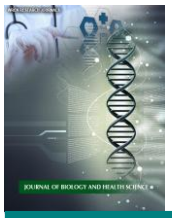

Journal of Biology and Health Science

ISSN: 3108-0189

Research Article

WRJBHS-25-041

Dose-Related Effects of Preconception Male Cocaine Use on Post-Weaning Voluntary Activity and Exploratory Behavioral Profiles of Offspring of Congenic, Naive Lean LA/Ntul//-cp Rats

Orien L Tulp*

Department of Medicine, University of Science Arts and Technology, Olveston, Montserrat and East West College of Natural Medicine, Sarasota Florida USA

***Correspondence:** Orien L Tulp, Department of Medicine, University of Science Arts and Technology, Olveston, Montserrat and East West College of Natural Medicine, Sarasota Florida USA, E-mail: o.tulp@usat.edu; DOI: https://doi.org/10.56147/jbhs.2.4.41

Citation: Tulp OL (2025) Dose-Related Effects of Preconception Male Cocaine Use on Post-Weaning Voluntary Activity and Exploratory Behavioral Profiles of Offspring of Congenic, Naive Lean LA/Ntul//-cp Rats. J Biol & Heal Sci 2: 41.

Abstract

Cocaine abuse has become a major public health problem in Western society, where it currently affects over 20 million adults and adolescents aged 12 and above. Previous studies indicate that mammalian spermatozoa contain opioid binding sites located on the head of the spermatozoa and upon direct exposure to genomic materials following fertilization and beyond, may induce epigenetic strand breaks in DNA. Thus, when exposed to opiates during spermatogenesis the opioid effects may become transmitted and incorporated into the female gamete during fertilization. Cocaine has been found to contribute to DNA strand breaks imposing epigenetic damage and the early-stage immature developing zygote would likely be more susceptible prior to development of DNA repair mechanisms to genomic damage as occurs in more mature cells. Unless the cocaine-induced DNA damage can be repaired during early crossover events, the epigenetic dysfunctions may conceivably remain present in offspring during periods of embryonic and fetal development and beyond. To determine the effects of chronic cocaine, use by males on the offspring of naïve females, groups of 60-day old lean LA/Ntul//-cp rats were reared from weaning on standard Purina chow and house water and administered 0 (Controls), 30 (Low Dose) or 60 (High Dose) mg/kg body weight of cocaine HCL daily for 90±2 days to fully encompass the duration of spermatogenesis. Opiate treated males were then mated with 82±3 day-old normally reared naive virgin females of the same strain that had never previously been exposed to the opioid or to a mating partner. Behavioral activity of each dosage level was assessed by subjecting the offspring postweaning at 21 days of age with a Stoelting activity wheel and a Calvin Hall open access exploratory field test. Offspring of pups were found to exhibit dose-related decreases in Stoelting wheel activity, with the greatest decrease at the highest dose administered (p=<0.01). Opiate treatment resulted in a latency in onset of exploratory activity at both dosage levels and decreased exploratory activit in bth inner squae s and outer squares at the low dose group; In contrast, the High Dose group demonstrated an increase in outer square exploration and in the total numbers of squares explored compared to the Low Dose groups or Controls. These results suggest that male cocaine exposure during spermatogenesis may result in longstanding dose related behavioral changes in the offspring of naive females and may predispose them to potential opioid-linked behavioral changes upon weaning and later in life.

Keywords: Cocaine; Behavior; Exploratory activity; Rat offspring; Prenatal exposure, Epigenetics

Received date: May 16, 2025; **Accepted date:** May 26, 2025; **Published date:** June 09, 2025

