Results

A preliminary analysis was conducted over the first 5-min period of observation (after SKF injection) to confirm that IH injection of SKF resulted in an increase in fetal motor activity. The two-factor repeated-measures ANOVA (four groups \times 5 min) assessing changes in overall activity indicated the significant main effect of time, $F(4, 144) = 103.3, p < 0.001$. SKF promoted a sharp increase in fetal activity that was similar to the effects reported in Experiment 1 (Fig. 5). Significant SKF-induced increases in head, $F(4, 144) = 97.0, p < 0.001$, forelimb, $F(4, 144) = 76.8, p < 0.001$, trunk, $F(4, 144) = 37.6, p < 0.001$, and wipe, $F(4, 144) = 3.7, p < 0.01$, also were indicated. Trunk movements were somewhat reduced in subjects that would later receive SCH injection compared to subjects that would receive SAL, as suggested by the main effect of group, $F(3, 36) = 4.5, p < 0.01$. There was no indication that SKF exerted different effects on fetal behavior when administered into the left or right hemisphere. Therefore, SKF administration into the left or right hemisphere of the fetus resulted in pronounced increases in motor activity that continued to be expressed immediately before the second IH injection.

To assess the effects of the second injection on SKF-induced increases in fetal movement, motor activity during minutes 8–12 was compared in a two-factor repeated-measures ANOVA (four groups \times 5 min of observation). This analysis revealed the significant interaction of groups \times time, $F(4, 144) = 94.3, p < 0.001$. Post hoc tests for simple main ef-

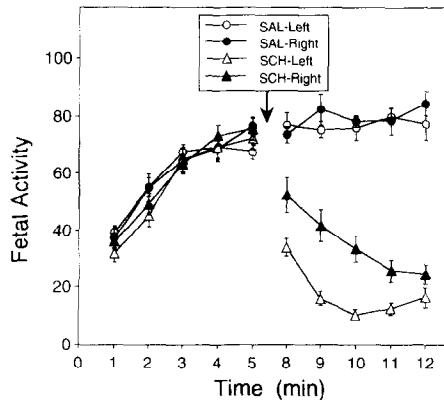


FIG. 5. Overall motor activity in Experiment 3. Fetuses received a left or right IH injection of SKF (5.5 μ g) at the beginning of the session. Five minutes later, subjects received an ipsilateral IH injection of SCH (5.5 μ g) or SAL. Fetal activity increased after IH administration of SKF-38393 (minutes 1–5), but decreased significantly after a second injection involving SCH-23390. The D_1 antagonist had a significantly greater effect when administered into the left hemisphere of the fetus, which was evident during minutes 8–11.

fects of group indicated significant differences in fetal activity during all 5 min after the second IH injection ($p < 0.001$). During minutes 8–12, fetal activity remained high in both control groups (SAL-Left, SAL-Right), suggesting that SKF continued to promote high levels of motor activity throughout the test session. Injection of SCH into the left hemisphere resulted in an immediate, pronounced decrease in fetal motor activity relative to SAL-injected controls. Injection of SCH into the right hemisphere initially resulted in a more modest decrease in motor activity (minutes 8–11), and reached a reduction in activity equivalent to the SCH-Left group by minute 12.

A series of two-factor repeated-measures ANOVAs was conducted for each of the seven categories of fetal movement (Fig. 6). The significant interaction of groups \times time was indicated for movements of head, $F(12, 144) = 7.6, p < 0.001$, and forelimb, $F(12, 144) = 5.5, p < 0.001$. The pattern of effects on these two behavioral categories was nearly identical to that described above for overall activity: SCH promoted a more pronounced decrease in movements of head (minutes 8–11) and forelimb (minutes 8–10) when injected into the left hemisphere ($p < 0.01$). The main effect of group was significant for trunk movements, $F(3, 36) = 89.4, p < 0.001$, with pronounced decreases in trunk movements evident in both the SCH-Left and SCH-Right groups. The main effect of groups also was indicated for mouth movements, $F(3, 36) = 8.7, p < 0.001$. Pairwise comparisons of mouthing between groups revealed that mouthing was significantly elevated only in subjects that received an IH injection of SCH into the right hemisphere ($p < 0.01$). The main effect of groups was indicated for lick, $F(3, 36) = 5.2, p < 0.01$, with more licking ex-

pressed by fetuses that received IH injection of SCH into the right hemisphere ($p < 0.01$). The main effect of groups was found for wipe, $F(3, 36) = 15.6, p < 0.001$, with more wiping movements expressed in subjects that received IH injection of SCH in the left hemisphere ($p < 0.01$).

