To study cocaine’s toxic effects in vitro, we have used primary mesencephalic and striatal cultures from rat embryonic brain. Treatment with cocaine causes a dramatic increase in DNA fragmentation in both primary cultures. The toxicity induced by cocaine was paralleled with a concomitant decrease in the microtubule associated protein 2 (MAP2) and/or neuronal nucleus protein (NeuN) staining. We also observed in both cultures that the cell death caused by cocaine was induced by an apoptotic mechanism, confirmed by TUNEL assay. Therefore, the present paper shows that cocaine causes apoptotic cell death and inhibition of the neurite prolongation in striatal and mesencephalic cell culture. These data suggest that if similar neuronal damage could be produced in the developing human brain, it could account for the qualitative or quantitative defects in neuronal pathways that cause a major handicap in brain function following prenatal exposure to cocaine.

1. Introduction

Drug abuse can have physiological, psychological, and social consequences [1]. Cocaine is a drug of abuse with reinforcing properties that can lead to the development of dependence. By binding to plasma membrane transporters, cocaine prevents the uptake of extracellular monoamines, consequently enhancing their extracellular levels, including norepinephrine and dopamine [2–4].

The vast majority of developmental studies investigating cocaine effects have focused on the dopaminergic system, presumably as a result of dopamine’s well-studied effects on reward and addiction [5]. The primary mesencephalic culture contains dopaminergic neurons from both the substantia nigra and ventral tegmental area, which expresses tyrosine hydroxylase (TH) [6, 7], the rate-limiting enzyme in dopamine synthesis. Dopaminergic afferents from substantia nigra pars compact provide dense innervations to the striatum [8, 9]. Given the reinforcing properties of cocaine such mesencephalic structures have been extensively investigated.

Besides its reinforcing properties, cocaine can cause damage to the CNS [10], being associated with cerebrovascular pathologies and convulsions that on occasion may be lethal [11]. More subtle functional and physical impairments may also be evident. Clinical and preclinical studies show learning and memory impairments, as well as the presence of movement disorders, following cocaine abuse, even after long periods of drug withdrawal [12, 13].

Cocaine can cross the placenta and accumulate in the fetus [14], with cocaine effects being especially evident in the newborns of females that abused cocaine during pregnancy. Maternal cocaine use during pregnancy is associated with significant impairment of cognitive development [15–17] that is detectable during the first two years of life and which may continue to contribute to learning difficulties and attentional dysfunction during later childhood [18]. In addition to the direct effects of cocaine, cocaine has a number of metabolites,
which will be present in the mother and fetus and which have a number of biological effects, including local anesthesia [19] and the inhibition of monoamine transporters [20], as well as vascular effects [21] and seizure induction [22].

Prior work on the effects of gestational cocaine has shown apoptosis in the fetal heart [23], decreased birth weight and head size, and deficits in cognition, attention, and language development in childhood [24, 25]. The prenatal cocaine exposure can result in molecular adaptations or anatomy changes in specific brain regions, including the hippocampus and cortex [26, 27]. The mechanisms underlying the damage caused by cocaine may involve a number of factors, including mitochondrial dysfunction, toxicity from dopamine metabolism, and/or reactive oxygen species (ROS) formation [28]. The nature of any subsequent cell death may be via either apoptotic or necrotic cell death processes.

The aim of this study was to determine the toxicity of cocaine in two different types of primary culture, striatal and mesencephalic. To our knowledge, this is the first study showing cocaine to cause cell death in such cultures. It is of note that the cocaine concentrations in this study are comparable to those of previous investigators, although in different cell types [29–32], as well as in the plasma of human drug abusers, ranging between 0.3 μM and 1 mM [30].

2. Materials and Methods

2.1. Primary Mesencephalic/Striatal Cultures. Primary cultures were cultured as previously described [33]. In brief, the mesencephalon or striatum of Sprague-Dawley rat embryos on day 17 was isolated and digested with 0.5 mg/mL trypsin in Earle’s Balanced Salt Solution (EBSS) (Life Scientific) for 2 hr at 37°C with 5% CO2 and plated on poly-L-lysine (Sigma) coated glass coverslips on plastic culture dishes (MatTek), at a density of 1 × 10⁶ cells/mL in high glucose Dulbecco’s minimum essential medium (DMEM) supplemented with 10% bovine calf serum, 25 U/mL penicillin, 25 mg/mL streptomycin, and 2 mM glutamine (Invitrogen). These mixed neuronal/glial cultures were treated with cocaine hydrochloride (Sigma) 1.0 mM or phosphate saline buffer (PBS) as control, on day 9 in vitro.

