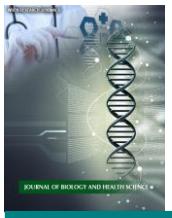

Journal of Biology and Health Science

ISSN: 3108-0189

Research Article

WRJBHS-25-040

Effect of Paternal Preconception Opiate Exposure on Exploratory Behavior and Thigmotaxis in Naive Offspring in Weanling 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.2.4.40

Citation: Tulp OL (2025) Effect of Paternal Preconception Opiate Exposure on Exploratory Behavior and Thigmotaxis in Naive Offspring in Weanling Rats. J Biol & Heal Sci 2: 40.

Abstract

Opiate use in Industrialized communities has reached near epidemic proportions and impacts the lives and activities of many who succumb to its use and abuse. In its native form, leaves of the coca tree have been in use for many centuries, particularly in the mountainous areas of South America and Andean culture where it was used to accommodate to the altitude and other issues. Because purified cocaine extracts common to Western culture have been reported to bind to surface domains of spermatozoa, it could readily become incorporated into ova upon their sperm penetration and fertilization. Alternatively, chronic paternal cocaine use could theoretically impose as yet unknown DNA damage to haploid DNA strands prior to fertilization. Cocaine is associated with proven damage to DNA, where it has been linked to disrupted DNA strands that when replicated in the developing blastocyst may undergo further replication during subsequent cell divisions and impact learning and behaviour effects soon after birth and weaning in the affected rat pups. Virgin male rats were administered 60 mg cocaine/kg BW, s.c. for >90 days to encompass the duration of de novo spermatozoa maturation (est. 54 days) and mated with never exposed, naive virgin females of a similar age. Offspring were observed for spontaneous activity and exploratory activity in a Stoelting Wheel (SWA) and a Calvin Hall Open Maze (CHOM) at 21 days of age. SWA was decreased by 33% and CHOM indicated a 40% decreased latency for initial exploration associated with greater outer and total square exploration. These observations are indicative of decreased onset but heightened exploratory activity in the outer square exploration activity squares. In conclusion, pre-fertilization cocaine exposure of spermatozoa may contribute to impaired learning and dopaminergic mediated thigmotaxic behavioral attributes of unclear origin in offspring of naive females.

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

Received date: May 15, 2025; **Accepted date:** May 21, 2025; **Published date:** June 05, 2025

Introduction

In a recent study, Zmitrovich et al. reported that exposure of pregnant females to daily subcutaneous injections of cocaine from 8 to 22 days of gestation resulted in a significant genomic change consisting of DNA breaks in selective brain tissues from the offspring after weaning. In addition, the authors also reported alterations in the exploratory behavior of offspring impacted by the cocaine during the immediate postweaning phase of their growth and development [1]. Cocaine is a highly addictive chemical, largely due to the intensity of the dopaminergic-induced euphoria in the limbic system experienced by the individual soon after exposure [2]. Moreover, the euphoric effects may increase over time due to progressive

Journal of Biology and Health Science

ISSN: 3108-0189

intracellular accumulation of ΔFosB, a silent intracellular promoter of euphoric dopamine actions that remains present for 6 to 8 weeks after cocaine exposure, thus extending and potentiating the direct effects of the dopaminergic trigger [3]. While excess dopaminergic actions may be partially counted by inhibitory actions of GABA over time, the mechanism of disinhibition following cocaine exposure remain unclear [4,5]. Moreover, Calkins et al proposed that cocaine exposed spermatozoa could bind cocaine and other opiates to their surface domains and therefore the opiate could potentially become incorporated in to a naive ova upon fertilization [6]. In as much as cocaine exposed zygotes have been demonstrated to undergo DNA strand breaks during early embryologic development in the rat and which remained present in neural tissues during early fetal development, the potential of the damaged DNA of paternal origin during neurologic development to impart physiologic or behavioral changes during later growth and development likely remains present for many subsequent cell divisions following its initial DNA encounter [1]. While some DNA strand breaks are known to undergo repair following postfertilization genomic crossover events, the ability for the DNA repair mechanisms to become effective during zygote formation from two haploid cells or later embryologic or fetal development of diploid cells remains unclear [1,6-8].