Journal of Biology and Health Science

ISSN: 3108-0189

Introduction

The current prevalence of cocaine abuse in Western Civilization is approaching epidemic proportions, with over 20 million individuals over the age of 12 now subject to succumbing to its non-medical use and abuse. Cocaine effects dopaminergic neurologic processes, in addition to its often-dire effects on norepinephrine and other catecholamines by impeding neuronal catecholamine reuptake mechanisms following heavier use [1-8]. Embryonic development during prenatal growth and development is subject to nutritional and toxicological exposure, where derangements in the fetal-maternal compartment may contribute to birth defects which may only become visibly apparent in the newborn. Prenatal nutrition, in addition to the presence of noxious chemicals have long been known to contribute to the embryonic processes of fetal growth and development in utero. Their combined embryonic manifestations are typically often only fully discerned *via* ultrasound or clinically apparent upon parturition when physical abnormalities may become better visualized [9-13]. Neurological or psychological deficits, however, may not be readily apparent at birth and may only become suspect later in the child's growth, neurological and psychological development. In Western Civilization, the incidence of birth defects has recently been estimated to impact 1 in 33 live births, often where the suspect causative agent is typically unknown with certainty [14]. The prenatal and early postnatal exposure may interfere with the normal development of multiple tissues depending on the magnitude, duration and chronological time of insult to the developing fetus [12,13]. Developmental insults that occur during the period of embryogenesis of the nervous system typically occur early in the life of the embryo, while insults delivered after the embryologic organ systems have developed tend to impose their primary impacts during the last semester, when the final growth of the fetus and its organ systems occur [9,10,12,13]. Gestation in the rat comprises a mere 21 days, compared to an average of approximately 280 days in humans, thereby providing an opportunity to gain insight into developmental processes in a shorter timeframe [11,15].

When nutritional insults are imposed during late gestation or postweaning growth, physical growth deficits typically occur in proportion to the duration and magnitude of the energy and nutrient insult [9-11]. In contrast to humans, where sensation of pain and external signals occurs by the third trimester, rat pups have little capacity for memory based on past learning experiences at the time of weaning when 21 days of age [16]. In humans, prenatal and early postnatal experiences to sounds and sensations likely also contribute to memory and learning processes present at birth [16]. Thus, the earlier in embryogenesis and early growth an insult may occur, the greater the potential impact may occur on epigenetic expression of later postweaning learning and developmental processes [7,10-13,17].

Opiates exert profound psychological and medical effects in humans, including aberrant behavior and potentially dire cardiovascular effects at higher exposure levels by impeding normal adrenergic neurotransmitter actions [7,8,18-20]. Cocaine produces its initial psychoactive and addictive effects primarily by acting on the brain's limbic system, where specific dopaminergic neurochemical regions that regulate pleasure and motivation are located. While short term effects of cocaine are likely reversible in humans and other mammalian species, when repeated, chronic exposure occurs, the short- and intermediate-term effects may cumulatively give rise to further negative effects that may persist for months or years, at which point they may become irreversible. The initial effects of cocaine exposure result in an accumulation of dopamine, a neurochemical that facilitates a sensation of euphoria in addition to an individual seeking additional exposures to enable a repeat or further enhancement of the euphoric process [22,23]. Following cocaine administration neuronal concentrations of of ΔFosB in the limbic system become increased and have been associated with addiction-like behaviors in mice. Similar to dopamine, ΔFosB functions as an intracellular, pace-setting chemical agent [4,17]. However, since ΔFosB remains in its original cell where it functions as a genetic transcription factor in the expression of multiple genes [4,17,19]. While cocaine effects impact multiple transcription factors, its effects on ΔFosB are considered the most long-lasting. Within the limbic system, the Nucleus Accumbens (NAc), is likely the primary focal point of the acute and chronic dopaminergic actions, as direct dopaminergic stimulation has been demonstrated to generate similar effects on pleasure and satisfaction as are typically observed during a cocaine high. Because of the long half-life of ΔFosB, which can persist for up to two months, prolonged cocaine exposure may precipitate increasing concentrations of the silent ΔFosB factor and contribute to long-lasting changes to nerve cell structure and function [17,19]. Although cocaine also inhibits the neuronal reuptake transporters for other neurotransmitter chemicals including norepinephrine and serotonin, its actions on the dopaminergic system are generally thought to be most important in the development of addiction-related behavior. In contrast, the later cumulative catecholamine effects are linked to the cardiovascular dysregulation and related toxicity [22]. Because Cocaine also alters the neurochemical reuptake system for all catecholamines in addition to the immediate euphoric effects, when cocaine is present, dopamine molecules that otherwise would be picked up and reenter neuronal dopamine stores remain neurophysiological active in the synaptic clefts, where they may accumulate and overstimulate dopamine receptors [6,7]. Once created, a molecule of ΔFosB lasts for 6 to 8 weeks before becoming biochemically inactivated [3,8]. Therefore, each new episode of cocaine abuse exacerbates the buildup of ΔFosB concentrations that have likely accumulated from previous episodes. Thus, if someone is abusing cocaine on a frequent basis, the cellular concentrations of ΔFosB will remain