2.2. Immunostaining. On day 10, after 24 hours in vitro, neurons were identified by staining with anti-MAP2 (1:100; Sigma) or anti-NeuN (1:100; Chemicon; MAB 377) [33, 35]. Unless otherwise stated, each experiment described below was repeated at least three times, and >100 neurons were scored for each condition on triplicate coverslips. After 24 hours of cocaine exposure, cultures were fixed with 4% paraformaldehyde (Sigma) in PBS and permeabilized with 0.1% Triton X-100. After blocking nonspecific binding with PBS plus 3% BSA and 3% fetal bovine serum, the cells were incubated with antibodies to identify neurons (anti-MAP2 or anti-NeuN) followed by secondary Alexa Fluor 594 goat anti-mouse antibodies (1:100; Molecular Probes). In the last wash step, Hoechst 33324 (1 g/mL) was added to assess nuclear morphology. Hoechst 33342 is a UV-excitable nucleic acid stain readily taken up by all cells. Its blue fluorescence is particularly bright in the condensed nuclei of apoptotic cells. Typically, several hundred cells were scored in each experiment using fluorescent microscopy.

2.3. TUNEL Assay. Cells with DNA fragmentation were detected by the terminal deoxynucleotidyl transferase-mediated biotinylated UTP nick end labeling (TUNEL) method using the “in situ cell death detection-fluorescein kit” (Roche).

2.4. Statistical Analysis. Data were obtained from three independent experiments. In each experiment three replicate samples were quantified. Statistical comparisons were made by Student’s t-test for single comparisons. All values of P < 0.05 were considered statistically significant.

3. Results

To characterize the primary striatum culture, on day 10 in vitro, we observed the expression of GABAergic neurons that were stained with anti-GAD65/67 [36]. Our results demonstrated that 90% of the neurons present in the culture were GABAergic neurons (data not shown). We also tested for the presence of dopaminergic neurons by antibody staining to identify tyrosine hydroxylase (TH), the rate-limiting enzyme in the dopamine synthetic pathway. The results showed that there was no sign of striatal neurons expressing TH (data not shown). The mesencephalic culture was positive for TH, indicating that our mesencephalic culture comprises 10% dopaminergic neurons, which is characteristic of mesencephalic cultures [6].

In the primary striatum culture, control neurons exhibited normal chromatin, showing only 3% cell death. In contrast, after cocaine treatment (1.0 mM, 24 hours), neurons manifested an increase in bright/condensed Hoechst 33342 fluorescence, with evidence of 10% cell death (Figures 1(a) and 1(b)). We also observed, as indicated by MAP2 and NeuN staining, that neurite extension was inhibited after cocaine treatment (Figure 1(a)).

Similarly, in the primary mesencephalic culture, treatment with cocaine (1.0 mM) for 24 hours caused a decrease in neuronal viability coupled to an inhibition of neurite prolongation (Figures 2(a) and 2(b)).

The TUNEL assay confirmed that cocaine caused apoptotic death in both striatal and mesencephalic cultures (Figures 3(a), 3(b), 4(a), and 4(b)).

