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Long-Term Reciprocal Changes in Dopamine Levels in Prefrontal Cortex versus Nucleus Accumbens in Rats Born by Caesarean Section Compared to Vaginal Birth

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Epidemiological evidence indicates a higher incidence of pregnancy and birth complications among individuals who later develop schizophrenia, a disorder linked to alterations in mesolimbic dopamine (DA) function. Two birth complications usually included in these epidemiological studies, and still frequently encountered in the general population, are birth by Caesarean section (C-section) and fetal asphyxia. To test the hypothesis that birth complications can produce long-lasting changes in DA systems, the present study examined the effects of Caesarean birth, with or without an added period of anoxia, on steady state monoamine levels and metabolism in various brain regions in a rat model. Pups born vaginally served as controls. At 2 months of age, in animals born by rapid C-section, steady state levels of DA were decreased by 53% in the prefrontal cortex and increased by 40% in both the nucleus accumbens and striatum, in comparison to the vaginally born group. DA turnover increased in the prefrontal cortex, decreased in the nucleus accumbens, and showed no significant change in the striatum, in the C-section group. Thus, birth by a Caesarean procedure produces long-term reciprocal changes in DA levels and metabolism in the nucleus accumbens and prefrontal cortex. This is consistent with the known inhibitory effect of increased prefrontal cortex DA activity on DA release in the nucleus accumbens. By contrast to birth by rapid C-section alone, young adult animals, that had been born by C-section with 15 min of added anoxia, showed no change in steady state DA levels in the prefrontal cortex, nucleus accumbens, or striatum and a significant decrease in DA turnover only in the nucleus accumbens, in comparison to the vaginally born group. Levels of norepinephrine, serotonin, and its metabolite, 5-hydroxyindole acetic acid, were unchanged in all groups, indicating relatively specific effects on DA systems. Although appearing robust at birth on gross observation, more subtle measurements revealed that rat pups born by C-section show altered respiratory rates and activity levels and increased levels of whole brain lactate, suggestive of low grade brain hypoxia, during the first 24 h of life, in comparison to vaginally born controls. Pups born by C-section with 15 min of added acute anoxia were pale, hypotonic, and inactive at birth and showed reduced respiration and high brain lactate levels. However, these alterations resolved by 1–5 h after birth and, with few exceptions, animals in the anoxic group remained normal with respect to these parameters during the remainder of the first 24 h of life. Immediately after birth, levels of plasma epinephrine, a hormone known to play a role in neonatal adaptation to extrauterine life and protection against hypoxia, were decreased in pups born by C-section but increased in pups born by C-section with 15 min added anoxia, in comparison to levels measured in vaginally born controls. These early developmental alterations could contribute to long-term alterations in dopaminergic parameters observed in rats born by C-section, with or without added anoxia. It is concluded that C-section birth is sufficient perturbation to produce long-lasting effects on DA levels and metabolism in the central nervous system of the rat. These findings highlight the sensitivity of DA pathways to variations in birth procedure and support the notion that birth complications might contribute to the pathophysiology of disorders involving central dopaminergic neurons, such as schizophrenia.

INTRODUCTION

Much evidence derived from clinical and animal studies has linked symptoms of schizophrenia and other psychoses to alterations of mesolimbic dopamine (DA) function (13, 21, 40). More specifically, a pathological enhancement of mesolimbic DA function related to a deficit in prefrontal cortical (PFC) DA function has been proposed to underlie positive symptoms of schizophrenia (13, 52). How such imbalances might arise is unknown; however, several lines of evidence point to

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faulty neural development as a contributing factor (4, 34, 37). Among other compelling evidence for the neurodevelopmental model, epidemiological studies have indicated an increased incidence of a variety of pregnancy and birth complications in those who later develop schizophrenia (9, 10, 18, 32). Two such birth complications, usually included in the epidemiological studies, are birth by Caesarean section (C-section) and fetal asphyxia. These birth complications are still quite common in the general human population. Recent U.S. studies place the frequency of C-section birth at approximately 23–27% in the early 1990s (16, 46). For full-term human infants, 0.2–0.4% have been estimated to suffer periods of birth asphyxia, while this figure is much higher in the case of prematurely born neonates (48). Despite this, the possibility that global fetal hypoxia or C-section birth may have lasting effects on CNS DA systems has not, as yet, been thoroughly investigated.

DA systems in the immature central nervous system are very reactive to hypoxia, at least acutely (36, 43, 47). A few recent studies have suggested that, in addition to acute changes, insult to perinatal rat brain could result in long-term alterations in DA transmission. To study this and other consequences of perinatal hypoxia (1, 3, 11, 14, 29), Bjelke, Andersson, and colleagues have developed a rat model of anoxia during a Caesarean birth (3, 29), in which the intact uterus, isolated from its blood supply, is removed from the pregnant dam at term via an abdominal incision and placed in a warm saline bath for several (14–22) min, mimicking an acute global anoxic event, before delivery of the pups. Using this model, Chen *et al.* have reported significant increases in the number of DA neurons, measured as an increased density of tyrosine hydroxylase immunoreactive cell bodies, in substantia nigra and ventral tegmental area of 4-week-old rats, that had been subjected to 19–20 min birth asphyxia, in comparison to controls born by C-section (11). These studies showing alterations in tyrosine hydroxylase immunoreactivity suggest that anoxia at birth could produce lasting changes in CNS DA levels and/or transmission. Unfortunately, a vaginally born control was not included in the above study, thus possible effects of a rapid C-section alone were not determined.

In recent studies, using the model of birth anoxia developed by Bjelke and Andersson *et al.*, our laboratory used *in vivo* voltammetry to test whether a period of anoxia during a C-section birth in the rat can alter the nucleus accumbens (NAcc) DA response to repeated stress in the adult animal (8). In animals born by C-section, either with or without added anoxia, tail pinch stress (15 min daily) elicited markedly longer lasting DA responses on Days 4 and 5 of testing, when compared to the initial stress response. In contrast, there was no significant day to day enhancement of the stress response in control, vaginally born animals. These results showed that a C-section birth procedure increases the sensitivity of mesolimbic DA neurons to effects of repeated stress in the adult rat.

In fact, it is known that C-section birth is not a normal birth, at least hormonally. Several studies have shown that human infants born vaginally have strikingly higher plasma levels of both catecholamines and cortisol at birth than do those born by elective C-section (22, 24, 35, 45). Circulating catecholamines and glucocorticoids are known to play important roles both in protecting the fetus from hypoxic episodes during the birth process and in adapting the fetus to extrauterine life at birth. For example, a surge in adrenal catecholamine release, elicited either by an uncomplicated vaginal delivery or even more potently by fetal hypoxia, serves to protect against hypoxia by promoting normal breathing, mobilizing fuel for energy, and redistributing blood flow to vital organs such as liver, heart, and brain (22, 24, 42). In addition, plasma catecholamines and glucocorticoids play a critical role in adaptative responses of the lungs at birth by increasing lung-liquid absorption, lung surfactant, and lung compliance (17, 24, 25, 51, 53). Thus the low levels of circulating adrenal medullary and cortical hormones consequent to C-section birth may contribute to transient hypoxia and/or susceptibility to hypoxic damage at early times after birth. In fact, in the case of human neonates, the incidence of transient mild respiratory distress (respiratory distress syndrome type II) has been reported to be higher following C-section in comparison to vaginal birth (19).