chronically elevated and present a potentially neuropathophysiological and euphoric state. In addition, epidemiological studies indicate that approximately half of an individual's potential risk for addiction to cocaine or other opiates or addictive agents has a significant genetic origin, qualitatively similar in magnitude to that which impacts one's susceptibility to disorders such as Type 2 diabetes or breast cancer [2,3,5]. Therefore, inappropriate prenatal exposure to cocaine and related agents presents an important risk for development of a substance abuse disorder later in the affected individual's lifespan. While specific genes that encode for ΔFosB or any of hundreds of other potential genes that may be impacted by cocaine that could conceivably contribute to the genetic risk for addiction remains unclear.

One possibility for the heritable risks is that at least some of them are the same genes that are affected by cocaine exposure. For example, variations in the genes encoding ΔFosB or any of hundreds of other genes affected by cocaine could conceivably contribute to the genetic risk for addiction. It is easy to speculate that an individual with a gene that expresses high concentrations of ΔFosB could become more prone to addiction; such an individual would be analogous to the experimental mice that are engineered to produce more ΔFosB and consequently, became more prone to addiction [4]. It is also possible that other genes that were unaffected by cocaine exposure may be contributory to the addiction potential. Research to more fully determine the neurobiology of cocaine addiction is critical because the available treatments may not always resolve the addiction. A pathway toward definitive treatments, potential cures, as well as early diagnosis, intervention and prevention may be ascertained *via* further exploration of the underlying neurobiological mechanisms as is apparent for numerous illnesses and disorders of mankind [2,18]. Glutamate receptors and receptors for the brain's natural opioid-like substances including κ opioid receptors are but two instances where deciphering the neurobiological basis had proven to be helpful in developmental therapeutics. Therapeutic approaches that could modulate the magnitude and duration of ΔFosB overstimulation could indeed become an effective strategy in addressing the addiction potential.

Cocaine abuse is currently a major public health problem, with an estimated 20 million users worldwide, based on the most recent United Nations World Drug Report [2,14]. The leaves of the coca plant Erythroxylon coca have been used as a stimulant in South American culture for over 4000 years. The traditional use of cocaine, as typically obtained from the native coca leaves, has been in use for many centuries however by indigenous peoples of the Andean and other populations and cultures. In those civilizations, it has often been used to combat the effects of hunger, environmental and altitude extremes including symptoms of altitude sickness common to individuals habituating the high altitudes of the Altiplano [24-26]. Unlike the ongoing cocaine usage trends in current Western civilization as cited above, where purified cocaine preparations may be taken by inhaling microcrystalline or vaporized forms of the drug, the native populations commonly consumed the agent orally in the form of a tea, by chewing coca leaves or by a sachet of coca leaves tucked in the cheek. Once cocaine has been absorbed *via* buccal mucosa, the opioid can bypass the initial hepatic first-pass phenomena to prolong the therapeutic or euphoric effects [25]. When consumed as such, it was generally deemed free of addictive or adverse acute psychotropic attributes. In the USA, cocaine is classed as a class II drug under the Controlled Substances Act, indicating that while it does have bona fide medical applications, continued use of cocaine and related opiates can become a risk factor for developing a high potential for abuse in large part due to the immediate and often persistent euphoric effects of repeated neurobiological exposure [22,23]. Among accepted applications include therapeutic use as a topical upper respiratory anesthetic and other uses related to analgesia. In 1961 the Single Convention on Narcotic Drugs supported the recreational use and non-medical distribution of cocaine and other opiates to be considered as a punishable crime. That classification was based at least in part due to its potential addictive and dependent properties in addition to cardiovascular and other pathophysiologic sequelae [22]. While recreational use of other, more potent opiates has long overshadowed cocaine in recent years, it remains on the contraband list and the mechanisms of absorption, distribution and molecular actions are similar to those of other members of the opiate drug class. As recently as in 2019, cocaine was used as a recreational substance by an estimated 20 million people globally by adults aged 15 to 64 years in several Westernized counties including Australia, New Zealand Western and Central Europe and North and South America [22-26]. Caulkins et al reported that cocaine had the capacity to bind to surface receptor domains of live spermatozoa during spermatogenesis [27]. Thus, spermatozoa may be able to transport the cocaine moieties into the ova during fertilization, where they could potentially impact of chemical embryogenesis from the origination of zygote formation with as yet unknown consequences [27,28]. Therefore, the purpose of the present investigation was to determine if prolonged male cocaine use throughout the duration of active spermatogenesis could influence the activities and behavioral characteristics of offspring of naive females conceived from the cocaine-exposed spermatids. The behavioral activities of offspring of naive females sired by addicted, cocaine treated males were subjected to activity and exploratory studies during their early postweaning development and demonstrated impairments in exploratory and activity patterns soon after weaning when assessed *via* both running wheel and open field exploratory activities and prior to the onset of gestational and neonatal memory activities.