4. Discussion

Cocaine abuse can lead to toxic effects, including causing damage in specific brain areas. Studies in humans [37, 38], animals [39], and cell cultures [40, 41] have shown the toxic effects of cocaine, which can lead to cell death. Neuronal death during CNS development can change the organization of synaptic connectivity, leading to developmental and behavioral abnormalities in the offspring. Previous work shows cocaine to modulate the development [42–44] and survival [43–45] of CNS cells.
The present study demonstrates that cocaine decreases neuronal survival in primary striatal and mesencephalic cultures, two different brain regions relevant to cocaine’s mechanism of action. Most neurons in striatal culture are GABAergic, with some cholinergic neurons. Also, striatal cultures of primary neurons express functional D₁ and D₂ dopamine receptors [46, 47] as well as the dopamine transporter [7]. We also observed morphological changes in both cultures, characterized by chromatin condensation and DNA fragmentation, which indicates a process of apoptosis. In our model, striatal neurons in cell culture do not express the TH enzyme, the rate-limiting enzyme in dopamine synthesis, suggesting that this culture cannot produce dopamine. However, mesencephalic neurons in culture did express TH and therefore produce dopamine. Given the cocaine toxicity in both cultures, this suggests that cocaine’s toxic effect may be regulated by dopamine, but also possibly by an array of signaling through multiple and diverse secondary messenger system(s).
Figure 3: (a) In situ histochemical evidence of DNA fragmentation after cocaine exposure. Striatal cultures were first established for 7 days and incubated with cocaine (1.0 mM) for 24 hours. After cells were fixed, the TUNEL method was performed. Cultures were photographed at the level of the neuronal layer. Note the labeling in the vast majority of treated cells, in contrast with the labeling of a few control cells. TUNEL positive cells were dUTP labeled (brown label). The neurons were labeled with MAP2 and NeuN (green label) and Hoechst 33342 (blue label) was added to monitor chromatin condensation. (b) Number of TUNEL positive cells observed by immunostaining of the striatal primary culture treated with PBS or cocaine for 24 hours. Values are mean ± SEM from five independent experiments. Cocaine treatment decreased neuronal viability. *Significantly different from the control (PBS) value: * P < 0.05 by Student’s t-test.

Figure 4: (a) Mesencephalic cultures were first established for 7 days and incubated with cocaine (1.0 mM) for 24 hours. After cells were fixed, the TUNEL method was used. Cultures were photographed at the level of the neuronal layer. Note the labeling in the vast majority of treated cells, in contrast with the labeling of a few control cells. TUNEL positive cells were dUTP labeled (brown label). The neurons were labeled with MAP2 and NeuN (green label) and Hoechst 33342 (blue label) was added to monitor chromatin condensation. (b) Number of TUNEL positive cells observed by immunostaining of the mesencephalic primary culture treated with PBS or cocaine for 24 hours. Values are mean ± SEM from five independent experiments. Cocaine treatment decreased neuronal viability. * Significantly different from the control (PBS) value: * P < 0.05 by Student’s t-test.

We showed that the exposure of both primary mesencephalic and striatal culture neurons to cocaine evoked an apoptotic process. Apoptosis has also been reported by some authors in other models but not always [48–50].

It might be that the great variability in the physiological and functional effects of cocaine on developing CNS is due to the multiple biochemical and pathophysiological routes of cocaine’s actions. For example, dopamine and 5-hydroxytryptamine (serotonin) transporter knock-out mice
still continue to exhibit drug seeking behavior, suggesting the involvement of additional molecular pathway for cocaine action, besides blocking monoamine neurotransmitter transporters [4]. Studies demonstrated that GABA transmission in the nucleus accumbens is also altered after withdrawal from repeated cocaine [51]. At higher concentrations, cocaine can act as a local anesthetic, interacting with a variety of targets in both specific and nonspecific manners.

Chronic cocaine treatment can modulate voltage-gated Na\(^+\) and Ca\(^{2+}\) channels activity via the production of cyclic AMP by DA \(_2\) receptor stimulation [52, 53]. Also, direct modulation of ion channels can be responsible for some cocaine effects. It has been shown that cocaine can block voltage-dependent Na\(^+\) channels [54] and Ca\(^{2+}\) channels [53]. Other ion channels are modified by cocaine including the K\(^+\) channels activated by acetylcholine and adenosine [55]. Ca\(^{2+}\) and K\(^+\) channels are involved in the repolarization and after-hyperpolarization phases of the action potential. The magnitude and duration of the after hyperpolarization phase determine the rate of neuronal firing. Blockade of the Ca\(^{2+}\)-activated K\(^+\) channels may facilitate repetitive neuronal firing that may enhance the propensity to induce seizures and neuronal function during cocaine overdose [56]. Blockade of K\(^+\) could also underline a variety of effects mediated by cocaine, including increased Ca\(^{2+}\) influx at the presynaptic terminal, which can augment neurotransmitter and hormone release and can contribute to neurodegenerative processes. As such ionic regulation may be a significant mediator of cocaine’s neurotoxicity. Although we have no evidence of the presence of cocaine metabolites (ecgonine, ethyl ecgonine and ecgonine methyl ester) in these cultures, we cannot rule out that they could also be involved in the mechanism of cell death. However, previous work showed that only cocaine significantly decreased MAP2 content in cortical culture [57]. This could suggest that the apoptotic cascade might require the intracellular penetration of cocaine.