Given this background, the current study aimed to extend our previous findings on long-term effects of birth condition on dopaminergic transmission in two ways. (1) The first objective was to test whether birth by C-section, either with or without an added period of acute anoxia, alters DA parameters in other brain regions in addition to the NAcc and to examine the specificity of effects with respect to monoamines; i.e., is the DA system particularly sensitive to birth insult. For this, rats born vaginally, by C-section, or by C-section with 15 min anoxia were raised to 2 months of age and steady state levels and metabolism of DA, serotonin (5-HT), and norepinephrine (NE) were quantitated in the PFC, NAcc, and striatum. (2) The second aim was to examine factors inherent in the C-section procedure which could contribute to long-term alterations in CNS DA function. For this, we measured brain lactate [an index of CNS hypoxia (30, 39)], respiratory rate, and other indices of neonatal well-being at early times after vaginal birth and birth by C-section or C-section with added acute anoxia, to determine if a transient condition of relative hypoxia might occur following an apparently innocuous C-section. Plasma catecholamines at birth were also measured to determine whether levels

of this hormone are reduced in the rat model of C-section birth, similar to the situation following C-section in the human.

MATERIALS AND METHODS

C-Section and Intrauterine Anoxia

Pregnant Sprague–Dawley rats (Charles River, St. Constant, Quebec) underwent acute anoxia during a C-section delivery using a modification of the procedure described by Bjelke *et al.* (3). Timed pregnant rats at 22 days of gestation (i.e., on the day of birth) were decapitated, an abdominal incision was made, and the uterus was quickly isolated from its blood supply and surrounding connective and fatty tissue (10–15 s). An acute anoxic episode was induced by immersing the intact uterus into a 37°C saline bath for 15 min (C-section + Anoxia group). The pups were then delivered and stimulated by gentle tapping until breathing became even (30–40 s). No other means of artificial resuscitation was employed. The umbilical cord was ligated and the animals were placed on a heating pad until given to their surrogate mothers. Survival was 90–95% following 15 min of birth anoxia. A second group of animals was delivered via C-section with no period of added anoxia (C-section group). Time between sacrifice of the dam and delivery of the last pup in the C-section group was <1.5 min and survival was 100% in the C-section group. Pups born vaginally served as controls (control group). Only male pups were retained for study. Pups from all groups were cross-fostered in mixed litters (12 pups/dam) by the same dam to minimize differential rearing effects. Animals in the C-section and C-section + Anoxia groups were placed with surrogate dams by 1–2 h after birth. Vaginally born animals were removed from their birth dams and placed with surrogate dams at 2–24 h after birth. All procedures with animals were performed in accordance with the guidelines of the Canadian Council on Animal Care and were approved by the McGill University Animal Care Committee.

At 2 months of age, rats from the three birth groups were sacrificed by decapitation and their brains were removed quickly and placed on an ice-cold plate. The PFC, NAcc, and striatum of the right and left hemispheres were dissected; right and left structures were alternately used for measurement of monoamine levels (or retained for measurements unrelated to the current study). Tissues were immediately frozen at −80°C for later chromatographic analysis. At 2 months of age, most dopaminergic markers, including DA levels, have reached adult levels in rat brain (20) and the rat is just past puberty (31). Thus, this age compares developmentally with the peak age of onset of schizophrenic symptomatology in humans, which occurs in early adulthood.

Levels of Catecholamines and Metabolites in CNS Regions

On the day of the analysis, tissues were thawed on ice and homogenized in an ice-cold medium of 0.1 *M* sodium acetate, 0.1 m*M* EDTA, and 4.3 ml/liter glacial acetic acid (pH 5.0). Samples were vortexed and centrifuged (13,000*g*, 25 min). The supernatant was transferred to vials for injection into the chromatographic system, and the pellet was retained for protein analysis. Catecholamines were measured by high pressure liquid chromatography coupled with electrochemical detection. Samples (40 µl) of supernatant were injected onto a C18 reverse phase column (5-µl spheres, Biophase ODS, Bioanalytical Systems, Inc., West Lafayette, IN), which was protected by a C18-packed precolumn. The electrochemical detector (LC4A, Bioanalytical Systems, Inc.) was equipped with a TL-5 glassy carbon electrode set at a potential of +0.74 V relative to a Ag/AgCl reference electrode. The mobile phase consisted of 0.3 *M* sodium acetate, 0.1 m*M* EDTA, 80 mg/liter octyl sodium sulfate, and 3.3% acetylnitrile (pH 3.8, with glacial acetic acid), pumped at 1.3 ml/min. Protein was measured using a bicinchoninic acid protein assay (44).

Plasma Catecholamines

Immediately following birth, male pups of each group were decapitated. Only the first drop of blood immediately following decapitation was collected in an EDTA-coated Eppendorf tube to avoid, as much as possible, measurement of adrenal catecholamines released as a consequence of decapitation. Each sample contained pooled blood from 6–8 pups. In fact, when samples containing only the first drop of blood were compared to those prepared by collecting most of the trunk blood, the former had significantly lower catecholamines levels (data not shown). Samples were centrifuged (1000*g*, 10 min, 4°C) and the supernatant stored at −80°C. On the day of the catecholamine analysis, samples were thawed on ice. An aliquot (150 µl) of the sample was added to a tube which contained 800 µl of extraction buffer, 340 µl of 0.1 *M* perchloric acid containing 1 m*M* EDTA, and 10 mg aluminum oxide (total assay volume = 1.29 ml). The extraction buffer consisted of 1.5 *M* Trizma hydrochloride, 20 m*M* sodium metabisulphite, 125 m*M* EDTA (pH 8.6). Epinine was used as an internal standard. Standards were routinely extracted with each group of samples and compared to nonextracted standards to calculate the percentage of recovery of catecholamines. For extraction, samples were shaken for 10 min. After this, the alumina was allowed to settle, the supernatant was discarded, and the alumina was washed 2× with 1 ml wash buffer containing 6 m*M* Trizma hydrochloride, 1 m*M* EDTA (pH 8.6), followed by 1 wash with 1 ml buffer containing 5 m*M*

sodium metabisulphite and 0.08 m*M* EDTA (pH 8.6). The alumina was then suspended in 200 µl of 0.1 *M* perchloric acid, the tubes were shaken for 6 min and centrifuged (3,000*g*, 15 min, 4°C), and the supernatant was retained for assay of catecholamines by HPLC. Recoveries of DA and dihydroxyphenylacetic acid (DOPAC) were 60% and that for norepinephrine and epinephrine, 75%.