Materials and Methods

Animals: Groups of Congenic homozygous Lean male

Journal of Biology and Health Science

ISSN: 3108-0189

and female LA/N-tul//-cp rats (n=6 rats/group) were obtained from the former Drexel University breeding colony by the author. Animals were maintained on commercially obtained Purina chow formula stock number 5054 and house water, both offered ad libitum, from birth and throughout the study. Housing consisted of Plexiglass enclosures, lined with 1 inch of fresh pine shavings, maintained at 22°C-24°C and 50% RH, on a reverse light cycle (light 2000-0800 daily). Lean rats of this strain were selected due to their healthy longevity resulting from their derivation from an aging-prone NIH strain of Lister/Albany (LA/N) rats. Lean phenotype of the LA/N rats have been observed to attain ages of 3 to 4 years for males and females respectively when reared under standard laboratory conditions of diet and environment equivalent to the housing condition applied in this study [29,30].

Experimental: Groups of naive female rats of reproductive age (82 ± 3 days of age). The cocaine was obtained *via* a special research use permit from the NIH, Bethesda, MD, USA [27]. Male and female rats were separated at weaning. Upon adulthood and attaining sexual maturity, naive males were then subjected to daily administration of cocaine HCL of zero (controls). 30 (low dose) or 60 (high dose) mg cocaine/kg BW, subcutaneously, from 60 days of age until 150 ± 3 days of age, to encompass the complete duration of maturation of spermatozoa in the rat [19]. After 90 consecutive days of cocaine administration, male rats were mated with never exposed, naive virgin females aged 82 ± 3 days. Cocaine administration in the males was continued throughout the duration of the breeding period and until pregnancy was visibly confirmed, at which time they addicted males were removed from the breeding cages. A similar control group of unexposed virgin female rats of the same age were mated with additional never exposed males of the same age as above and maintained in separate cages. Upon weaning, when offspring were 21 days of age, they were removed from the dams and subjected to behavioral testing (n=8 rats/group obtained from 4 or more dams). Exploratory activity was determined by exposure to a Calvin Hall Maze and physical activity levers determined with a Stoelting activity wheel (Stoelting Co., Wood Dale IL USA) and recorded as the number of rotations completed per animal within a timed, 3-minute testing period [31-34]. Data was analyzed by Students 't' test. Litter size was unaffected by the cocaine treatment (n=8 ± 1 pup/litter in both treatment and control groups (p=n.s.) [35]. Each test group had equal number of male and female pups, of similar body weights (40 g ± 2 g BW) and were visually confirmed free of stigmata of early onset obesity, which was assured by utilizing breeding pairs that were confirmed homozygous lean offspring. In addition, offspring were tested only once, so as to preclude the chronologic opportunity for learned behaviour following repeated trials.

Ethics and study approval: The study was approved by the Institutional Animal Care and Use Committee and was consistent with AVMA procedures for animal experimentation [36].

Results

The effects of male cocaine pre-fertilization exposure throughout the duration of spermatogenesis compared to naive, never exposed prepubertal pups are depicted in **Figure 1** and indicate that the activity of pups born to addicted males were moderately less active than were pups born to naive, never cocaine exposed male breeders at the Low Dose treatment and were impacted yet further with the high Dose regimen, where voluntary activity was reduced by an average of 30%.