We also observed an inhibition of the neurite outgrowth in the cells exposed to cocaine. This could be due to the influence of cocaine on cytoplasmic calcium, thereby affecting the cytoskeletal network and altering neuronal regulation. Cocaine may target cytoskeleton proteins, particularly microtubule associated proteins (MAPs) [58] and actin filaments, altering the process of initiation, elongation, and turning of neuritic branches [59]. Cocaine can also act to modulate integrin structure and functions, thereby contributing to decreased neurite outgrowth. Nonintegrin ligands can alter neuronal integrin expression, with consequences for neurite outgrowth [60].

Maternal gestational cocaine abuse can cause damage to their offspring. Since the migration of neurons ultimately determines their connectivity, synaptic potential, and viability, altered neuronal migration may be a significant determinant of the consequences of maternal gestational cocaine use in the offspring. Here we demonstrate, for the first time, that an acute dose of cocaine can cause the apoptosis of primary striatal and mesencephalic culture cells after 24 hours. Further investigation as to the biological underpinnings of cocaine’s effects is likely to contribute to the etiology, course, and treatment of the consequences of maternal gestational cocaine abuse in the offspring.

**Conflict of Interests**

The authors declare that there is no conflict of interests for any of the authors.

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**References**

[1] E. J. Nestler and G. K. Aghajanian, “Molecular and cellular basis of addiction,” *Science*, vol. 278, no. 5335, pp. 58–63, 1997.

[2] L. T. Kennedy and I. Hanbauer, “Sodium-sensitive cocaine binding to rat striatal membrane: possible relationship to dopamine uptake sites,” *Journal of Neurochemistry*, vol. 41, no. 1, pp. 172–178, 1983.

[3] M. E. A. Reith, B. E. Meisler, H. Sershen, and A. Lajtha, “Structural requirements for cocaine congener to interact with dopamine and serotonin uptake sites in mouse brain and to induce stereotyped behavior,” *Biochemical Pharmacology*, vol. 35, no. 7, pp. 1123–1129, 1986.

[4] M. C. Ritz, R. J. Lamb, S. R. Goldberg, and M. J. Kuhar, “Cocaine receptors on dopamine transporters are related to self-administration of cocaine,” *Science*, vol. 237, no. 4819, pp. 1219–1223, 1987.

[5] R. A. Wise, “Brain reward circuitry: Insights from unsensed incentives,” *Neuron*, vol. 36, no. 2, pp. 229–240, 2002.

[6] B. A. Bennett, C. E. Hyde, J. R. Pecora, and J. E. Clodfelter, “Differing neurotoxic potencies of methamphetamine, mazindol, and cocaine in mesencephalic cultures,” *Journal of Neurochemistry*, vol. 60, no. 4, pp. 1444–1452, 1993.

[7] G. D. Lyng, A. Snyder-Keller, and R. F. Seegal, “Dopaminergic development of prenatatal ventral mesencephalon and striatum in organotypic co-cultures,” *Brain Research*, vol. 1133, no. 1, pp. 1–9, 2007.

[8] P. Voorn, A. Kalsbeek, B. Jorritsma-Byham, and H. J. Groenewegen, “The pre- and postnatal development of the dopaminergic cell groups in the ventral mesencephalon and the dopaminergic innervation of the striatum of the rat,” *Neuroscience*, vol. 25, no. 3, pp. 857–887, 1988.
M. A. Gates, V. M. Coupe, E. M. Torres, R. A. Fricker-Gates, and S. B. Dunnett, “Spatially and temporally restricted chemoattractive and chemorepulsive cues direct the formation of the nigrostriatal circuit,” *European Journal of Neuroscience*, vol. 19, no. 4, pp. 831–844, 2004.

C. S. Planeta, L. B. Lepsch, R. Alves, and C. Scavone, “Influence of the dopaminergic system, CREB, and transcription factor-xB on cocaine neurotoxicity,” *Brazilian Journal of Medical and Biological Research*, vol. 46, no. 11, pp. 909–915, 2013.

