

Effect of Cocaine on Cell Proliferation in the Cerebral Wall of Monkey Fetuses

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This study examined the effect of cocaine on cell proliferation in the fetal monkey cerebral wall. Pregnant monkeys received cocaine daily (10 mg/kg, orally, in fruit treats, at 07.00 h and 19.00 h) beginning on the 40th day of pregnancy (E40). The control animals received fruit treats only. One set of monkeys was used to examine the state of cell proliferation in the fetal cerebral wall at peak cocaine levels. These animals were injected with [³H]thymidine intravenously on E73, 1.5 h after the morning drug or placebo administration. Another set of monkeys was used to determine the state of cell proliferation after cocaine concentration declined to ineffective levels. These animals were injected with [³H]thymidine on the same day of pregnancy 10 h after the treatment. Cesarean sections were performed 40 min after the radioisotope injection. The right hemispheres were processed for autoradiography. The left hemispheres were used for biochemical analysis of the radioisotope incorporation into DNA. The third set of monkeys was used to determine whether chronic cocaine treatment extends the timing of neocortical neuronogenesis. These monkeys received their final cocaine treatment on E102 (the last day of normal neocortical neuronogenesis) and were injected with [³H]thymidine 24 h later. On E113, the fetal brains were processed for emulsion autoradiography. We found a significant decrease in the density of [³H]thymidine-labeled cells and in the levels of this radioisotope incorporation into DNA in the fetal cerebral wall 1.5 h after cocaine administration. In contrast, 10 h after cocaine administration we detected a significantly elevated density of radiolabeled cells, and abnormally high levels of [³H]thymidine incorporation into DNA. This suggests that chronic intermittent administration of cocaine results in significant periodic fluctuations in cell production within the fetal cortical proliferative zones. We detected no cocaine-induced extension in neocortical neuronogenesis.

Introduction

Cocaine abuse is a significant problem not only in the general population but also among pregnant women (Keller and Snyder-Keller, 2000). This raises the question of the effects of this drug on fetal development. In pursuit of an answer to this question, we developed a primate model of prenatal cocaine exposure. In our model, cocaine is administered to pregnant rhesus monkeys orally twice daily during the second trimester, which is the time of neocortical neuronogenesis in primates (Rakic, 1982, 1994). This developmental period has been previously identified as one of the most teratogen-sensitive phases of brain development (Kameyama, 1991). The oral administration of cocaine to monkeys models the snorting of cocaine by human addicts because the pharmacokinetics of cocaine administered orally closely resembles that achieved through intranasal application (Wilkinson *et al.*, 1980; Jatlow, 1988; Jufer *et al.*, 1998; Fattinger *et al.*, 2000).

We observed that monkeys born from the mothers receiving cocaine in accordance with our model displayed abnormal neocortical cytoarchitecture, with many neurons being unable

to assume their proper position within the cortical plate (Lidow, 1995, 1998; Lidow and Song, 2001). These findings were quite similar to those reported for mice exposed to cocaine *in utero* (Gressens *et al.*, 1992; Kosofsky *et al.*, 1994). Furthermore, our investigations went on to demonstrate that prenatally cocaine-exposed monkeys had a significantly reduced density and number of neocortical neurons (Lidow and Song, 2001). In search of the mechanisms of the latter effect of prenatal cocaine administration, we examined its ability to induce cell death in the fetal brain. This examination showed that the presence of cocaine increased the occurrence of cell death in the fetal monkey cerebral wall during corticogenesis (He *et al.*, 1999). However, the rise in cell death may not be the sole reason for the cocaine-induced decrease in density and number of neocortical neurons. It is conceivable that the aforementioned decrease may also reflect the apparent ability of this drug to interfere with cell proliferation. Such ability of cocaine has been demonstrated in cultures of fibroblasts (Di Francesco *et al.*, 1990), pheochromocytoma PC12 cells (Zachor *et al.*, 1994; Tosk *et al.*, 1996), C6 glioma cells (Grag *et al.*, 1993) and splenic T lymphocytes (Klein *et al.*, 1988; Berkeley *et al.*, 1994; Piccotti *et al.*, 1997). In addition, cocaine-induced suppression of cell proliferation has been reported in the cerebellum and cerebral wall of neonatal rats (Anderson-Brown *et al.*, 1990). Therefore, the present study was designed to determine whether the cocaine treatment used in our nonhuman primate model of prenatal cocaine exposure indeed affects cell proliferation in the fetal cerebral wall. Specifically, we addressed the following questions: (i) whether there is a reduction in cell proliferation during peak levels of cocaine in the organism; (ii) whether an initial reduction in cell proliferation is followed by a burst in proliferative activity when the concentration of cocaine declines to ineffective levels; and (iii) whether the chronic cocaine treatment used in our model extends the timing of neocortical neuronogenesis, as has been seen during development of the cerebral cortex in ethanol-exposed animals (Miller, 1986, 1987).