Indeed, according to the CDC, the prevalence of birth defects currently impacts as many as 1 in 33 live births in the United States, many with unknown or proven origins or other causative embryologic factors [8]. Nutritional factors, including folic acid deficiency and substrate availability are well known to interfere with physiological or neurological development, often with lifelong after effects if left untreated from early life [9-11]. While nutritional insults during early gestation may impact neurologic development, nutritional or energy deficits in later stages of pregnancy and gestation exert profound impacts on fetal growth from which recovery may or may not become complete in man and animals [9-11]. The recovery of neurologic deficits is often more difficult to quantify, however, as the full range of their neurophysiologic impact may may not be readily apparent in their physical growth and chronologic development, but may take many months or years to become fully expressed in the behavioral actions of offspring of addicted parents [8-11].

In rats, spermatid development and gestation is of remarkably shorter duration than in humans (21 days *vs.* 280 days, respectively) and learning experiences typically do not commence or become significantly imprinted prior to 28 days of age in the rat [9-12]. Thus, the purpose of the present investigation was to determine if chronic paternal cocaine exposure, a well-established addictive opiate, could impact behavioral or exploratory changes in the offspring of naive, never exposed dams at the time of weaning, *via* timed trials in a Stoelting Wheel or Calvin Hall Open Maze apparatus, to which the offspring or their parents had never previously been exposed [13-15]. Studies were undertaken in homozygous lean phenotype breeding pairs of congenic LA/Ntul//-cp rats, a strain noted for its healthful longevity and absence of any known predispositions for metabolic disorders [16].

Materials and Methods

Animals: Groups of congenic lean male 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 rats of unknown origin, often attaining up to 3 to 4 years of age for males and females respectively when reared under standard laboratory conditions of diet and environment [17].

Experimental: Groups of naive female rats of reproductive age (82 ± 3 days of age). Males and females of planned breeding partners were separated at weaning. Upon adulthood and attaining sexual maturity, males were then subjected to daily administration of cocaine HCL (60 mg/kg BW), subcutaneously as reported previously [1]. The cocaine was obtained *via* a special research use permit from the NIH from 60 days of age for 90 ± 3 consecutive days, to encompass the complete duration of maturation of spermatozoa in the rat [6,12]. After 90 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=6-8 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) as the number of rotations completed within a timed, 3-minute testing period [13-16]. Data were analyzed by Students 't' test. Litter size was unaffected by the cocaine treatment (n=6-8 pups/litter in both treatment and control groups (p=n.s.) [18]. Each test group had equal number of male and female pups, of similar body weights (40 g ± 2 g BW).

Ethics and study approval: The study was approved by the Institutional Animal Care and Use Committee and

Journal of Biology and Health Science

ISSN: 3108-0189

was consistent with AVMA procedures for animal experimentation [19].

Results

The effects of voluntary running activity in 21-day old weanling rats are depicted in **Figure 1** and depicts the number of rotations per minute for control and pre-fertilization exposed rats. Control rats completed approximately 5 rotations per minute, while cocaine exposed averaged only 3.3 rotations per minute, indicating a significant reduction in voluntary locomotor activity in the running wheel. After a brief, 30-45 minutes rest and recovery period, rats were then presented with a Calvin Hall Maze to estimate their exploratory activity **(Figures 2,3)**. Initial entry of cocaine exposed offspring was significantly delayed as depicted in **Figure 2**, as cocaine exposed pups took almost twice as long to embark on exploration activity. While there was a delay in the trend of cocaine treated rats to initiate exploratory activity, once they entered the squares they migrated to outer squares of the maze where they exhibited greater levels of activity while in the outer squares than their untreated controls, consistent with the observations of Zmitrovich et al, in gestationally treated dams in a similar rodent model [1].