M. C. Ritz and F. R. George, “Cocaine toxicity: concurrent influence of dopaminergic, muscarinic and sigma receptors in mediating cocaine-induced lethality,” *Psychopharmacology*, vol. 129, no. 4, pp. 311–321, 1997.

S. S. O’Malley and F. H. Gawin, “Abstinence symptomatology and neuropsychological impairment in chronic cocaine abusers,” *NIDA Research Monograph Series*, no. 101, pp. 179–190, 1990.

S. O’Malley, M. Adams, R. K. Heaton, and F. H. Gawin, “Neuropsychological impairment in chronic cocaine abusers,” *The American Journal of Drug and Alcohol Abuse*, vol. 18, no. 2, pp. 131–144, 1992.

S. Schenker, Y. Yang, R. F. Johnson et al., “The transfer of cocaine and its metabolites across the term human placenta,” *Clinical Pharmacology and Therapeutics*, vol. 53, no. 3, pp. 329–339, 1993.

G. A. Richardson, M. L. Conroy, and N. L. Day, “Prenatal cocaine exposure: effects on the development of school-age children,” *Neurotoxicology and Teratology*, vol. 18, no. 6, pp. 627–634, 1996.

M. D. Schroder, P. J. Snyder, I. Sielski, and L. Mayes, “Impaired performance of children exposed in utero to cocaine on a novel test of visuospatial working memory,” *Brain and Cognition*, vol. 55, no. 2, pp. 409–412, 2004.

L. T. Singer, S. Minnes, E. Short et al., “Cognitive outcomes of preschool children with prenatal cocaine exposure,” *Journal of the American Medical Association*, vol. 291, no. 20, pp. 2448–2456, 2004.

L. T. Singer, R. Arendt, S. Minnes et al., “Cognitive and motor outcomes of cocaine-exposed infants,” *Journal of the American Medical Association*, vol. 287, no. 15, pp. 1952–1960, 2002.

W. W. Just and J. Hoyer, “The local anesthetic potency of norcocaine, a metabolite of cocaine,” *Experientia*, vol. 33, no. 1, pp. 70–71, 1977.

M. C. Ritz, E. J. Cone, and M. J. Kuhar, “Cocaine inhibition of ligand binding at dopamine, norepinephrine and serotonin transporters: a structure-activity study,” *Life Sciences*, vol. 46, no. 9, pp. 635–645, 1990.

J. A. Madden and R. H. Powers, “Effect of cocaine and cocaine metabolites on cerebral arteries in vitro,” *Life Sciences*, vol. 47, no. 13, pp. 1109–1114, 1990.

R. J. Konkol, B. A. Erickson, J. K. Doerr, R. G. Hoffman, and J. A. Madden, “Seizures induced by the cocaine metabolite benzoylecgonine in rats,” *Epilepsia*, vol. 33, no. 3, pp. 420–427, 1992.

Y. Xiao, D. Xiao, J. He, and L. Zhang, “Maternal cocaine administration during pregnancy induces apoptosis in fetal rat heart,” *Journal of Cardiovascular Pharmacology*, vol. 37, no. 6, pp. 639–648, 2001.

L. C. Mayes, C. Grillon, R. Granger, and R. Schottenfeld, “Regulation of arousal and attention in preschool children exposed to cocaine prenatally,” *Annals of the New York Academy of Sciences*, vol. 846, pp. 126–143, 1998.

J. A. Harvey, “Cocaine effects on the developing brain: current status,” *Neuroscience and Biobehavioral Reviews*, vol. 27, no. 8, pp. 751–764, 2004.

A. M. Thompson, J. Swant, and J. J. Wagner, “Cocaine-induced modulation of long-term potentiation in the CA1 region of rat hippocampus,” *Neuropharmacology*, vol. 49, no. 2, pp. 185–194, 2005.

B. L. Thompson, P. Levitt, and G. D. Stanwood, “Prenatal cocaine exposure specifically alters spontaneous alternation behavior,” *Behavioural Brain Research*, vol. 164, no. 1, pp. 107–116, 2005.

T. G. Hastings, D. A. Lewis, and M. J. Zigmend, “Role of oxidation in the neurotoxic effects of intrastriatal dopamine injections,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 93, no. 5, pp. 1956–1961, 1996.

M.-C. Nassogne, P. Evrard, and P. J. Courtoy, “Selective direct toxicity of cocaine on fetal mouse neurons. Teratogenic implications of neurite and apoptotic neuronal loss,” *Annals of the New York Academy of Sciences*, vol. 846, pp. 51–68, 1998.