Materials and Methods

Animals

For this study, healthy time-pregnant rhesus monkeys (*Macaca mulatta*), 5–7 years of age, were purchased from the Oregon Regional Primate Research Center (Beaverton, OR, USA). The monkeys arrived at the University of Maryland Animal Facilities between pregnancy days 35 and 37 (E35–E37). Eleven of these animals were administered cocaine hydrochloride (Research Technology Branch, National Institute of Drug Abuse, Rockville, MD, USA) daily beginning on E40, the day when neocortical neurons are first generated in the transient fetal proliferative zones (Rakic, 1982, 1994). This is also the day of pregnancy on which we began cocaine treatment in our previous studies (Lidow, 1995; He *et al.*, 1999; Lidow and Song, 2001). Cocaine (10 mg/kg) was administered orally (in fruit treats) twice daily: at 07.00 h and 19.00 h. Previously, we demonstrated that cocaine presented in this way to pregnant monkeys

achieves its peak plasma concentration in the fetus within 1.5 h (Zhou *et al.*, 2001). The fetal plasma concentration of the drug then declines to the levels undetectable by chromatographic-mass spectrometric technique within the next 8 h (Zhou *et al.*, 2001). While the pharmacokinetics of cocaine in the fetal brain was not examined, the available literature indicates that it should closely follow the one observed in the fetal circulation (Wiggins, 1992). Eleven control pregnant monkeys received fruit treats only. During the study, all monkeys received High Protein Monkey Chow (Ralston Purina Co., St Louis, MO, USA) and were given fresh fruits twice a day. Water was available *ad libitum*. As cocaine is a known appetite suppressant (Lidow *et al.*, 1999), the daily food consumption was monitored for all pregnant monkeys involved in the present study from the time of their arrival to the University of Maryland until the time when their fetuses were removed for examination. The body weight of these animals was also recorded on a weekly basis. We did not observe any decline either in food consumption or in body weight among cocaine-treated pregnant monkeys. Furthermore, food consumption and body weight of the latter monkeys were well within the range of those seen in drug-naive pregnant animals. The failure of cocaine to suppress appetite in pregnant monkeys was also observed in all of our previous studies (Lidow, 1995; He *et al.*, 1999; Lidow and Song, 2001) and in studies of Morris *et al.* (Morris *et al.*, 1996, 1997).

[³H]Thymidine Injections

On E73, eight cocaine-treated and eight drug-naive pregnant animals received intravenous injections of 10 mCi/kg [³H]thymidine in 5 ml of sterile distilled water (New England Nuclear Co., Boston, MA, USA). This embryonic age was chosen because on this day both ventricular and subventricular transient proliferative zones are active in most developing neocortical regions (Rakic, 1982, 1994). It has been demonstrated that alcohol (Miller and Nowakowski, 1991) and such neurotransmitters as GABA and glutamate (Haydar *et al.*, 2000) can differentially regulate the rate of cell proliferation in these two zones. Therefore, it was reasonable for us to wish to evaluate the effect of cocaine exposure on proliferative activity in each of these zones. In half of the animals, [³H]thymidine injection was administered 1.5 h after the morning cocaine or placebo treatment. As was mentioned earlier, this is the time at which we detected maximal concentration of cocaine in the fetal circulation (Zhou *et al.*, 2001). The other half of the animals received [³H]thymidine injection 10 h after the morning treatment. By that time, cocaine could no longer be detected in the plasma of fetuses (Zhou *et al.*, 2001). This allowed us to evaluate [³H]thymidine incorporation into the DNA of proliferating cells under the peak levels of cocaine, achieved in our model of prenatal cocaine exposure and after the drug had been eliminated from the fetal circulation. Forty minutes after the [³H]thymidine injection, all fetuses were removed by Cesarean section, and their brains dissected out. The right cerebral hemispheres were blocked and fixed by immersion into 4% paraformaldehyde for 6 h at 4°C. This was followed by immersion overnight in phosphate buffer containing 10% sucrose and, then, for 24 h in solution containing 20% sucrose. Finally, the tissue was frozen to -30°C in isopentane and stored at -70°C prior to processing for emulsion autoradiography. The cerebral wall of the left hemispheres was dissected out and homogenized in 10 volumes of ice-cold water to access the incorporation of the radioisotope into DNA.