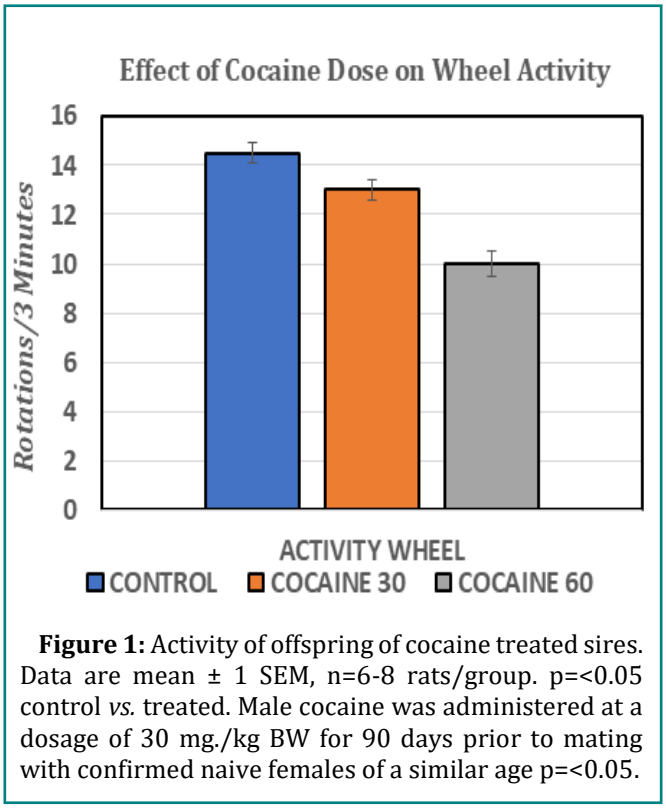

Figure 1: Activity of offspring of cocaine treated sires. Data are mean ± 1 SEM, n=6-8 rats/group. p=<0.05 control *vs.* treated. Male cocaine was administered at a dosage of 30 mg./kg BW for 90 days prior to mating with confirmed naive females of a similar age p=<0.05.

The effect of dose related, preconception male cocaine use on exploration activity of offspring in a Calvin Hall Maze is depicted in **Figure 2** below and indicates that pups exposed to preconception low dose cocaine in the male sire exhibited a delayed latency in both initiating exploration and in final exploration compared to pups born to naive sire and dam mattings. The cocaine measures are significant at the p=<0.05 level as indicated for each parameter examined. Pups from cocaine treated males and naive virgin females were slower to initiate exploratory behavior and exhibited less total exploratory behavior indicative of a cocaine mediated effect upon fertilization. When offspring of high dose cocaine were evaluated, the initial latency was less pronounced than with the low dose offspring, but were greater than controls or low dose offspring in both outer ring and total exploratory activity squares, suggestive of hyperactivity in the high dose offspring and similar to the results reported by Zmitrovich

Journal of Biology and Health Science

ISSN: 3108-0189

et al. when early gestation female rats were administered an equivalent dose of cocaine (60 mg/kg BW, s.c.) during pregnancy [37].

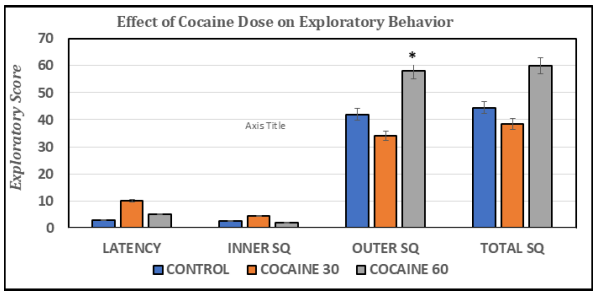

Figure 2: Effect of preconception cocaine dose on exploratory open field activity parameters of offspring of cocaine treated sires. Data are mean ± 1 SEM. N=6-8 rats/group. P=<0.05 for latency, initial squares, outer squares and final total squares explored; n.s.=not significant, p=>0.05.