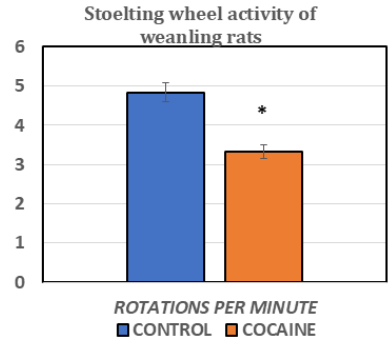

Figure 1: Effect of cocaine on Stoelting wheel activity of weanling rats. Data are mean ± 1 SEM, n=6-8 rats/group, *=p=<0.05.

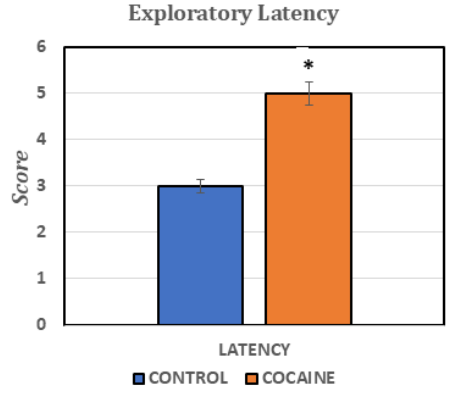

Figure 2: Effect of cocaine on exploratory latency of weanling rats. Data are mean ± 1 SEM, n=6-8 rats/group, *=p=<0.05.

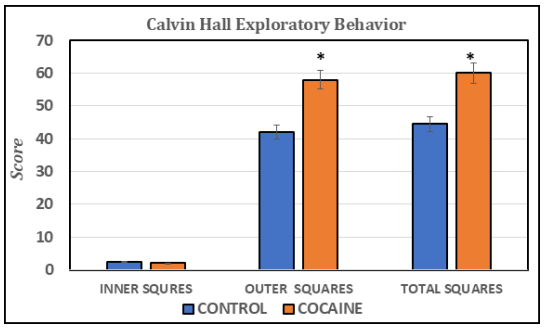

Figure 3: Effect of cocaine on Calvin Hall exploratory Behavior of weanling rats. Data are mean ± 1 SEM, n=6-8 rats/group, *=p=<0.05; ns=not significant; p=>0.05.

Animals such as rats and mice normally tend to exhibit an aversion to brightly lit open areas, seeking less brightly illuminated areas often perceived as being nearer the outer margins of the maze. However, they may also seek to explore a perceived threatening stimulus in inner areas of the maze that they may sense as potentially less secure areas of the maze [13-16]. In a Calvin Hall maze, it has been observed that decreased levels of anxiety tend to lead to increased exploratory behavior in outer, less threatening squares. Increased apparent anxiety as occurred in the present study resulted in less initial onset of locomotion and an apparent preference to stay closer to the outer boundaries of the field as occurred in this study, a phenomenon commonly known as thigmotaxis and representing the sum total of the dopaminergic and GABA mediated neurologic actions contributing to factors of reward cognition, motivation and learning experiences [2-5,16].

Discussion

The results of this study indicate that pre-fertilization cocaine exposure of spermatozoa may contribute to impaired learning and dopaminergic mediated thigmotaxic behavioral attributes of unclear origin in the offspring of naive cocaine-free females. While the biological mechanism of the impaired behavioral actions remains unclear, chronic cocaine use has been reported to result in confirmed DNA strand breakage during gestational exposure and in an atypical behaviour pattern of offspring upon weaning and prior to the recognized onset of learning patterns regarding exploratory behaviour of weanling rat pups. Calkins et al proposed that opiates including cocaine could bind to surface domains on the head of spermatozoa, thereby enabling the opiate to enter the haploid ova upon fertilization [6]. In addition, chronic exposure to the male breeders throughout the duration of the known spermatogenesis process may also have impacted the DNA integrity of the spermatids, enabling injured haploid chromosomes to undergo fertilization of bona fide naive ova and thereby becoming subject to cellular reproduction during subsequent cell divisions in the developing blastocyst [1,6]. During

ISSN: 3108-0189

chromosome crossover events, the reconfiguration and DNA annealing of the alleles readily occurs with extraordinary precision.