The remaining three experimental and three control pregnant animals were used for examination of the ability of the chronic cocaine treatment employed in our model to produce a significant extension of the period of neocortical neuronogenesis beyond E102, which is normally the last embryonic day for generation of neocortical neurons in rhesus monkeys (Rakic, 1982, 1994). For this purpose, the monkeys received their last treatment on the evening of E102, and on the evening of E103 they were injected with [³H]thymidine (10 mg/kg; intravenously). The fetuses were allowed to survive until the morning of E113. At that time, they were removed by Cesarean section. Their brains were dissected out, blocked and fixed for emulsion autoradiography as described above. A 10-day period between [³H]thymidine injection and Cesarean section was chosen to allow the labeled cells to reach their final position within the cerebral wall as well as for their nuclei to assume a size and shape that could be easily recognized in cresyl violet-stained sections according to

the criteria of Williams and Rakic (Williams and Rakic, 1988) and Selemon *et al.* (Selemon *et al.*, 1999).

Autoradiography

For autoradiographic visualization of [³H]thymidine labeling, the fetal brain tissue was sectioned serially on a cryostat into 25 µm thick coronal sections, which were mounted on gelatin-subbed slides. Sections were then defatted in a series of graded alcohols and xylene. The defatted sections were processed for autoradiography by dipping them in an Ilford Nuclear Research Emulsion K5D (Polysciences, Inc., Warrington, PA, USA). This emulsion produces silver grains of large size, which enhances the visualization of the radiolabel. The emulsion-coated sections were stored in the dark in a refrigerator for 10–18 weeks before being developed in a Kodak D-19 developer, fixed with 24% sodium thiosulfate, and stained with cresyl violet. The examination of the tissue sections was performed using a Zeiss Aristoplane microscope (Oberkochen, Germany). For each fetus, the counting of the labeled cell nuclei was conducted in the cerebral walls of the primary visual cortex of the calcarine sulcus (VCW) and the prefrontal cortex of the principal sulcus (PCW) of the right hemisphere. Within both VCW and PCW, coronal sections for counting were selected using uniform random method (Gundersen *et al.*, 1988), with the first section chosen at random and every third consecutive section being taken for analysis. This provided ~50 sections from each region of every animal examined. On each of these sections, the counting included labeled nuclei under 1 mm of the pial surface selected randomly, but within VCW or PCW. The selection of tissue sections as well as cell counting were performed by investigators blind to whether the tissue came from the cocaine-exposed or drug-naive fetuses. The detection of shrinkage associated with the tissue processing was performed as described by O'Kusky and Colonier (O'Kusky and Colonier, 1982). In agreement with these investigators, we found the shrinkage to be very similar for section thickness and section areas. Most importantly, we detected no significant differences between the shrinkage of the experimental and control sections, which was $13.5 \pm 4.3\%$ and $13.9 \pm 6.0\%$ (mean \pm SD), respectively.

Analysis of the Radioisotope Incorporation into DNA of the Proliferating Cells

The analysis of [³H]thymidine incorporation into DNA was performed as described by Anderson-Brown *et al.* (Anderson-Brown *et al.*, 1990) with modifications. After the homogenates of the brain tissue were generated, 0.5 ml aliquots (three per sample) of the these homogenates were used to assess the total uptake of the radiolabel with a Beckman LS5811 Scintillation Counter (Fullerton, CA, USA). Six additional 0.5 ml aliquots per sample were precipitated with 10% trichloroacetic acid, sedimented at 1000 g for 15 min, and the resultant pellets washed twice by resuspension and centrifugation. Three of these aliquots were used to measure radioisotope incorporation into DNA. The pellets from these aliquots were digested with hyamine hydroxide, and counted on a Beckman LS5811 Scintillation Counter (Fullerton, CA, USA). The remaining three aliquots were used for the determination of the amount of DNA with a Hitachi U-1100 Spectrophotometer (Tokyo, Japan). For the comparative analysis, the [³H]thymidine incorporation into DNA from the tissue of each animal was expressed as: $\text{DPM}_{\text{of the sample DNA}} / (\text{mean of three aliquots}) / \text{DPM}_{\text{of the tissue}} / (\text{mean of three aliquots}) / \mu\text{g DNA}_{\text{in the sample}} / (\text{mean of three aliquots}) \times 10^{-3}$ (Kornblum *et al.*, 1987), where DPM is disintegrations of the radioisotope per minute in a given sample. This expression takes into consideration the fact that the incorporation of radiolabel into macromolecules is dependent upon the amount of the radiolabel taken up by the tissue (Kornblum *et al.*, 1987; Anderson-Brown *et al.*, 1990).