Indices of Neonatal Well-Being

At various times after birth from 10 min until 24 h, rat pups were rated on the following measures: Skin color, the animal's color was rated on a scale of 0 to 3, where 3 indicates a pink, well-oxygenated color and 0 indicates a pale bluish cast; Gasping, the animal was rated for the presence or absence of mouth and thoracic movements indicative of gasping; Vocalization, the animal was rated for the presence or absence of vocalization while it was being picked up; Muscle tone, the animal's muscle tone was rated on a scale of 0 to 3, where 3 indicates a strong general body tonus when the animal was lifted and strong resistance of a hind limb when the limb was flexed, and 0 indicates a limp or flaccid tonus; Activity/movement, activity/movement was rated on a scale of 0 to 4, as described by Loidl *et al.* (29), where 0 indicates akinesia and/or rigidity, 1 indicates movement of one of the following body parts, either front legs, hind legs, or head alone, 2 indicates movement of two of the body parts above, 3 indicates movement of all body parts, and 4 indicates intensive movements shown by wriggling or walking; Respiratory rate, respiratory movements of the thorax and abdomen were counted for 1 min.

Whole Brain Lactate

Animals were decapitated at 1, 5, 12, or 24 h after birth. Brains were immediately placed in isopentane cooled over dry ice, left for 10 s, and then stored at −80°C. Whole brain lactate was extracted by modification of methods described by Magal *et al.* (30). Each brain was homogenized in 150 µl of solution of 20 m*M* potassium phosphate buffer, pH 6.0, to which had been added Tris–HCl, pH 8.0, to a final concentration of 0.1 *M* and dithiothreitol to a final concentration of 0.5 m*M*. Homogenates were extracted with ice-cold 8% perchloric acid (1:1 vol/vol), vortexed, and centrifuged (1500*g*, 10 min, 4°C). Lactate was determined in 10-µl aliquots of the supernatant, using a kit purchased from Sigma Chemical Co. (St. Louis, MO).

Statistical Analysis

Data obtained from measures taken at sequential time points (respiratory rate, whole brain lactate, indices of neonatal well-being) were analyzed using two-way analysis of variance with birth group and time designated as between subject variables. Significance of differences among birth groups at a single time point were analyzed by one-way analysis of variance with post-hoc Neuman–Keul's tests, except in the case of data for plasma catecholamine levels. Because data for plasma catecholamine levels (Table 4) showed inhomogeneity of variance, nonparametric tests (Kruskalis–Wallis one-way analysis of variance by ranks with post-hoc Mann–Whitney *U* tests), were used to analyze significance of differences within this data set.

RESULTS

Monoamine Levels in Brain at Young Adulthood

As young adults, rats born by rapid C-section (with no added anoxia) showed a significant decrease (−53%) in DA levels in the PFC and increased DA levels in the NAcc (+40%) and striatum (+41%) in comparison to vaginally born controls (Figs. 1A and 1B). There were no significant effects of birth group on levels of DOPAC or homovanillic acid (HVA) in PFC, NAcc, or striatum (Table 1). As a result, in the C-sectioned group, DA metabolism, as assessed by the ratio of DA metabolites to DA [(DOPAC + HVA)/DA] increased in the PFC (+57%) and decreased in the NAcc (−20%), compared to values for vaginally born controls (Figs. 2A and 2B). Although DA metabolism tended to decrease in the striatum, this difference was not significant.

In contrast to results for the C-section group, young adult rats born by C-section with 15 min of added acute anoxia showed no significant change in DA levels in any brain region studied, when compared to vaginally born controls (Fig. 1). Rats born by C-section with 15 min of anoxia showed a decrease in DA metabolism only in the NAcc (−20%), with no significant change in DA metabolism in the PFC or striatum (Fig. 2).

There were no group differences in levels of norepinephrine (NE), serotonin (5-HT), or its metabolite, 5-hydroxyindole acetic acid (5-HIAA), in the brain regions examined (Table 1).

Indices of Neonatal Well-Being, Respiratory Rate, and Whole Brain Lactate during the First 24 h after Birth

Rat pups born vaginally, by C-section, or by C-section with 15 min of acute anoxia were rated at several time intervals, beginning at 10 min until 24 h after birth, on indices of neonatal well-being. Several of these indices are analogous to measures included in the APGAR score used to rate neonatal condition in humans (e.g., color, muscle tone, respiratory rate). On cursory gross observation, rat pups born by C-section with 15 min of acute anoxia clearly showed pale bluish skin color immediately at birth in comparison to the pinker color shown by animals born vaginally or by C-section.

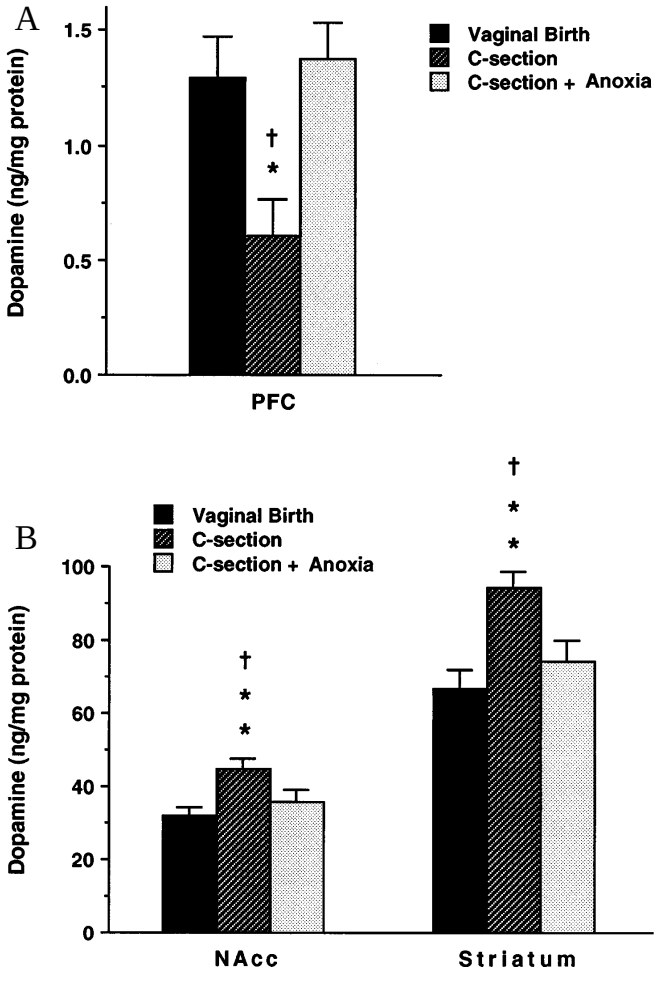

FIG. 1. Dopamine levels in prefrontal cortex (A) and nucleus accumbens and striatum (B) of adult rats born vaginally, by C-section, or by C-section with 15 min of acute anoxia. Rats were born vaginally, by C-section, or by C-section with 15 min of added anoxia (C-section + Anoxia). At 2 months of age, levels of dopamine were measured in the prefrontal cortex (PFC), nucleus accumbens (NAcc), and striatum. Data represent the mean ± SEM from 7–10 animals for PFC and from 8–15 animals for other regions. One-way analysis of variance indicated a significant effect of birth group on dopamine levels in PFC [$F(2, 23) = 6.08$, $P = 0.008$], NAcc [$F(2, 31) = 5.25$, $P = 0.011$], and striatum [$F(2, 33) = 6.80$, $P = 0.003$]. Post-hoc Neuman–Keul's tests showed significant differences as indicated: (**) different from Vaginal Birth at $P < 0.01$; (*) different from Vaginal Birth at $P < 0.05$; (†) different from C-section + Anoxia at $P < 0.05$.