In contrast, previously altered DNA replication from the cocaine impacted allele may escape DNA correction processes and thus continue to remain present in the emerging DNA profiles of the impacted chromosomes, where the epigenetic expression of numerous aspects of progressive growth, development and emerging behavioral patterns are likely determined [20,21]. The incidence of physical and neurological birth defects has recently been reported to impact up to one in 33 or over 3% of live births in the USA, most without proven or otherwise confirmed causative factors [8].

While cocaine use has been in existence for hundreds of years it was previously used to combat the vicissitudes of altitude sickness and other maladies, predominantly in the Andean civilizations [22-25]. Cocaine intoxication invokes strong euphoric effects in humans, which contribute to repeated reward-seeking behaviour [26-31]. The euphoric effects are directly attributed to the neurotransmitter dopamine and its neuropharmacological effects on the limbic system and the silent factor ΔFosB which accumulates over repeated exposures and further enhances the euphoric effects. Because the duration of the ΔFosB is biologically deactivated only slowly and can persist for up to 6 to 8 weeks following exposure, the effects of repeated cocaine exposure can become exaggerated over time and precipitate fulminant effects *via* impeding neurohormone reuptake for dopamine and other catecholamines including norepinephrine and serotonin. The impaired neurohormone reuptake may eventually overstimulate cardiovascular responses, often with dire effects. Cocaine overstimulation may also enhance the release of γ-Aminobutyric acid (GABA), an inhibitory neurotransmitter that under normal circumstances helps to balance dopamine actions in the reward processing domains of mesolimbic and associated neuroprocessing systems and where the GABAergic inhibitory effects on behavior can contribute to hyperactivity in behavioral activities including thigmotaxis related actions. While under normal circumstances, it is speculative that GABA can inhibit dopamine release and the excess dopamine can in turn modulate GABA receptors, the progressive increase in ΔFosB may inflict a dysregulation of the balance between heightened dopamine actions on reward behavior. The dysregulation of the neurochemical balance may thereby contribute to the exacerbation of dopaminergic actions induced by repeated opiate exposure and its toxicological effects on behavior irritability, excitability and cardiovascular function. Regardless of the epigenetic mechanisms involved, the paternal preconception effects on early behavior of naive offspring remain of concern, as their implications for dysfunctional behavioral attributes during later stages of growth and development, in addition to potential links to future susceptibility to substance abuse patterns during adolescence and adulthood is unclear. This is especially relevant considering that maternal substance abuse during pregnancy has been associated with a greater predisposition to substance abuse among the surviving children as they approach adolescence and adulthood, suggestive of an epigenetic dysregulation of those attributes that modulate reward behavior. In summary, dopaminergic and GABA pathways in the human CNS including the brain and limbic system are involved in both physiological and behavioral processes including voluntary and involuntary movement, cognition, executive functions, reward and euphoric reward enhancement, individual motivation and neuroendocrine control pathways. Each pathway consists of a set of projection neurons, consisting of individual dopaminergic neurons each with discrete functions and physiologic effects and which may become beneficial or non-beneficial depending the circumstances of their involvement. Although the assessments undertaken in the present study may be open to interpretation, the conduct of the studies immediately after weaning virtually precludes inadvertent conflicts of prior learning activity [32]. In addition, while sex hormones have been shown to impact opiate use in adult rats, the young age of the animals studied in the present report occurred prior to gender maturation and thus likely were not impacted by gender specific hormonal factors [33]. Only future research may be able to more fully clarify the genomic mechanisms that contribute to such behavioral attributes and may ultimately guide clinicians toward more effective preventive and therapeutic strategies.