Statistical Analysis

For all experimental paradigms used in this study, the comparison between values generated by the cocaine-exposed and drug-naive fetuses was performed with two-tailed Student's *t*-test. The differences were considered significant when $P < 0.05$.

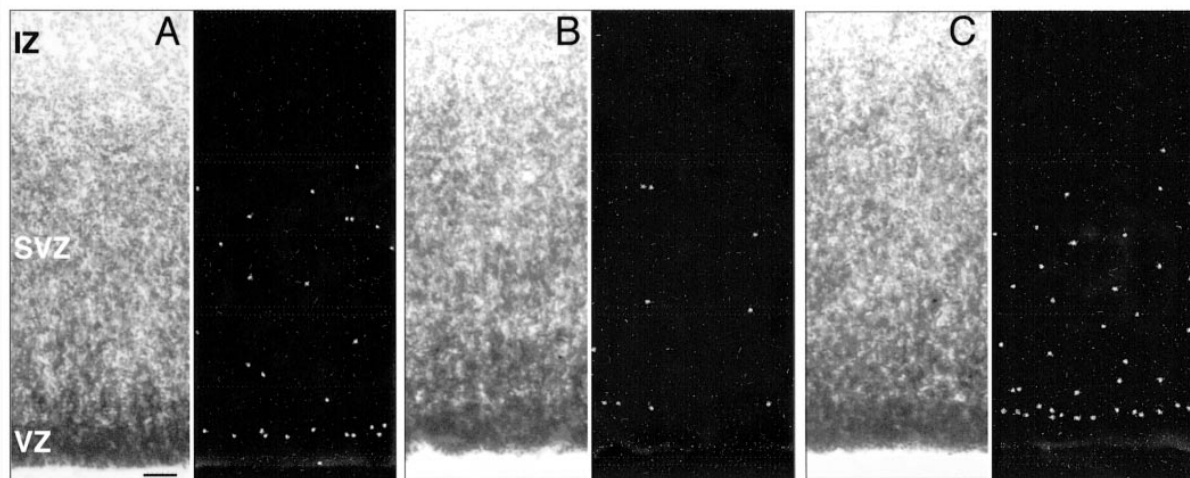


Figure 1. Micrographs of coronal sections through the deep portion of the occipital cerebral wall of 73-day-old monkey fetuses. (A) Section from a fetus of a drug-naive mother receiving [^3H]thymidine injection 1.5h after placebo treatment. (B) Section from a fetus of a cocaine-treated mother receiving [^3H]thymidine injection 1.5h after cocaine treatment. (C) Section from a fetus of a cocaine-treated mother receiving [^3H]thymidine injection 10h after cocaine treatment. All fetuses were delivered by Cesarean section 40min after the radionucleotide injection. Their right cerebral hemispheres were fixed, sectioned and processed for emulsion autoradiography followed by staining with cresyl violet. The right images show cresyl violet staining of the sections. The left images show the distribution and density of radiolabeled nuclei on the same sections in the dark field. VZ – ventricular proliferative zone; SVZ – subventricular proliferative zone; IZ – intermediate zone between the proliferative zones and the cortical plate. Scale bar = 200 μm . Note that the section of the cerebral wall of a fetus which received [^3H]thymidine 1.5h after cocaine administration displays much less radiolabeled nuclei than the section of the cerebral wall of a fetus receiving [^3H]thymidine 1.5h after placebo treatment. In contrast, [^3H]thymidine injected 10h after cocaine administration produced an excess of radiolabeled nuclei.

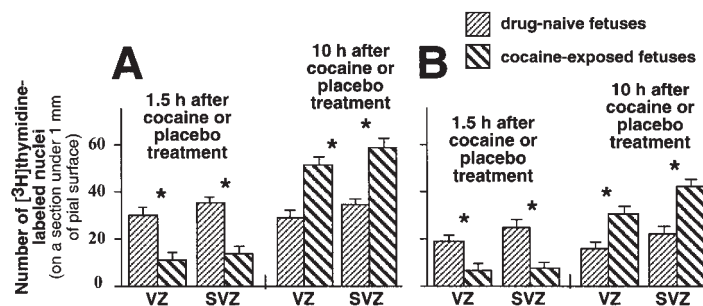


Figure 2. Histograms representing the number of [^3H]thymidine-labeled nuclei under 1mm of the pial surface on sections of the ventricular (VZ) and subventricular (SVZ) proliferative zones of cerebral wall containing the developing primary visual cortex (A) and the developing prefrontal cortex (B) in cocaine-exposed and drug-naive 73-day-old fetuses. The radionucleotide was administered to pregnant monkeys either 1.5 or 10h after cocaine or placebo treatment. Forty minutes after radionucleotide administration, the fetuses were delivered by Cesarean section. Their right cerebral hemispheres were fixed, sectioned and processed for emulsion autoradiography followed by counting of radiolabeled nuclei. Each column represents a mean value for four control or four experimental animals \pm SD. The statistically significant differences between drug-naive and cocaine-exposed fetuses are marked by asterisks ($P < 0.05$; two-tailed Student's t -test). Note that cocaine produced a decrease in the number of the nuclei labeled with [^3H]thymidine administered 1.5h after drug treatment. In contrast, 10h after cocaine treatment, [^3H]thymidine administration labeled an excessive number of nuclei.