TABLE 1

Levels of Dihydroxyphenylacetic Acid, Homovanillic Acid, Norepinephrine, Serotonin, and 5-Hydroxyindole Acetic Acid in Brain Regions of Adult Rats Born Vaginally, by C-Section or by C-Section with 15 Min of Acute Anoxia

	Vaginal birth	C-section	C-section + anoxia
PFC			
DOPAC	2.4 ± 0.2	2.4 ± 0.3	3.4 ± 0.5
HVA	1.6 ± 0.2	1.0 ± 0.1	1.3 ± 0.1
NE	n.d.	n.d.	n.d.
5-HT	2.4 ± 0.2	2.1 ± 0.2	2.2 ± 0.2
5-HIAA	5.3 ± 0.3	5.2 ± 0.3	5.6 ± 0.3
NAcc			
DOPAC	19.1 ± 1.3	20.4 ± 2.1	16.3 ± 1.8
HVA	6.3 ± 1.0	8.1 ± 1.3	5.3 ± 0.7
NE	15.5 ± 1.1	16.6 ± 1.2	16.1 ± 1.5
5-HT	8.6 ± 0.5	8.9 ± 0.8	7.9 ± 0.8
5-HIAA	14.7 ± 0.9	16.4 ± 2.8	13.7 ± 1.4
Striatum			
DOPAC	21.4 ± 2.4	21.3 ± 1.5	19.6 ± 1.6
HVA	10.3 ± 0.7	12.6 ± 1.0	10.4 ± 1.3
NE	3.1 ± 0.4	3.8 ± 0.3	2.9 ± 0.5
5-HT	5.8 ± 0.8	7.8 ± 0.8	6.2 ± 1.2
5-HIAA	8.9 ± 0.8	10.5 ± 1.0	10.1 ± 0.8

Note. Rats were born vaginally, by C-section or by C-section with 15 min of added anoxia (C-section + anoxia). At 2 months of age, levels of dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), norepinephrine (NE), serotonin (5-HT), and 5-hydroxyindole acetic acid (5-HIAA) were measured in the prefrontal cortex (PFC), nucleus accumbens (NAcc), and striatum. Data are expressed as ng/mg protein and represent the mean ± SEM from 6–11 animals for PFC and from 9–14 animals for other regions. One-way analysis of variance indicated no significant effect of birth group in PFC on levels of DOPAC [$F(2, 23) = 2.80$, $P = 0.082$], HVA [$F(2, 22) = 2.14$, $P = 0.142$], 5-HT [$F(2, 25) = 0.34$, $P = 0.713$], or 5-HIAA [$F(2, 23) = 0.43$, $P = 0.657$]; no effect in NAcc on levels of DOPAC [$F(2, 30) = 1.45$, $P = 0.251$], HVA [$F(2, 26) = 2.03$, $P = 0.151$], NE [$F(2, 31) = 0.21$, $P = 0.816$], 5-HT [$F(2, 33) = 0.60$, $P = 0.557$], or 5-HIAA [$F(2, 32) = 0.64$, $P = 0.532$]; and no effect in striatum on levels of DOPAC [$F(2, 32) = 0.24$, $P = 0.786$], HVA [$F(2, 33) = 1.59$, $P = 0.218$], NE [$F(2, 27) = 1.04$, $P = 0.367$], 5-HT [$F(2, 33) = 1.42$, $P = 0.256$], or 5-HIAA [$F(2, 31) = 0.94$, $P = 0.401$]. n.d., not detectable.

However, by 10–30 min after birth, the time at which systematic rating of animals began, there was no significant differences in skin color among pups born vaginally, by C-section, or by C-section with 15 min of anoxia (Table 2). There were no significant differences in ratings of skin color among the three birth groups at later times (1, 5, 12, and 24 h) during the first day of life. Similarly, while gasping behavior was observed immediately after birth in pups born by C-section with anoxia, but rarely in animals born vaginally or by C-section alone, by 10–30 min after birth until 24 h, animals from all three birth groups rarely showed gasping behavior (Table 2). With the exception of the occasional animal, almost all animals vocalized when picked up during the first 24 h after birth (Table 2).

At 10–30 min after birth, pups born by C-section showed excellent muscle tone similar to that of vaginally born animals (Table 2), and tone remained similar to that of the vaginally born group throughout the first 24 h. By contrast, at 10–30 min after birth, rat pups born by C-section with added anoxia were hypotonic in comparison to either the vaginally born or C-sectioned groups. By 1 h after birth, muscle tone in the anoxic group was similar to that in the vaginally born group and remained so when tested at later times. At 10–30 min after birth, activity levels of pups born by C-section were not significantly different from those of vaginally