Discussion

The results of Experiment 3 confirm that IH injection of SCH-23390 is effective in reversing the behavioral effects of the D_1 agonist, SKF-38393, administered into the same cerebral hemisphere. SKF injection into either the left or right hemisphere promoted large-magnitude increases in overall activity, including movements of head, forelimbs, and body trunk, which were sharply reduced by IH injection of SCH. However, this experiment also provides further evidence for asymmetries between left and right hemispheres in the effects of the D_1 antagonist. IH injection of SCH into the left hemisphere resulted in more immediate and more pronounced decreases in head, forelimb, and trunk movements (the principal patterns increased after SKF injection), and a significant increase in wiping movements. Conversely, injection of SCH into the right hemisphere resulted in elevated mouth and lick movements relative to subjects receiving left hemisphere injection of SCH or control injections of SAL. Because the asymmetric effects of SCH-23390 in Experiment 3 were observed following pharmacological stimulation of D_1 receptors with SKF-38393, it seems unlikely that they can be attributed to differences in endogenous dopamine activity in the left and right hemispheres. Rather, alternative explanations should be

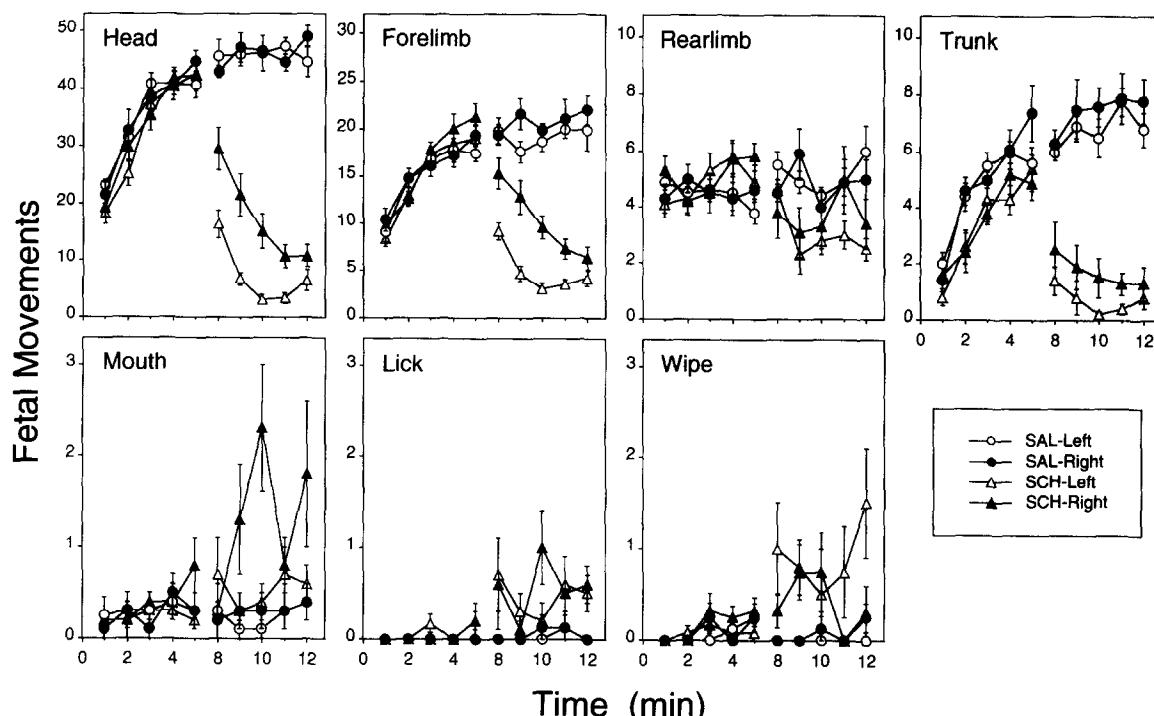


FIG. 6. Changes in seven categories of fetal behavior after left or right IH injection of SKF followed by ipsilateral IH injection of SCH or SAL in Experiment 3. Data for head, forelimb, rearlimb, trunk, mouth, lick, and wipe are shown in separate graphs. The asymmetrical effects of SCH-23390 were evident in a significantly greater reduction of head and forelimb movements after IH injection into the left hemisphere, a greater increase of mouth and lick movements after injection into the right hemisphere, and a greater increase in wipe movements after injection into the left hemisphere.

sought to explain differences in the effects of SCH-23390 in the left and right hemispheres of the rat fetus.