Results

Analysis of [^3H]Thymidine Labeling in Tissue Sections from 73-day-old Fetuses

The examination of the [^3H]thymidine labeling in sections of the developing cerebral wall of 73-day-old fetuses revealed no differences in the laminar position of the radiolabeled cell nuclei between drug-naive and cocaine-exposed tissue. On all sections, the radiolabeled nuclei were situated predominantly in the ventricular and subventricular proliferative zones. In the ventricular zone, these nuclei were largely positioned near its border with the subventricular zone (Fig. 1), while the labeled nuclei were observed throughout the entire thickness of the subventricular zone (Fig. 1).

The qualitative examination of tissue sections from the fetuses of the placebo- and cocaine-treated mothers receiving [^3H]thymidine injection 1.5 h after the treatment showed that

the latter fetuses displayed a clearly detectable decrease in the density of radiolabeled nuclei in both the ventricular and subventricular proliferative zones across the entire cerebral wall. This observation was further supported by the counting of the radiolabeled nuclei conducted in the VCW and PCW sections. Counting demonstrated that the number of labeled nuclei under 1 mm of pial surface on the sections through these two regions of the cerebral wall of the cocaine-exposed fetuses contained less than half of the labeled nuclei seen in comparable sections from the drug-naive fetuses (Fig. 2). This decrease was equally strong and statistically significant in the ventricular and subventricular zones of both VCW and PCW.

A comparison of the sections of cocaine-exposed fetuses from the mothers receiving [^3H]thymidine injections 1.5 and 10 h after drug treatment showed that the latter fetuses contained a much higher density of radiolabeled nuclei (Fig. 1). Moreover, the cerebral wall of these fetuses contained a much higher

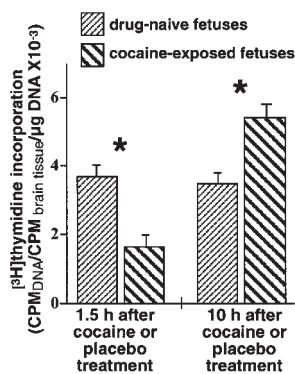


Figure 3. Histogram representing the effects of cocaine administration on the incorporation of [³H]thymidine into the DNA of cells of the cerebral wall of 73-day-old fetuses. The radionucleotide was administered to pregnant monkeys 1.5 or 10h after cocaine or placebo treatment. Forty minutes after radionucleotide administration, the fetuses were delivered by Cesarean section. The cerebral wall of their left cerebral hemispheres was homogenized and processed for analysis of incorporation of the radioisotope into DNA. Each column represents a mean value for four control or four experimental animals \pm SD. The statistically significant differences between drug-naive and cocaine-exposed fetuses are marked by asterisks ($P < 0.05$; two-tailed Student's *t*-test). Note that cocaine produced a decrease in incorporation of [³H]thymidine administered 1.5h after cocaine treatment and an increase in incorporation of this radioisotope when it was administered 10h after cocaine treatment.

density of labeled nuclei than the cerebral wall of the drug-naive fetuses. Counting of radiolabeled nuclei in the sections from the fetuses receiving [³H]thymidine 10 h after cocaine treatment revealed nearly twice as many such nuclei under 1 mm of pial surface as compared with the sections from the fetuses receiving [³H]thymidine 10 h after placebo treatment (Fig. 2). This increase was equally strong and statistically significant in the ventricular and subventricular zones of both VCW and PCW.

Analysis of [³H]Thymidine Incorporation into DNA of Cells of the Fetal Cerebral Wall

In agreement with the results of our autoradiographic studies, we found that DNA from the cerebral wall of 73-day-old fetuses from the mothers receiving [³H]thymidine injections 1.5 h after cocaine administration contained less than half of the isotope detectable in DNA from corresponding control fetuses (Fig. 3). The difference was statistically significant. When [³H]thymidine incorporation into DNA was examined in fetuses receiving this radionucleotide 10 h after the drug or placebo administration, we found that the cocaine-exposed fetuses displayed nearly 50% higher levels of radioisotope incorporation than their drug-naive counterparts (Fig. 3). This increase in radioisotope incorporation was statistically significant.