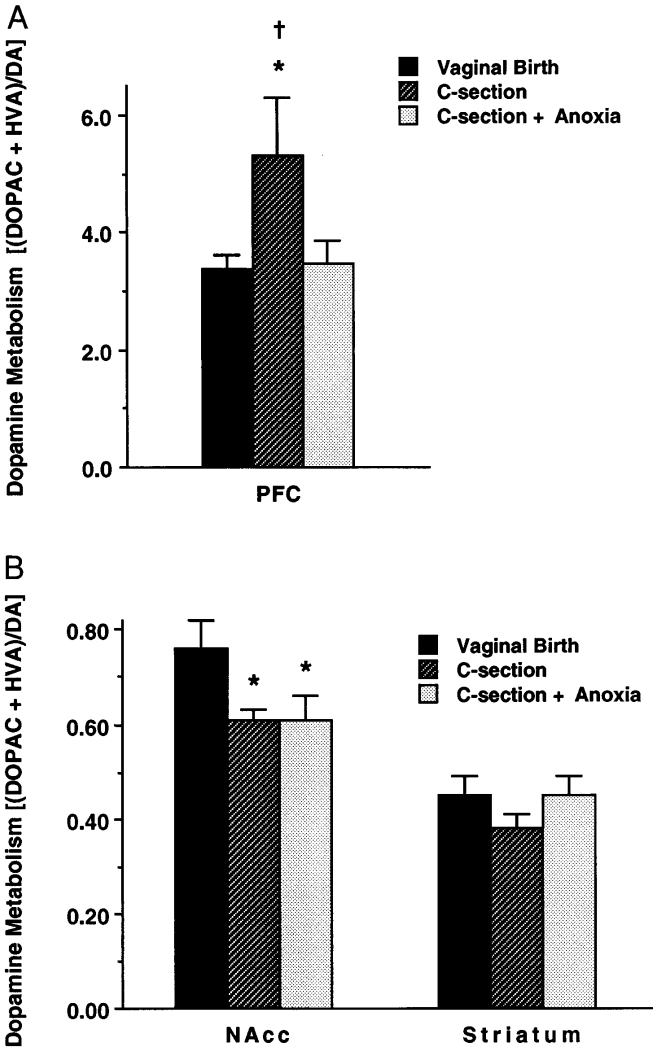

FIG. 2. Dopamine metabolism in prefrontal cortex (A) and nucleus accumbens and striatum (B) of adult rats born vaginally, by C-section, or by C-section with 15 min of acute anoxia. Rats were born vaginally, by C-section, or by C-section with 15 min of added anoxia (C-section + Anoxia). At 2 months of age, levels of dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and dopamine (DA) were measured in the prefrontal cortex (PFC), nucleus accumbens (NAcc), and striatum. Data represent the mean ± SEM from 8–11 animals for PFC and from 8–14 animals for other regions. One-way analysis of variance indicated a significant effect of birth group on dopamine metabolism in PFC [$F(2, 21) = 5.08$, $P = 0.016$] and NAcc [$F(2, 25) = 3.60$, $P = 0.042$], but no effect in striatum [$F(2, 34) = 1.10$, $P = 0.345$]. Post-hoc Neuman–Keul's tests showed significant differences as indicated: (*) different from Vaginal Birth at $P < 0.05$; (†) different from C-section + Anoxia at $P < 0.05$.

born animals (Table 2). However, the profile of activity in the C-sectioned animals differed from that of vaginally born controls at later times: C-sectioned animals were hyperactive at 1 h and hypoactive at 12 and 24 h. Pups born by C-section with 15 min added anoxia were significantly underactive at 10–30 min after birth, and also at 12 h, compared to vaginally born animals, although activity levels were similar to those of vaginally born controls at other time points.

With respect to body weight, animals born by C-section with or without added anoxia and assessed at 10–30 min or at 1 h had not been placed with a dam; thus these pups had not begun to feed and their body weights reflect birth weights. Vaginally born animals had access to their dams from birth and feeding may have begun by 10–30 min after birth. At 10–30 min after birth, the vaginally born group weighed slightly but significantly more than did animals born by C-section either with or without anoxia (Table 2). This may be due to a slight degree of prematurity and/or lack of feeding in the C-section and anoxic groups. However, in the larger groups of animals assessed at 1 h after birth, there was no significant difference in birth weight among the three birth groups. By 5 h all animals had begun feeding, and all showed visible milk contents in their stomachs. There were no significant group differences in body weight at 5, 12, and 24 h, with the exception of the anoxic groups at 5 and 24 h, whose weight was significantly greater than that of the vaginally born or C-sectioned groups.

Shortly after birth (i.e., 10–30 min), respiratory rate was similar in C-sectioned compared to vaginally born animals (Fig. 3A). However, the profile of respiration over the next 24 h differed in the C-sectioned group, compared to that of vaginally born controls. C-sectioned animals showed significantly increased respiratory rates at 1 and 12 h and reduced respiratory rates at 24 h, in comparison to vaginally born controls. By contrast, pups born by C-section with 15 min of added anoxia showed reduced respiratory rates at 10–30 min after birth in comparison to vaginally born controls (Fig. 3B). Thereafter the profile of respiration in the anoxic group was not significantly different from that of vaginally born controls, except at 24 h when respiratory rate was lower in the anoxic group.

In vaginally born control animals, whole brain lactate levels at 1 h after birth averaged 1970 nmol/g and fell to values in the range of 800 nmol/g wet weight at 5, 12, and 24 h after birth (Table 3). At 1 h after birth, values for brain lactate were not significantly different in C-sectioned animals versus vaginally born controls. However, the brain lactate level in the C-sectioned group was significantly greater than that measured in the vaginally born group at 5 h after birth; a similar trend, which did not reach statistical significance, was observed at 12 h. By 24 h after birth, the brain lactate in the C-sectioned group was reduced to values similar to those measured in vaginally born controls. In pups born by C-section with 15 min of added anoxia, the brain lactate level at 10–30 min after birth was significantly greater than that measured in either vaginally born or C-sectioned animals. This high level of brain lactate fell quite rapidly, such that the brain lactate

TABLE 2

Ratings of Indices of Neonatal Well-Being at Various Times after Vaginal Birth, Birth by C-Section, or by C-Section with 15 Min of Acute Anoxia

	Time after birth				
	10–30 min	1 h	5 h	12 h	24 h
Skin Colour (rated from 0–3)					
Vaginal birth	3.0 ± 0	2.9 ± 0.1	3.0 ± 0	2.9 ± 0.1	2.8 ± 0.1
C-section	3.0 ± 0	2.7 ± 0.2	2.9 ± 0.1	2.4 ± 0.2	2.6 ± 0.2
C-section + anoxia	2.7 ± 0.2	2.9 ± 0.1	2.9 ± 0.1	2.8 ± 0.1	2.9 ± 0.1
Gasping (No. of animals showing behavior/total *n*)					
Vaginal birth	0/7	0/15	1/16	0/16	0/13
C-section	0/7	0/14	0/15	0/15	0/13
C-section + anoxia	1/7	0/13	0/15	1/15	0/15
Vocalization (No. of animals showing behavior/total *n*)					
Vaginal birth	7/7	15/15	16/16	13/16	12/13
C-section	7/7	14/14	15/15	14/15	13/13
C-section + anoxia	7/7	13/13	15/15	15/15	15/15
Muscle tone (rated from 0–3)					
Vaginal birth	3.0 ± 0	2.9 ± 0.1	2.9 ± 0.1	2.8 ± 0.1	2.8 ± 0.1
C-section	3.0 ± 0	3.0 ± 0	2.9 ± 0.1	2.7 ± 0.1	3.0 ± 0*
C-section + anoxia	1.9 ± 0.3**,††	2.8 ± 0.1	3.0 ± 0	2.9 ± 0.1	3.0 ± 0*
Activity/movement (rated from 0–4)					
Vaginal birth	2.4 ± 0.2	2.4 ± 0.3	1.9 ± 0.2	2.0 ± 0.2	1.1 ± 0.2
C-section	3.1 ± 0.3	3.5 ± 0.2**	2.3 ± 0.3	1.2 ± 0.1**	0.3 ± 0.1**
C-section + anoxia	0.3 ± 0.2**,††	2.2 ± 0.4††	2.7 ± 0.3	1.2 ± 0.2**	0.9 ± 0.1†
Body weight (g)					
Vaginal birth	6.2 ± 0.03	5.7 ± 0.2	5.8 ± 0.1	5.9 ± 0.1	5.9 ± 0.1
C-section	5.8 ± 0.1**	5.6 ± 0.2	5.5 ± 0.1	6.0 ± 0.1	6.1 ± 0.2
C-section + anoxia	5.7 ± 0.1**	5.4 ± 0.1	6.2 ± 0.1*,††	6.4 ± 0.1**,††	6.0 ± 0.1