CONCLUSIONS

The present study contributes to a growing literature that the dopamine system is functional during the prenatal period and can exert effects on motor behavior of the developing rat fetus. Administration of the selective D₁ agonist SKF-38393 into cisterna magna (18) or laterally into one of the cerebral hemispheres (this study) results in immediate, sustained, large-magnitude increases in motor activity. The principal behavioral categories that contribute to this increase in movement are head, forelimbs, and body trunk. In fact, the behavioral effects resulting from IH or intracisternal administration of SKF-38393 are very similar. One possible interpretation of this similarity is that the agonist engages the same population of D₁ receptors to bring about changes in fetal motor activity after left IH, right IH, or intracisternal injection. It is unlikely that the similarity in effects of left and right IH injection are due to ceiling effects; higher doses of SKF-38393 have been shown to promote motor activity 50% greater than the levels reported in this study (18). Central administration of the selective D₁ antagonist SCH-23390 also produces behavioral effects in the E21 rat fetus. However, blockade of D₁ receptors appears to exert slightly different behavioral effects that depend upon the site of injection. In a previous study, we reported that intracisternal injection of SCH-23390 increases mouth, lick, and wipe movements, but exerts little influence on other categories of fetal activity. Specifically, a range of doses of the D₁ antagonist (1.0–20.0 µg per subject) administered into the cisterna magna provided no evidence for reduced fetal activity. In contrast, Experiment 2 of this study demonstrated that injection of SCH-23390 into the left or right cerebral hemisphere resulted in significant reductions of head, forelimb, rearlimb, and trunk movements, with slightly greater reductions occurring after IH injection into the left hemisphere. Qualitative differences between IH and intracisternal administration of SCH-23390, and quantitative differences between left and right IH injections of this drug, suggest that the D₁ antagonist may engage different populations of D₁ receptors.

The results of Experiment 3 corroborated earlier findings that SCH-23390 exerts different effects when administered into the left or right cerebral hemisphere of the fetal rat. All fetal subjects in this experiment showed pronounced increases in motor activity as a result of IH injection of the D₁ agonist SKF-38393. Experimental subjects that received a second IH injection of SCH-23390 showed a subsequent reduction in activity that differed in magnitude and time course as a function of whether the injection was delivered into the left or right hemisphere. Specifically, fetuses that received SCH-23390 into

the left hemisphere showed an immediate, pronounced reduction in activity, whereas fetuses that received a right injection showed a slower decline in movement. Somewhat surprising was the finding that fetuses receiving right IH injections showed higher levels of mouth and lick movements than fetuses receiving left IH injections. The results of this study therefore provide intriguing evidence that D₁ receptors of the dopamine system exhibit functional asymmetries during the prenatal period. One hypothesis to explain asymmetries in the effects of IH injections of dopaminergic drugs is that the two hemispheres exhibit a transient difference in their rates of development, with one hemisphere somewhat more developed on E21 than the other (e.g., more receptors, more neurons bearing D₁ receptors, better connections with effector systems). Alternatively, lateral asymmetries may suggest that the two hemispheres exhibit qualitative differences in structure that emerge during the prenatal period and persist into adulthood.

Various studies have documented that early behavior or experience can influence or predict lateral asymmetries in the adult brain. Neonatal tail posture (a preference for holding the tail to the left or right of the midline) is predictive of lateralized behavior expressed by rats in adulthood, such as spontaneous rotation (13). Rotational behavior of adults is related to asymmetries in the striatal dopamine system (3,6). A left bias in tail posture of neonatal rats (1 day after birth) also is correlated with lateral asymmetry in the dopamine system of adult rats, with more dopamine in the right striatum (11). Even earlier links to adult dopamine asymmetry have been reported to result from prenatal stress resulting from maternal exposure to acoustic and visual stimulation, which amplify left-right differences in dopamine activity in the cortex, nucleus accumbens, and striatum of adult rats (5). The present study suggests that the dopamine system of the rat fetus exhibits a functional asymmetry on E21 of gestation, and that prenatal or neonatal manipulations that influence lateralized behavior of adults may result from influences on a dopamine system that is already lateralized. By extension, the present study has implications for the effects of prenatal exposure to drugs of abuse, such as cocaine, that affect the dopamine system. Future investigations of prenatal drug effects should consider that prenatal exposure to drugs may promote lateralized effects on behavior by exerting an asymmetrical influence on dopamine activity in the fetal brain.

ACKNOWLEDGEMENTS

E.I.V. and E.S.P. were supported as visiting scientists by the Center for Developmental Psychobiology at Binghamton University. W.P.S. is supported by NICH&HD (NIH) MERIT Award HD 16102. W.P.S. and S.R.R. are supported by NIH grants HD 28231 and HD 28014.