Analysis of Radiolabeled Nuclei in the Cerebral Wall of Fetuses Receiving [³H]Thymidine Shortly After the Last Neocortical Neuron Should Have Been Generated in the Course of the Normal Corticogenesis

In this part of the study, pregnant monkeys were subjected to daily cocaine or placebo treatment from E40 to E102. On the evening of E103, they received a single injection of [³H]thymidine, and the position, density and type of cells containing the label within the fetal cerebral wall were examined 10 days later (on E113) with the help of the emulsion autoradiography.

As was expected based on our previous studies (Lidow, 1995; Lidow and Song, 2001), the overall histological appearance of the neocortex of the cocaine-exposed 113-day-old fetuses dif-

fered significantly from the neocortex of the drug-naive fetuses. In particular, the neocortex of the drug-exposed fetuses lacked detectable lamination (Fig. 4). The only clearly recognizable cortical lamina was layer I. In addition, the cortical border with the white matter was poorly defined in the cocaine-exposed brains, with the white matter containing an abnormally high cell density (Fig. 4).

Nevertheless, we detected no differences between the distribution of the [³H]thymidine-labeled cell nuclei in the cerebral wall of the drug-naive and cocaine-exposed fetuses. In all fetuses, the radiolabeled nuclei were situated largely in the deep white matter and in cortical layer I. Only occasional labeled nuclei were observed within cortical layers II-VI. In addition, the cresyl violet-stained radiolabeled nuclei within the cerebral wall of both cocaine-exposed and control brains displayed characteristic features of glial nuclei (Williams and Rakic, 1988; Selemon *et al.*, 1999) such as small size, a dark nuclear membrane, and a dispersed pattern of nuclear chromatin (Fig. 4 insert).

The counting of radiolabeled nuclei in the VCW and PCW sections under 1 mm of the pial surface revealed no significant differences between the drug-naive and cocaine-exposed fetuses (Fig. 5).

Discussion

The present study demonstrates that cocaine can interfere with cell proliferation in the ventricular and subventricular transient zones of the fetal primate cerebral wall. This is shown by both the autoradiographic visualization of [³H]thymidine-labeled nuclei and by biochemical examinations of the incorporation of this radionucleotide into DNA. These findings are in agreement with the earlier reports of cocaine-induced inhibition of DNA synthesis in cultures of several cell types (Klein *et al.*, 1988; Di Francesco *et al.*, 1990; Zachor *et al.*, 1994; Tosk *et al.*, 1996; Piccotti *et al.*, 1997) and in the brains of neonatal rats (Anderson-Brown *et al.*, 1990).

Most importantly, we have found that the initial suppression of cell proliferation produced by an individual cocaine administration is followed by a significant compensatory burst of proliferative activity when the levels of the drug decline to ineffective levels. The data obtained in the present study do not allow us to discern exactly how cocaine administration leads to this fluctuation in cell proliferation. However, earlier investigations in fibroblast cultures demonstrated that addition of cocaine causes an accumulation of dividing cells at the G1/S border of the cell cycle (Di Francesco *et al.*, 1990). We speculate that a similar accumulation of cells poised to enter the S phase also takes place in the cerebral wall of monkey fetuses during the several hours after cocaine administration. In such case, the eventual decline in cocaine levels would release the accumulated cells to enter the S phase of the cell cycle in numbers exceeding those in control brains.

While the chronic intermittent cocaine treatment employed in our model induces fluctuations in the proliferation of cortical cells, the ability of this treatment to produce changes in the overall number of cells generated by the cortical proliferative zones is much less certain. Indeed, each cocaine-induced reduction in cell proliferation during the course of the treatment is likely followed by a compensatory increase in cell division. Furthermore, on average, the magnitude of a decrease in the density of [³H]thymidine-labeled cells seen during peak cocaine levels is matched in magnitude by an increase in the density of such cells observed 10 h later. This suggests that a reduction in actual cell production may not be among the main causes of a