Note. Rats born vaginally, by C-section, or by C-section with 15 min of added anoxia (C-section + anoxia) were rated on indices of neonatal well-being as described under Materials and Methods, at the time after birth as indicated. Numbers of animals rated for all parameters are the numbers shown as total *n* for gasping and vocalization behaviors. Two-way analysis of variance with birth group and time as between subject factors indicated, for skin color, no significant effect of group [$F(2, 181) = 2.85$, $P = 0.060$] or time [$F(4, 181) = 1.64$, $P = 0.165$] and no interaction [$F(8, 181) = 1.20$, $P = 0.303$]; for muscle tone, a significant effect of group [$F(2, 181) = 7.21$, $P = 0.001$] and of time [$F(4, 181) = 5.57$, $P = 0.0003$] and a significant interaction [$F(8, 181) = 10.63$, $P < 0.0001$]; for activity/movement, a significant effect of group [$F(2, 181) = 9.57$, $P = 0.0001$] and of time [$F(4, 181) = 28.42$, $P < 0.0001$] and a significant interaction [$F(8, 181) = 10.82$, $P < 0.0001$]; for body weight, no significant overall effect of group [$F(2, 181) = 2.34$, $P = 0.099$] but a significant effect of time [$F(4, 181) = 6.55$, $P = 0.0001$] and a significant interaction [$F(8, 181) = 3.90$, $P < 0.0003$]. One-way analysis of variance at individual time points with post-hoc Neuman Keul's tests showed significant differences as indicated: (**) different from vaginal birth at $P < 0.01$; (*) different from vaginal birth at $P < 0.05$; (††) different from C-section at $P < 0.01$; (†) different from C-section at $P < 0.05$.

levels in the anoxic group were not significantly different from those measured in vaginally born controls at 5, 12, or 24 h after birth.

Plasma Catecholamines at Birth

Immediately after delivery, the C-section group showed significantly lower levels of plasma epinephrine (−60%) compared to vaginally born controls (Table 4). Plasma NE levels were similar in the C-section and vaginally born groups, as were DOPAC levels. DA levels in plasma from C-sectioned animals were greater (+64%) than those in vaginally born controls. The effect of 15 min anoxia during the C-section procedure was a marked overall increase in plasma catecholamine levels in comparison to either vaginally born control or C-section groups; levels of NE, epinephrine, and DA were significantly increased (by +360%, +272%, +95%, respectively), compared to levels measured in vaginally born animals. Plasma DOPAC levels following anoxia, however, remained close to values for vaginally born controls.

DISCUSSION

The present study demonstrates that young adult rats delivered by C-section alone showed decreased DA levels in the PFC and increased DA levels in the NAcc and striatum, compared to vaginally born control animals. Furthermore, DA metabolism increased in PFC and decreased in NAcc. Since no significant changes in DOPAC or HVA levels were observed, this suggests that the changes in DA metabolism may be due mainly to altered intracellular accumulation of DA rather than to changes in DA release. This is consistent with reports

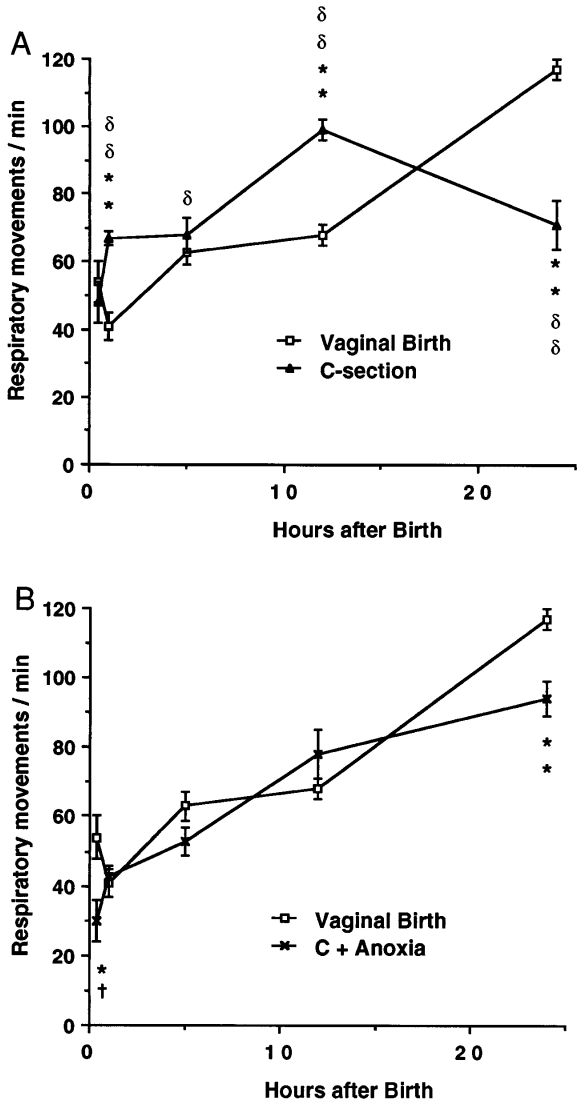

FIG. 3. Respiratory rates at various times after vaginal birth, birth by C-section, or by C-section with 15 min of acute anoxia (C + Anoxia). Numbers of animals rated in each group are the numbers shown as total *n* for gasping and vocalization behaviors in Table 2. Two-way analysis of variance with birth group and time as between subject factors indicated significant effects of group [$F(2, 181) = 7.92$, $P = 0.0005$] and of time [$F(4, 181) = 58.93$, $P < 0.0001$] and a significant interaction between group and time [$F(8, 181) = 11.56$, $P < 0.0001$]. One-way analysis of variance at individual time points with post-hoc Neuman–Keul's tests showed significant differences as indicated: (**) different from Vaginal Birth at $P < 0.01$; (*) different from Vaginal Birth at $P < 0.05$; (†) different from C-section at $P < 0.05$; (δδ) different from C + Anoxia at $P < 0.01$; (δ) different from C + Anoxia at $P < 0.05$.

by Loidl *et al.* that, in comparison to vaginally born control animals, 6-month-old rats born by C-section showed no change in extracellular levels of striatal DA (DA release) monitored by microdialysis under basal (no stress) conditions (29). The C-section procedure seems to have a relatively specific effect on the DA system in the brain regions studied as there was no significant change in NE, 5-HT, or its metabolite, 5-HIAA, when compared to values for vaginally born controls.