C. Yuan and D. Acosta Jr., “Effect of cocaine on mitochondrial electron transport chain evaluated in primary cultures of neonatal rat myocardial cells and in isolated mitochondrial preparations,” *Drug and Chemical Toxicology*, vol. 23, no. 2, pp. 339–348, 2000.

A. Zaragoza, C. Diez-Fernández, A. M. Alvarez, D. Andrés, and M. Cascales, “Mitochondrial involvement in cocaine-treated rat hepatocytes: effect of N-acetylcysteine and deferoxamine,” *British Journal of Pharmacology*, vol. 132, no. 5, pp. 1063–1070, 2001.

T. Cunha-Oliveira, A. C. Rego, M. T. Morgadinho, T. Macedo, and C. R. Oliveira, “Differential cytotoxic responses of PC12 cells chronically exposed to psychostimulants or to hydrogen peroxide,” *Toxicology*, vol. 217, no. 1, pp. 54–62, 2006.

M. Digicaylioglu and S. A. Lipton, “Erythropoietin-mediated neuroprotection involves cross-talk between Jak2 and NF-xB signalling cascades,” *Nature*, vol. 412, no. 6847, pp. 641–647, 2001.

L. B. Lepsch, C. D. Munhoz, E. M. Kawamoto et al., “Cocaine induces cell death and activates the transcription nuclear factor kappa-b in pc12 cells,” *Molecular Brain*, vol. 2, article 3, 2009.

S.-I. Okamoto, K. Sherman, G. Bai, and S. A. Lipton, “Effect of the ubiquitous transcription factors, SP1 and MAZ, on NMDA receptor subunit type 1 (NR1) expression during neuronal differentiation,” *Molecular Brain Research*, vol. 107, no. 2, pp. 89–96, 2002.

M.-T. Herrero, C. Barcia, and J. M. Navarro, “Functional anatomy of thalamus and basal ganglia,” *Child’s Nervous System*, vol. 18, no. 8, pp. 386–404, 2002.

R. E. Mittelman and C. V. Wettl, “Death caused by recreational cocaine use. An update,” *Journal of the American Medical Association*, vol. 252, no. 14, pp. 1889–1893, 1984.

L. L. Cregler and H. Mark, “Medical complications of cocaine abuse,” *The New England Journal of Medicine*, vol. 315, no. 23, pp. 1495–1500, 1986.

M. A. Bozarth and R. A. Wise, “Toxicity associated with long-term intravenous heroin and cocaine self-administration in the rat,” *Journal of the American Medical Association*, vol. 254, no. 1, pp. 81–83, 1985.

J. He, Y. Xiao, and L. Zhang, “Cocaine-mediated apoptosis in bovine coronary artery endothelial cells: role of nitric oxide,” *Journal of Pharmacology and Experimental Therapeutics*, vol. 298, no. 1, pp. 180–187, 2001.
BioMed Research International

[41] Y. Xiao, J. He, R. D. Gilbert, and L. Zhang, “Cocaine induces apoptosis in fetal myocardial cells through a mitochondria-dependent pathway,” *Journal of Pharmacology and Experimental Therapeutics*, vol. 292, no. 1, pp. 8–14, 2000.

[42] T. L. Foltz, D. M. Snow, B. J. Strupp, R. M. Booze, and C. F. Mactutus, “Prenatal intravenous cocaine and the heart rate-orienting response: a dose-response study,” *International Journal of Developmental Neuroscience*, vol. 22, no. 5-6, pp. 285–296, 2004.

[43] S. Dey, C. F. Mactutus, R. M. Booze, and D. M. Snow, “Cocaine exposure in vitro induces apoptosis in fetal locus coeruleus neurons by altering the Bax/Bcl-2 ratio and through caspase-3 apoptotic signaling,” *Neuroscience*, vol. 144, no. 2, pp. 509–521, 2007.

[44] S. Dey, C. F. Mactutus, R. M. Booze, and D. M. Snow, “Specificity of prenatal cocaine on inhibition of locus coeruleus neurite outgrowth,” *Neuroscience*, vol. 139, no. 3, pp. 899–907, 2006.