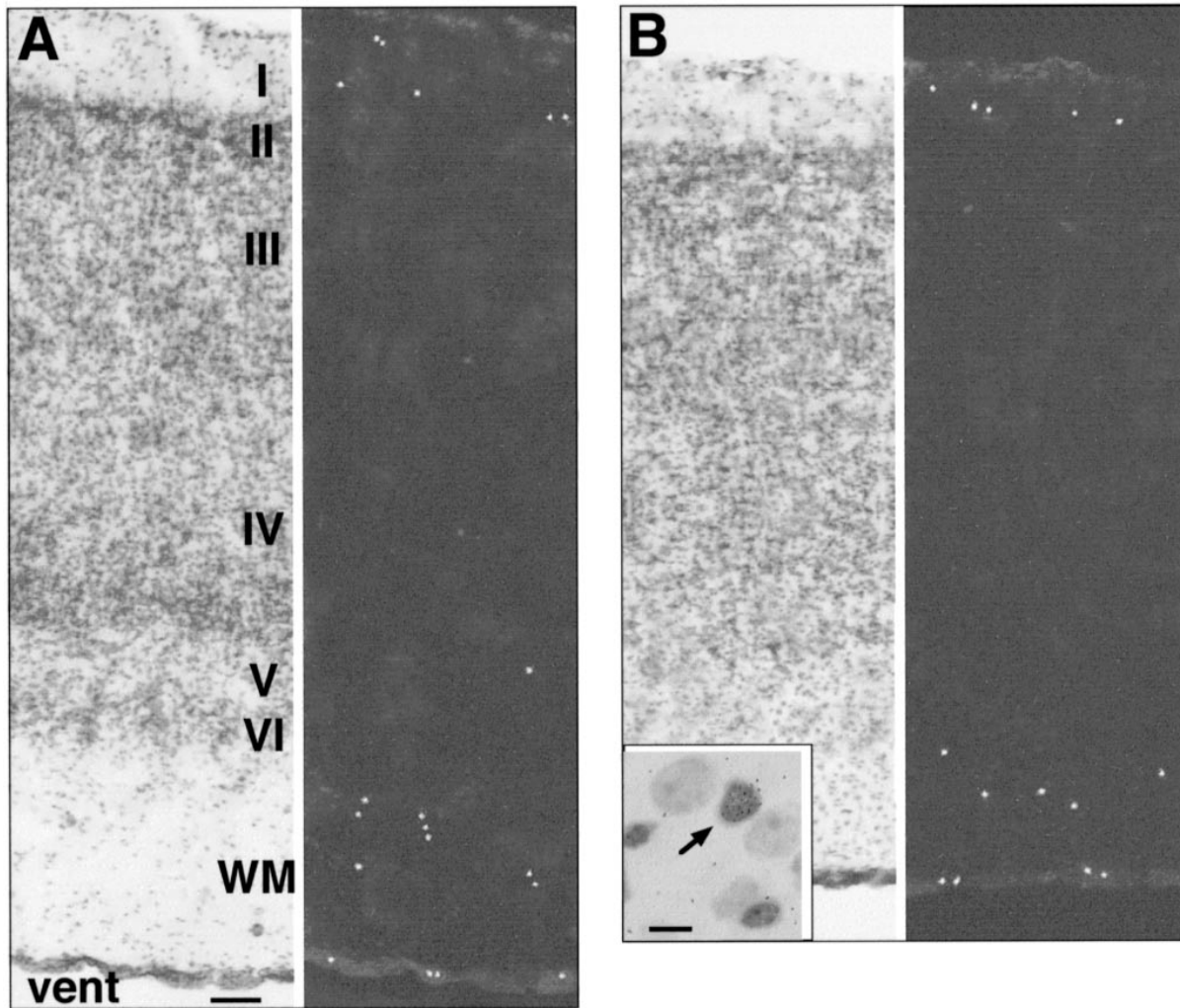
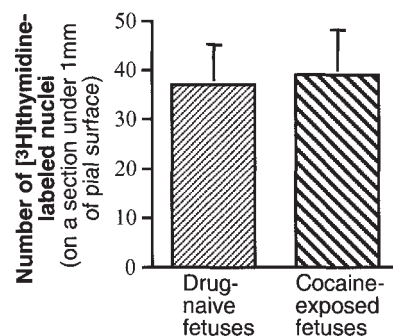


Figure 4. Micrographs of the coronal sections through the cerebral wall containing the primary visual cortex of 113-day-old fetuses from either a drug-naive mother (A) or a mother receiving cocaine between E40 and E102 (B). The right images show the cresyl violet staining of the sections. The left images show the same sections in dark field, which allows a visualization of nuclei labeled with ^3H thymidine administered on E103. VENT – lateral ventricle; WM – white matter; I–VI – cortical layers. Scale bar = 200 μm . Note the absence of clearly detectable differences in the distribution and density of the radiolabeled nuclei between cocaine-exposed and drug-naive fetuses. The insert shows a typical cresyl violet image of a radiolabeled nucleus (pointed to by the arrow). The nucleus is small with a dispersed chromatin pattern indicating that this nucleus belongs to a glial cell (Williams and Rakic, 1988; Selemon *et al.*, 1999). For the insert, scale bar = 10 μm .