Discussion

The epigenetic control of neuronal gene expression patterns has been reported to have emerged as contributors to underlying regulatory mechanisms for neuronal function, neuronal identity, neuronal plasticity, neuro cellular regeneration and neuronal survival. Their impact on multiple aspects of behavioral health, in which short- to long-lasting adaptation is required to dynamically respond and process external stimuli in response in the presence of opiate-induced influences remains unclear and incompletely elucidated. The results of this study indicate that male cocaine use over a prolonged duration resulted in impaired behavior of offspring born from previously naive, virgin females of a similar age who had never been exposed to cocaine, analogs of cocaine or other noxious substances. In addition, the cocaine induced effects appear to be dose related, with the greater dose studied demonstrating the greatest negative impact. While the molecular mechanism(s) could not be determined from the present study, the male exposure to cocaine occurred throughout and beyond the projected duration of spermatogenesis, recently reported to be approximately 54 days in rats, although minor variation in the duration of spermatogenesis may occur due to a variety of nutritional, strain, age and other factors [28]. Thus, the duration of mail cocaine exposure well exceeded the anticipated duration of spermatogenesis in this study. Rats have been observed to attain reproductive age soon after puberty, from approximately 42 days of age onward in our experience in this unique strain [38]. The head of the spermatozoa has been reported to bind to opioids that can be carried into the ova upon fertilization, thereby providing a simple explanation for inclusion of cocaine into the fertilized zygote [27]. In a clinical study, however, opiate users were found to have decreased sperm counts and significant DNA fragmentation compared to sperm obtained from healthy, cocaine-free volunteers [37]. Thus, the emerging possibility of preconception DNA damage from the male gamete prior to conception may have occurred in this study. In another study, Telese et al noted that cocaine addicted rats had different neuronal levels of GABA signaling, an inhibitory neurochemical in found in the amygdala and which could have contributed to the greater exploratory activity with the higher dosages studied in addition to impaired neuronal reuptake of excitatory norepinephrine and other catechol's common in opiate use [20]. In addition, cocaine binding to other cellular proteins may have occurred and which ultimately impacted cellular aspects of energy metabolism in their studies. While the rat pups in this study were likely not addicted of their own accord per se, the multiple possibilities of male gamete damage point to an alteration in intracellular epigenesis of neuronal properties early in the life of the developing offspring when paired with naive ova. The extent to which such DNA damage may be repaired later in gestational or postnatal growth and development remains unclear from the present study, since later observations were not undertaken. What is clear however, is preconception cocaine exposure of the male gamete resulted in developmental behavior deficits in the offspring of virgin, naive females. In this study, all rats who gave birth were considered to be healthy young adults with no visible nutritional or developmental deficits and the offspring while of apparent normal size, mass and dimensions, only demonstrated the developmental changes in physiological and exploratory behavior. The reported onset of memory-based learning in the rat occurs after 4 weeks of age, thus potential effects of prebirth memory influences were likely unaffected in this study [10,11,27]. A recent study reported that female sex hormones impacted opioid use in adult rats, but the epigenetic effects noted in the present study preceded the onset of gender maturation in the weanling rats.38 Thus, since the only variable deemed important in the current study was the opiate exposure throughout the duration of spermatogenesis, the likelihood of dose related opiate contamination during zygote formation contributed to the behavioral aberrations in the weanling pups, prior to the known age when neural inputs into rat memory or sex hormone influences would normally have occurred [20,21,37,38].

Conclusions

The epigenetic control of neuronal gene expression patterns has emerged as an underlying regulatory mechanism for neuronal function, identity and plasticity, in which short- to long-lasting memory, learning and adaptation is required to dynamically respond and process external stimuli. The results of this study indicate that the 21-day old offspring of chronic, cocaine treated male rats mated to naive, virgin rats of a similar age, demonstrated impairments in exploratory and activity patterns as determined by exposure to a Calvin Hall Maze and a Stoelting activity wheel. Virtually all parameters examined

were deemed significant at the p=<0.05 level by statistical analysis. While the biological mechanism of the behavioral impact could not be determined, the effects were likely secondary to intraova transport at the time of fertilization, followed by molecular events during the initial formation of the xygote, where the parental DNAs of both parental haploid lines merge to form the diploid zygote. Thus, should the chronic cocaine exposure damage of the paternal parental DNA remain compromised upon fertilization, the resulting immature zygote would likely inherit and retain the damaged DNA throughout early development of the developing blastocyst and later fetus. While the blastocyst is often considered to be protected from maternal chemical insults prior to implantation, potential DNA damage prior to implantation would likely already be present with each succeeding cell division. The onset of the development of neurogenesis in rats begins soon after fertilization and continues for approximately 2 weeks, while glial cells necessary for support functions continue to form soon after birth of the newborn pups. During the early stages of neurogenesis, epigenetic errors due to chemical or nutritional injury present at fertilization may survive thereafter and likely impact the behavior of the offspring later in life. Similar results were reported by Zmitrovich et al. in pregnant females that were treated with cocaine during early to mid-gestation in the rat, implying that the damaging epigenetic effects during neuronal embryogenesis may originate from either paternal or maternal alleles during neuronal formation [26]. Regardless of the neurochemical mechanism, the effects of cocaine exposure on the male gamete prior to conception may bring about behavorial and activity changes in the offspring prior to memory development and with unknown long-term significance during the early postweaning life of the affected offspring.