REFERENCES

1. Afonso, D.; Santana, C.; Rodriguez, M. Neonatal lateralization of behavior and brain dopaminergic asymmetry. *Brain Res. Bull.* 32:11–16; 1993.
2. Babine, A. M.; Smotherman, W. P. Uterine position and conditioned taste aversion. *Behav. Neurosci.* 98:461–466; 1984.
3. Carlson, J. N.; Glick, S. D. Cerebral lateralization as a source of interindividual differences in behavior. *Experientia* 45:788–798; 1989.
4. Castellano, M. A.; Diaz-Palarea, M. D.; Barroso, J.; Rodriguez, M. Behavioral lateralization in rats and dopaminergic system: Individual and population laterality. *Behav. Neurosci.* 103:46–53; 1989.
5. Fride, E.; Weinstock, M. Prenatal stress increases anxiety related behavior and alters cerebral lateralization of dopamine activity. *Life Sci.* 42:1059–1065; 1988.
6. Glick, S. D.; Jerussi, T. P.; Waters, P. H.; Green, J. P. Amphetamine-induced changes in striatal dopamine and acetylcholine levels and relationship to rotational (circling) behavior in rats. *Biochem. Pharmacol.* 23:3223–3225; 1974.
7. Holson, R. R.; Pearce, B. Principles and pitfalls in the analysis of

prenatal treatment effects in multiparous species. *Neurotoxicol. Teratol.* 14:221-228; 1992.

8. Moody, C. A.; Robinson, S. R.; Spear, L. P.; Smotherman, W. P. Fetal behavior and the dopamine system. Activity effects of D_1 and D_2 receptor manipulations. *Pharmacol. Biochem. Behav.* 44: 844-850; 1993.
9. Paxinos, G.; Tork, I.; Tecott, L. H.; Valentino, K. L. *Atlas of the developing rat brain*. New York: Academic Press; 1991.
10. Petrov, E. S.; Varlinskaya, E. I.; Robinson, S. R.; Smotherman, W. P. de Costa, B. R.; Rice, K. C. Kappa opioid effects on fetal behavior: Central administration of U50,488. *Physiol. Behav.* 56: 175-182; 1994.
11. Rosen, G. D.; Finklestein, S.; Stoll, A. L.; Yutzy, D. A.; Denenberg, V. H. Neonatal tail posture and its relationship to striatal dopamine asymmetry in the rat. *Brain Res.* 297:305-308; 1984.
12. Rosen, G. D.; Finklestein, S.; Stoll, A. L.; Yutzy, D. A.; Denenberg, V. H. Neurochemical asymmetries in the albino rat's cortex, striatum, and nucleus accumbens. *Life Sci.* 34:1143-1148; 1984.
13. Ross, D. A.; Glick, S. D.; Meibach, R. C. Sexually dimorphic brain and behavioral asymmetries in the neonatal rat. *Proc. Natl. Acad. Sci. USA* 78:1958-1961; 1981.
14. Smotherman, W. P.; Robinson, S. R. Response of the rat fetus to acute umbilical cord occlusion: An ontogenetic adaptation? *Physiol. Behav.* 44:131-135; 1988.
15. Smotherman, W. P.; Robinson, S. R. Accessibility of the rat fetus for psychobiological investigation. In: Shair, H. N.; Barr, G. A.; Hofer, M. A., eds. *Developmental psychobiology: New methods and changing concepts*. New York: Oxford University Press; 1991:148-163.
16. Smotherman, W. P.; Robinson, S. R.; Varlinskaya, E. I.; Petrov, E. S.; Orlowski, M.; de Costa, B. R.; Rice, K. C. Central administration of the endopeptidase 24.15 inhibitor cFP-AAF-pAB suggests dynorphin as the endogenous ligand underlying the behavioral effects of milk in the fetal rat. *Physiol. Behav.* 47:715-719; 1994.
17. Varlinskaya, E. I.; Petrov, E. S.; Robinson, S. R.; Smotherman, W. P. Behavioral effects of centrally administered arginine vasopressin in the rat fetus. *Behav. Neurosci.* 108:000-000; 1994.
18. Varlinskaya, E. I.; Petrov, E. S.; Robinson, S. R.; Smotherman, W. P. Intracisternal administration of SKF-38393 and SCH-23390: Behavioral effects in the rat fetus. *Pharmacol. Biochem. Behav.* 48:741-748; 1994.