Figure 5. Histogram representing the number of ^3H thymidine-labeled nuclei under 1mm of the pial surface on sections of the cerebral wall of the right hemisphere containing the developing primary visual cortex from 113-day-old fetuses of drug-naive mothers and mothers treated with cocaine between E40 and E102. ^3H Thymidine in both animal groups was injected on E103. Each column represents a mean value for three control or three experimental animals \pm SD. Note a lack of significant differences between experimental and control fetuses ($P > 0.05$; two-tailed Student's *t*-test).



decrease in cell number in the neocortex of animals born from cocaine-treated mothers (Lidow, 1995; Lidow and Song, 2001). In such case, this reduction may largely reflect the unusually high levels of postmitotic death of cortical cells that were previously detected in cocaine-exposed monkey fetuses (He *et al.*, 1999). At this time, we do not know whether and how the cocaine-induced fluctuations in cell proliferation may affect corticogenesis. Presently, we are conducting studies to address this question.

In addition to cocaine, several other widely abused substances, including marijuana, amphetamine, alcohol, morphine and nicotine, are known to be capable of suppressing the

proliferation of neural cells (Huot, 1974; Bendek and Hahn, 1981; Miller and Nowakowski, 1991; Stiene-Martin and Hauser, 1993; Levin and Slotkin, 1998). However, the present study, to our knowledge, is the first that demonstrates the ability of the developing brain to support a significant compensatory burst of proliferative activity following suppression of cell division by one such drug. We believe that this is because very few previous studies were designed to test this possibility. We expect future investigations to show that such compensatory aftereffect can also accompany administration of at least some of the other drugs of abuse listed above.

At this time, however, the only other well-documented substantial compensatory response to suppression of cell proliferation in the developing brain is an extension of the timing of neocortical neuronogenesis in the fetuses of rats chronically receiving an alcohol-containing liquid diet (Miller, 1986, 1987). Interestingly, we found that cocaine exposure employed in our model did not result in such protraction of the period of generation of neocortical neurons. Specifically, we detected no differences between cocaine-exposed and drug-naive animals in the number of cells in the cerebral wall labeled with [³H]thymidine administered one day after the end of the normal neocortical neuronogenesis. Furthermore, the labeled cells in all animals represented glia positioned in the white matter and layer I of the cortex. This may indicate that, as suggested above, the intermittent cocaine treatment employed in the present study does not result in a large enough overall reduction in the number of generated neurons to trigger an extension of the period of neocortical neuronogenesis. This is in contrast to a paradigm of essentially continuous suppression of cell proliferation in studies employing alcohol-containing liquid diet (Miller, 1986, 1987), which does not allow for a periodic compensation of a drug-induced reduction in cortical cell production. Alternatively, cocaine may affect proliferative activity in the fetal cerebral wall by mechanisms that do not allow for compensation by extending the period of neuronogenesis.

The mechanisms involved in the ability of cocaine to influence cell division in the fetal cerebral wall are now open for discussion. Several such mechanisms can be identified. For example, the fact that cocaine can block proliferation in cell cultures (Klein *et al.*, 1988; Di Francesco *et al.*, 1990; Grag *et al.*, 1993; Zachor *et al.*, 1994; Tosk *et al.*, 1996; Piccotti *et al.*, 1997) points to its direct effect on dividing cells. This effect is probably related to the ability of cocaine to induce elevation in the levels of intracellular free Ca²⁺, which has been shown to be responsible for cocaine-instigated suppression of proliferation in cultures of human T lymphocytes (Matsui *et al.*, 1993). In addition, cocaine may affect cell proliferation in the fetal cerebral wall by modulating the extracellular levels of monoaminergic neurotransmitters (Lidow, 1998; Lidow *et al.*, 1999), which are likely involved in regulating the rate of cell proliferation in the fetal brain (Lidow and Wang, 1995; Wang and Lidow, 1997; Wang *et al.*, 1997). The inhibition of cell proliferation in the fetal brain following cocaine administration may also be related to hypoxia, which has been shown to accompany the cocaine-induced constriction of the uterine artery and fetal brain vessels (Moore *et al.*, 1986; Woods *et al.*, 1987; Morgan *et al.*, 1991). The ability of hypoxia to affect the progression of cells throughout the cell cycle has been well documented (Spiro *et al.*, 1984; Amellem and Pettersen, 1991). We hope that future studies will address the contribution of each of these mechanisms to the ability of cocaine to interfere with cell proliferation in the fetal primate cerebral wall.

Notes

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