The reciprocal changes in DA levels in the PFC and NAcc due to the C-section procedure are consistent with the known inhibitory effect of PFC DA activity on DA release in NAcc and striatum. For example, Vezina *et al.* have reported that *d*-amphetamine administered into PFC inhibits locomotion induced by *d*-amphetamine injected into NAcc (50). Moreover, intra-PFC injection of SCH23390, a DA antagonist, potentiates the locomotion elicited by intra-NAcc injection of *d*-amphetamine (50), and 6-hydroxydopamine lesion of PFC DA terminals potentiates the increase in NAcc DA release elicited by stress, amphetamine, or naturally reinforcing stimuli such as food or sex-related olfactory cues (2, 15, 33). Manipulations of structures other than the PFC itself can also result in reciprocal alterations in DA turnover in the PFC and NAcc in adult animals. For example, Lipska *et al.* have reported long-term alterations in DA levels and metabolism in NAcc and PFC in adult rats given ibotenate lesions of the ventral hippocampus (27), while neonatal rats given similar ventral hippocampal lesions show enhancement of DA-mediated behaviors when tested after puberty (26, 28). These observations that hippocampal lesions can alter dopaminergic function are of interest in view of *in vivo* imaging as well as histopathological studies demonstrating abnormalities in the hippocampal formation in patients suffering from schizophrenia (34). The current study shows that modelling of some aspects of a common variation in human birth, i.e., C-section birth, also produces lasting reciprocal changes in DA levels in PFC and NAcc. Experiments are currently underway in our laboratory to determine whether alterations in hippocampal neuronal number accompanies the alterations in DA levels and/or metabolism in rats born by C-section in comparison to vaginally born controls.

In contrast to results for animals born by rapid C-section, rats delivered by C-section with 15 min of added acute anoxia showed no overall long-term changes in brain DA levels and altered DA metabolism only in the NAcc, in comparison to vaginally born controls. Although this finding alone seems to imply that anoxia during birth might protect DA systems from the effects of C-section, previous studies by our group indicate that rats born by C-section either with or without added anoxia all show deficits in dopaminergic function when challenged. Specifically, using *in vivo* voltammetry to measure DA release, we have found that, in comparison to vaginally born controls, adult rats born by C-section with 0, 5, or 15 min of added anoxia, show a marked enhancement of DA release from the NAcc in response to repeated stress (8). Previous studies in our laboratory have also shown long-term alteration in another component of the stress response, the hippocam-

TABLE 3

Whole Brain Lactate Levels at Various Times after Vaginal Birth, Birth by C-Section, or by C-Section with 15 Min of Acute Anoxia

	Brain lactate (nmol/g wet weight) at			
	1 h	5 h	12 h	24 h
Vaginal birth	1970.3 ± 236.9 (17)	830.4 ± 74.0 (29)	767.5 ± 87.3 (12)	796.0 ± 91.7 (26)
C-section	1370.6 ± 151.7 (20)	1226.7 ± 102.6 (33)*	1140.6 ± 116.9 (14)	715.9 ± 57.0 (23)
C-section + anoxia	3716.1 ± 310.7 (10)**,†	986.1 ± 72.6 (14)	992.7 ± 105.4 (9)	562.0 ± 86.8 (13)

Note. Rats born vaginally, by C-section, or by C-section with 15 min of added anoxia (C-section + anoxia) were sacrificed for measurement of whole brain lactate levels at the time after birth as indicated. Numbers of animals in each group are shown in parentheses. Two-way analysis of variance with birth group and time as between subject factors indicated significant effects of group [$F(2, 208) = 14.96$, $P < 0.0001$] and of time [$F(3, 208) = 87.24$, $P < 0.0001$] and a significant interaction between group and time [$F(6, 208) = 22.54$, $P < 0.0001$]. One-way analysis of variance at individual time points with post-hoc Neuman–Keul's tests showed significant differences as indicated: (**) different from vaginal birth at $P < 0.01$; (*) different from vaginal birth at $P < 0.05$; (†) different from C-section at $P < 0.01$.

pal-hypothalamic-pituitary-adrenal response, following C-section birth. Specifically, as adults, rats born by C-section, either with no added anoxia or with 10 or 15 min of added anoxia, show reduced affinity of hippocampal and hypothalamic corticosteroid type I receptors, increased basal AM plasma corticosterone levels, and decreased stress-induced corticosterone responses (7).

The mechanism(s) by which C-section delivery induces long-term alterations in DA levels and metabolism is unknown. However, our results indicate altered early developmental profiles in both animals born by C-section alone and those born by C-section with added anoxia, in comparison to vaginally born controls, and the profiles for the two C-sectioned groups differ. At birth and up to the first 30 min after birth (see Table 2 and Fig. 3A, ratings at 10–30 min), pups born by rapid C-section (with no added anoxia) show ratings for skin color, vocalization, muscle tone, activity levels, and respiratory rates similar to those scored for vaginally born animals and thus appear to be in excellent condition. However, C-sectioned animals showed abnormalities at subsequent times throughout the first 24 h. In comparison to vaginally born controls, C-sectioned animals were hyperactive at 1 h and hypoactive at 12 and 24 h of age. C-sectioned animals also showed increased respiratory rates at 1 and 12 h and decreased respiratory rates at 24 h, indicating alterations in respiratory regulation.

TABLE 4

Plasma Catecholamine Levels Immediately after Birth in Rats Born Vaginally, by C-Section, or by C-Section with 15 Min of Acute Anoxia

	Vaginal birth	C-section	C-section + anoxia
NE	1138.7 ± 135.5	1184.4 ± 130.8	4862.1 ± 677.3**,†
E	1756.0 ± 171.8	708.3 ± 135.8**	8535.3 ± 1130.6**,†
DA	1202.7 ± 172.3	1974.1 ± 182.0*	2276.3 ± 316.2*
DOPAC	1786.0 ± 131.9	1346.0 ± 158.2	1661.9 ± 209.8

Note. Rats were born vaginally, by C-section, or by C-section with 15 min of added anoxia (C-section + anoxia). Immediately after birth, pups were decapitated and the first drop of trunk blood from 6–8 pups was pooled for measurement of plasma levels of norepinephrine (NE), epinephrine (E), dopamine (DA), and dihydroxyphenylacetic acid (DOPAC). Data are expressed as pg/ml plasma and represent the mean ± SEM from 10–14 separate samples of pooled plasma for each group of animals. Kruskal–Wallis one-way analysis of variance by ranks indicated a significant effect of birth group on plasma NE [$H(2) = 18.96$, $P < 0.0001$], E [$H(2) = 26.26$, $P < 0.0001$], and DA [$H(2) = 9.32$, $P = 0.0095$], but no effect on DOPAC [$H(2) = 5.15$, $P = 0.076$]. Post-hoc Mann–Whitney U tests showed significant differences as indicated: (**) different from vaginal birth at $P < 0.001$; (*) different from vaginal birth at $P < 0.01$; (†) different from C-section at $P < 0.001$.