Acknowledgement

The author thanks the University of Science Arts and Technology for the resources to prepare this manuscript and to Professor James Caulkins, Eemeritis of Drexel University for inspiration and encouragement to conduct this study.

Disclaimer (Artificial Intelligence)

Authors hereby declare that no generative AI technologies such as Large Language Models (ChatGPT, COPILOT, *etc.*) and text-to-image generators have been used during the writing or editing of this manuscript.

Consent

It is not applicable.

Ethical Approval

The study was approved by the Institutional Animal Care and Use Committee.

Competing Interests

Author has declared that no competing interests exist.

References

1. Nestler EJ (2005) The neurobiology of cocaine addiction. Sci Pract Perspect 3: 4-10. [Crossref] [Google Scholar] [Indexed]
2. Goldstein A (2001) Addiction: From biology to drug policy (2nd edn). Oxford University Press: Oxford. [Google Scholar]
3. Nestler EJ (2001) Molecular basis of neural plasticity underlying addiction. Nat Rev Neurosci 2: 119-128. [Crossref] [Google Scholar] [Indexed]
4. Nestler EJ, Barrot M, Self DW (2001) ΔFosB: A molecular switch for addiction. Proc Natl Acad Sci USA 98: 11042-11046. [Crossref] [Google Scholar] [Indexed]
5. Nestler EJ, Malenka RC (2004) The addicted brain. Scientific American 290: 78–85. [Google Scholar]
6. Hyman SE, Malenka RC (2001) Addiction and the brain: The neurobiology of compulsion and its persistence. Nat Rev Neurosci 2: 695-703. [Crossref] [Google Scholar] [Indexed]
7. Kalivas PW, McFarland K (2003) Brain circuitry and the reinstatement of cocaine-seeking behavior. Psychopharmacology 168: 44-56. [Crossref] [Google Scholar] [Indexed]
8. Koob GF, Sanna PP, Bloom FE (1998) Neuroscience of addiction. Neuron 21: 467-476. [Crossref] [Google Scholar] [Indexed]
9. Bleker L, de Rooij S, Painter R, Ravelli A, Roseboom T (2021) Cohort profile: The Dutch Famine Birth Cohort (DBFC)-a prospective birth cohort study in the Netherlands. BMJ Open 11. [Crossref] [Google Scholar] [Indexed]
10. Fleming TP, Watkins AJ, Velazquez MA, et al. (2018) Origins of lifetime health around the time of conception: Causes and consequences. Lancet 391: 1842-1852. [Crossref] [Google Scholar] [Indexed]
11. Marshall N, Abrams B, Barbour L, et al. (2021) The importance of nutrition in pregnancy and lactation: Lifelong consequences. Am J Obstet Gynecol. [Crossref] [Google Scholar] [Indexed]
12. Wang C, Yang H, Chen S, et al. (2024) Early and late place cells during postnatal development of the hippocampus. Nat Commun 15: 10075. [Crossref] [Google Scholar] [Indexed]
13. Farooq U, Dragoi G (2024) Experience of Euclidean geometry sculpts the development and dynamics of rodent hippocampal sequential cell assemblies. Nat Commun 15: 8417. [Crossref] [Google Scholar] [Indexed]
14. https://www.cdc.gov/birth-defects/data-research/facts-stats/index.html.