Conclusions

The effects of chronic paternal cocaine exposure resulted in behavioral changes in offspring of naive, never cocaine exposed dams when 21 days of age, before significant postweaning learning attributes had an opportunity to occur. Offspring were tested only once, thereby further diminishing the potential for previous learning experiences to impact their behavior upon weaning. The physiologic mechanisms that may have contributed to the impacted behavior of cocaine exposed *vs.* never exposed offspring remain unclear, but likely occurred due to epigenetic effects resulting from cocaine induced in DNA integrity of paternal alleles initiated during fertilization of naive ova and which effects appear to have remained present at weaning. Thus, these results contribute to the body of evidence that reinforce the importance of damaging effect of opiate exposure, whether incurred by the paternal or maternal alleles and likely impacting the integrity of the DNA of the offspring impacted by the opiate exposure during early, formative stages of their neurologic and physiologic development.

Acknowledgement

The author thanks the University of Science Arts and Technology for the resources to prepare this manuscript and to Professor James Caulkins, Emeritis, of Drexel

University for inspiration and encouragement to conduct this study.

Disclaimer Regarding Applications of Artificial Intelligence (AI)

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. 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]
2. Brunton L, Knollmann B (2024) Goodman and Gilman's The Pharmacological Basis of Therapeutics (14th edtn). McGraw-Hill Pubs. [Crossref] [Google Scholar]
3. 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]
4. Creed MC, Ntamati NR, Tan KR (2014) VTA GABA neurons modulate specific learning behaviors through the control of dopamine and cholinergic systems. Front Behav Neurosci 8: 8. [Crossref] [Google Scholar] [Indexed]
5. VanZessen R, Phillips JL, Budygin EA, et al. (2012) Activation of VTA GABA neurons disrupts reward consumption. Neuron 73: 1184-1194. [Crossref] [Google Scholar] [Indexed]
6. 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.
7. 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]
8. Centers for Disease Control and Prevention (2024) Birth defects facts and stats.
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. 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]
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. Matsumura T, Katagiri K, Yao T, et al. (2007) The open field test: Reinventing the wheel. J Psychopharmacol 21: 134-135. [Crossref] [Google Scholar] [Indexed]
13. 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]
14. 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]
15. Stanford SC (2007) The open field test: Reinventing the wheel. J Psychopharmacol 21: 134-135. [Crossref] [Google Scholar] [Indexed]
16. Crusio WE (2013) The genetics of exploratory behavior. In: Crusio WE, Sluyter F, Gerlai RT, Pietropaolo S (eds) Behavioral Genetics of the Mouse. Cambridge University Press, Cambridge, UK: 148-154. [Crossref] [Google Scholar]
17. Tulp OL (1990) Characteristics of thermogenesis, obesity and longevity in the LA/N–cp rat. ILAR J 32: 32-39. [Crossref] [Google Scholar]
18. Nie N, Hull CH, Jenkins K, et al. (1981) Statistical package for the social sciences (2nd edtn). McGraw Hill Pubs: New York.
19. AVMA (2010) Use of animals in research, testing and education. J Vet Med Educ 37: 115.
20. 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]
21. 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]
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. 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]

27. Kalivas PW, McFarland K (2003) Brain circuitry and the reinstatement of cocaine-seeking behavior. Psychopharmacology (Berl) 168: 44-56. [Crossref] [Google Scholar] [Indexed]

28. Koob GF, Sanna PP, Bloom FE (1998) Neuroscience of addiction. Neuron 21: 467-476. [Crossref] [Google Scholar] [Indexed]

29. Nestler EJ (2005) The neurobiology of cocaine addiction. Sci Pract Perspect 3: 4-10. [Crossref] [Google Scholar] [Indexed]

30. Goldstein A (2001) Addiction: From biology to drug policy (2nd edtn). Oxford University Press: Oxford. [Google Scholar]

31. Nestler EJ (2001) Molecular basis of neural plasticity underlying addiction. Nat Rev Neurosci 2: 119-128. [Crossref] [Google Scholar] [Indexed]

32. Ennaceur A (2014) Tests of unconditioned anxiety pitfalls and disappointments. Physiol Behav 135: 55-71. [Crossref] [Google Scholar] [Indexed]

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