[45] D. M. Snow, J. D. Smith, R. M. Booze, M. A. Welch, and C. F. Mactutus, “Cocaine decreases cell survival and inhibits neurite extension of rat locus coeruleus neurons,” *Neurotoxicology and Teratology*, vol. 23, no. 3, pp. 225–234, 2001.

[46] S. Weiss, M. Sebben, J. A. Garcia-Sainz, and J. Bockaert, “D2-Dopamine receptor-mediated inhibition of cyclic AMP formation in striatal neurons in primary culture,” *Molecular Pharmacology*, vol. 27, no. 6, pp. 595–599, 1985.

[47] S. Schinelli, M. Paolillo, and G. L. Corona, “Opposing actions of D1- and D2-dopamine receptors on arachidonic acid release and cyclic AMP production in striatal neurons,” *Journal of Neurochemistry*, vol. 62, no. 3, pp. 944–949, 1994.

[48] B. C. Jones, A. D. Campbell, R. A. Radcliffe, and V. G. Erwin, “Cocaine actions, brain levels and receptors in selected lines of mice,” *Pharmacology, Biochemistry and Behavior*, vol. 40, no. 4, pp. 941–948, 1991.

[49] H.-Y. Wang, J. M. Yeung, and E. Friedman, “Prenatal cocaine exposure selectively reduces mesocortical dopamine release,” *Journal of Pharmacology and Experimental Therapeutics*, vol. 273, no. 3, pp. 1211–1215, 1995.

[50] X.-H. Wang, P. Levitt, A. O. Jenkins, and E. H. Murphy, “Normal development of tyrosine hydroxylase and serotonin immunoreactive fibers innervating anterior cingulate cortex and visual cortex in rabbits exposed prenatally to cocaine,” *Brain Research*, vol. 715, no. 1-2, pp. 221–224, 1996.

[51] Z.-X. Xi, S. Ramamoorthy, H. Shen, R. Lake, D. J. Samuvel, and P. W. Kalivas, “GABA transmission in the nucleus accumbens is altered after withdrawal from repeated cocaine,” *Journal of Neuroscience*, vol. 23, no. 8, pp. 3498–3505, 2003.

[52] X.-F. Zhang, X.-T. Hu, and F. J. White, “Whole-cell plasticity in cocaine withdrawal: reduced sodium currents in nucleus accumbens neurons,” *The Journal of Neuroscience*, vol. 18, no. 1, pp. 488–498, 1998.

[53] X.-F. Zhang, D. C. Cooper, and F. J. White, “Repeated cocaine treatment decreases whole-cell calcium current in rat nucleus accumbens neurons,” *Journal of Pharmacology and Experimental Therapeutics*, vol. 301, no. 3, pp. 1119–1125, 2002.

[54] M. E. A. Reith, “Cocaine receptors on monoamine transporters and sodium channels,” *NIDA Research Monograph Series*, no. 88, pp. 23–43, 1988.

[55] R. G. Tsushima, J. E. Kelly, and J. A. Wasserstrom, “Characteristics of cocaine block of purified cardiac sarcoplasmic reticulum calcium release channels,” *Biophysical Journal*, vol. 70, no. 3, pp. 1263–1274, 1996.

[56] L. S. Premkumar, “Block of a Ca+-activated potassium channel by cocaine,” *Journal of Membrane Biology*, vol. 204, no. 3, pp. 129–136, 2005.

[57] M.-C. Nassogne, J. Louahed, P. Evrad, and P. J. Courtoy, “Cocaine induces apoptosis in cortical neurons of fetal mice,” *Journal of Neurochemistry*, vol. 68, no. 6, pp. 2442–2450, 1997.

[58] M. Tanaka and T. Nishikawa, “Effects of clonidine premedication on the pressor response to α-adrenergic agonists,” *British Journal of Anaesthesia*, vol. 75, no. 5, pp. 593–597, 1995.

[59] E. W. Dent and K. Kalil, “Axon branching requires interactions between dynamic microtubules and actin filaments,” *Journal of Neuroscience*, vol. 21, no. 24, pp. 9757–9769, 2001.

[60] M. L. Condic, D. M. Snow, and P. C. Letourneau, “Embryonic neurons adapt to the inhibitory proteoglycan aggrecan by increasing integrin expression,” *The Journal of Neuroscience*, vol. 19, no. 22, pp. 10036–10043, 1999.