15. Tulp OL, Horton ES (1981) The effect of prolonged experimental protein malnutrition and of refeeding on growth and adipose tissue development in the rat. J Nutr 111: 1145-1156. [Crossref] [Google Scholar] [Indexed]

16. McClung CA, Nestler EJ (2003) Regulation of gene expression and cocaine reward by CREB and ΔFosB. Nature Neuroscience 6: 1208-1215. [Crossref] [Google Scholar] [Indexed]

17. O'Brien CP (2003) Research advances in the understanding and treatment of addiction. American Journal on Addictions 12: S36-S47. [Crossref] [Google Scholar] [Indexed]

18. McClung CA, et al. (2004) ΔFosB: A molecular switch for long-term adaptation in the brain. Molecular Brain Research 132: 146-154. [Crossref] [Google Scholar] [Indexed]

19. Telese F, Gamliel A, Skowronska-Krawczyk D, Garcia-Bassets I, Rosenfeld MG (2013) Insights into the epigenetics of neuronal gene regulation. Neuron 77: 606-623. [Crossref] [Google Scholar] [Indexed]

20. Zhou JL, de Guglielmo G, Ho AJ, et al. (2023) Single-nucleus genomics in outbred rats with divergent cocaine addiction-like behaviors reveals changes in amygdala GABAergic inhibition. Nat Neurosci 26: 2035. [Crossref] [Google Scholar] [Indexed]

21. Brunton L, Knollmann B (2024) Goodman and Gilman's the pharmacological basis of therapeutics (14th edn). McGraw-Hill Pubs. [Google Scholar]

22. Warner EA (1993) Cocaine abuse. Ann Intern Med 119: 226-235. [Crossref] [Google Scholar] [Indexed]

23. Karch SB (1989) The history of cocaine toxicity. Hum Pathol 20: 1037-1039. [Crossref] [Google Scholar] [Indexed]

24. Novák M, Salemink CA, Khan I (1984) Biological activity of the alkaloids of *Erythroxylum coca* and *Erythroxylum novogranatense*. J Ethnopharmacol 10: 261-274. [Crossref] [Google Scholar] [Indexed]

25. Plowman T (1982) The identification of coca (*Erythroxylum* species): 1860-1910. Bot J Linn Soc 84: 329-353. [Crossref] [Google Scholar]

26. Calkins J, Winfrey A, Peisong H, Tulp O (1994) Effects of male cocaine use on behavioral attributes of F1 offspring in rats. FASEB J 8: A631.

27. Matsumura T, Katagiri K, Yao T, et al. (2023) Generation of rat offspring using spermatids produced through *in vitro* spermatogenesis. Sci Rep 13: 12105. [Crossref] [Google Scholar] [Indexed]

28. Stanford SC (2007) The open field test: Reinventing the wheel. Journal of Psychopharmacology 21: 134-135. [Crossref] [Google Scholar] [Indexed]

29. Tulp OL () Impact of aging and obesity on insulin parameters, t4/t3 ratios, metabolic rates, sirtuins and longevity in congenic la/ntul//-cp (corpulent) rats. Medical Science: Trends and Innovations 5: 12-38. [Crossref] [Google Scholar]

30. Robinson TE, Berridge KC (2001) Incentive-sensitization and addiction. Addiction 96: 103-114. [Crossref] [Google Scholar]

31. Volkow ND, Fowler JS, Wang GJ (2003) The addicted human brain: Insights from imaging studies. Journal of Clinical Investigation 111: 1444-1451. [Crossref] [Google Scholar] [Indexed]

32. Denenberg VH (1969) Open-field behavior in the rat: What does it mean? Ann N Y Acad Sci 159: 852-859. [Crossref] [Google Scholar] [Indexed]

33. Hall CS, Ballachey EL (1932) A study of the rat's behavior in a field: A contribution to method in comparative psychology. Univ Calif Publ Psychol 6: 1-12. [Google Scholar]

34. Stanford SC (2007) The open field test: Reinventing the wheel. J Psychopharmacol 21: 134-135. [Crossref] [Google Scholar] [Indexed]

35. Nie N, Hull CH, Jenkins K, et al. (1981) Statistical package for the social sciences (2nd edn). McGraw Hill Pubs: New York.

36. AVMA (2010) Use of animals in research, testing and education. J Vet Med Edu 37: 115.

37. Zmitrovich AC, Hutchins DE, Dow-Edwards DL, Malowany S, Chirch S (1992) Effects of prenatal exposure to cocaine on the rest-activity cycle of the preweaning rat. Pharmacology, Biochemistry and Behavior 43: 1059-1064. [Crossref] [Google Scholar] [Indexed]

38. Tulp OL (1990) Characteristics of thermogenesis, obesity and longevity in the LA/N–cp rat. ILAR J 32: 32-39. [Crossref] [Google Scholar]

39. Bradford S (2025) Female sex hormones reduced fentanyl use in rats. Reviewed in The Scientist: 1-6.