Measurement of whole brain lacate levels suggest that this altered respiratory profile (or other factors) does have consequences for the oxygenation and/or metabolism of the brain in C-sectioned animals. The profile of brain lactate in vaginally born rats was one of high lactate at birth, rapidly decreasing to a steady level by 1 h after birth, in agreement with findings previously reported by Vannucci and Duffy (49). Spontaneous vaginal birth has been shown to be associated with fetal hypoxemia and hypercapnia in a lamb model in which blood gases were continuously monitored (12). The hypoxemia is thought to arise due to fetal compression by uterine contractions and passage through the birth canal and may be responsible for the transiently increased brain lactate levels observed following spontaneous vaginal birth. At 10–30 min after birth, brain lactate levels in C-sectioned rats were not significantly different from and tended to be lower than levels measured for vaginally born controls. However, at 5 h, C-sectioned animals showed significantly increased brain lactate, which was not completely resolved by 12 h, in comparison to vaginally born controls. This suggests that C-sectioned animals may undergo an hypoxic episode, that has consequences for the CNS and that lasts for several hours during the first day of life.

By contrast, animals born by C-section with 15 min of added acute anoxia, who looked quite poor at birth, seemed to fare somewhat better than did the C-section group during the first 24 h of life. As expected, at birth and at 10–30 min after birth, the anoxic group showed poor color and were hypotonic and hypoactive. They also had reduced respiratory rates compared to vaginally born controls and the highest brain lactate levels measured in any group in the study. However, by 1 h these animals born by C-section with added anoxia had good color and muscle tone and normal activity levels and respiratory rates. From 1–24 h of age, animals in the anoxic group rated similarly to vaginally born controls on all measures of neonatal well-being, with the exception of hypoactivity at one point and decreased respiratory rate at one point. Importantly, the high levels of brain lactate resolved by 5 h and remained at control levels for the first 24 h in animals that had been born by C-section with added acute anoxia. It should be noted that the rat is able to sustain longer acute periods of anoxia at birth than is the human neonate (23). Since rats undergoing 15 min of acute anoxia during C-section began breathing at birth without the aid of any form of artificial resuscitation other than palpation and exhibited normal behavior as adults [except upon specialized testing (6)], this period of anoxia in the rat may represent a mild to moderate, as opposed to severe, anoxic episode.

Overall our results indicate that the rat models of C-section and C-section with added acute anoxia used in this study are both models of perinatal hypoxia. C-section alone appears to produce more prolonged low grade hypoxia, in contrast to the acute episode of more severe hypoxia produced in the C-section plus added anoxia group. Our results raise the possibility that the long-term alterations in dopaminergic parameters observed in both animals born by C-section and those born by C-section with added acute anoxia may be due to episodes of perinatal hypoxia suffered by these animals. The observation that the C-section group is not a normal control group is important in relation to several recent studies (1, 3, 11) where CNS effects have been attributed to acute birth anoxia in the rat by comparison only to a C-sectioned control, not to a vaginally born control. It is also noteworthy that increased brain lactate was found in C-sectioned animals shortly after birth, at times when the animals did not appear distressed. In parallel to this, a recent study by Poets *et al.* reported that a proportion of apparently well preterm human infants exhibited episodes of severe prolonged hypoxemia without any evidence of alterations in monitored rate of breathing movements or heart rate (38). These observations raise the possibility that, in some cases, the neonate whose birth deviates from an uncomplicated term vaginal delivery might suffer undetected hypoxic insult, unless subtle measures of detection are used.

As has been described for human neonates, rat pups born by C-section had significantly lower plasma epinephrine levels at birth than did vaginally born control animals. We have also recently reported that rat pups born by C-section show reduced levels of plasma corticosterone 1 h after delivery (5). Thus the rat C-section procedure models the human condition in terms of these hormonal alterations known to occur following human C-section. Of the different known functions of plasma catecholamines and corticosteroids at birth, the promotion of lung function is one of the most well-characterized (24, 25, 51). Epinephrine is much more potent than norepinephrine in inhibiting secretion of lung fluid and initiating its absorption in the mature fetus (51), and epinephrine also plays a central role in stimulating surfactant synthesis and efflux following delivery (25). It is thus possible that the low levels of adrenal medullary and cortical hormones consequent to C-section birth contributed to the alterations in respiration, CNS lactate, and behavior observed in these animals during the first day of life. Hypoxia in the neonate is known to be a potent stimulus to secretion of adrenal catecholamines, which activate cardiovascular, respiratory, and metabolic mechanisms serving to protect against the hypoxic episode (22, 25, 42). In agreement with this, rat pups born by C-section with added anoxia in the current model showed greatly elevated levels of plasma epinephrine, NE, and DA at birth, compared to levels measured for vaginally born controls. These high levels of plasma catecholamines may contribute to the quick resolution, by 1–5 h after birth, of abnormalities in skin color, muscle tone, activity, respiration, and brain lactate observed at birth in the anoxic group.

In addition to hormonal and respiratory considerations, other differences between the C-section procedure and vaginal birth in our experiments could play a role in producing altered DA levels and metabolism in the adult animal. These differences include rapid maternal decapitation, absence of labor, slight prematurity of the neonate, removal of the neonate from the mother for the first 1 to 2 h of life, and possible altered maternal–infant interactions in the case of the C-sectioned animals. In regard to the first of these, rapid maternal decapitation does not appear to stress the neonate, at least as assessed hormonally, since C-section birth was not associated with increases in neonatal plasma catecholamines (present study) or corticosterone (5), in comparison to vaginal birth. C-sectioned animals had similar birth weights compared to vaginal controls, indicating that their birth is only slightly premature. Impaired feeding may play a role in long-term CNS alterations in C-sectioned animals, as we have observed a modest reduction in body weight in

in some but not all groups of C-sectioned animals in comparison to vaginally born cohorts (5, 6). However, animals born by C-section with 15 min of acute anoxia also show a tendency to reduced body weight compared to vaginally born controls (5, 6), although the anoxic group did not show alterations in CNS DA levels as young adults.

In conclusion, in the rat, birth by C-section produces long-term changes in steady state DA levels and DA metabolism in the PFC, NAcc, and striatum, with no change in 5-HT or NE. Thus, the birth process may be viewed as a critical period for the normal development of DA pathways in the rat. By contrast to C-section alone, rats born by C-section with 15 min of acute anoxia show control levels of steady state DA as young adults. However, our previous studies have demonstrated that adult rats born by C-section, both with and without an added period of acute anoxia, show altered NAcc DA responses to repeated stress (8). At birth, C-sectioned animals appeared robust while those born by C-section with 15 min of acute anoxia were pale, hypotonic, and inactive and showed reduced respiration. However, evaluation of whole brain lactate levels, respiratory rate, and measures of neonatal well-being, such as muscle tone and activity levels, during the first day of life suggest that both C-section birth and C-section with added acute anoxia may be considered models of perinatal hypoxia in the rat.

It should be recalled that rat brain at birth is generally thought to be at a less mature stage of development than that of the human neonate (41). Thus, inasmuch as parallels can be drawn between rat and human systems, the rat model of C-section birth, with or without added periods of acute anoxia, may relate more to the condition of the premature rather than the term human infant. Our findings highlight the sensitivity of DA pathways to variations in birth procedure and support the notion that perinatal insult could play a role in the development of disorders postulated to involve altered dopaminergic function, such as schizophrenia.

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