British Journal of Healthcare and Medical Research - Vol. 12, No. 03
Publication Date: May 26, 2025
DOI:10.14738/bjhr.1203.18846.
Tulp, O. L. (2025). Effect of Preconception Male Cocaine Use on Post-Weaning Thigmotaxic Behavioral Activity of Offspring in Congenic, Lean LA/Ntul//-cp Rats: Thigmotaxis in Cocaine Exposed Offsping. *British Journal of Healthcare and Medical Research*, Vol - 12(03). 157-164.

Effect of Preconception Male Cocaine Use on Post-Weaning Thigmotaxic Behavioral Activity of Offspring in Congenic, Lean LA/Ntul//-*cp* Rats: Thigmotaxis in Cocaine Exposed Offsping

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ABSTRACT

Because mammalian sperm have been found to contain opioid binding sites, there exists a potential for opioids to impact on early embryonic development of offspring in zygote exposed naïve, opioid-free dams. To determine the effects of chronic cocaine use by males on the offspring of naïve females, groups of adult 60 day old lean male LA/Ntul//-*cp* rats were reared from weaning on standard Purina chow and house water, and administered 0 or 30 mg of cocaine HCL for 90 ±2 days to encompass the duration of spermatogenesis, then mated with 82±3 day old naïve, normally reared virgin females of the same strain that had never been exposed to the opioid or related compounds. Behavioral activity of offspring was assessed by subjecting the postweaning offspring at 21 days of age with a Stoelting activity wheel and a Calvin Hall open access field test. Offspring were found to exhibit fewer open field square and exploratory activity at 21 days of age than offspring sired by similar aged and reared non-cocaine treated drug free males. These observations suggest that opioid addiction of male partners may impact the early post-weaning thigmotaxis behavioral activity of offspring of naive, drug free dams compared to drug free controls, and may predispose the offspring of opiate treated males to additional opiate-linked behavioral deficits during later stages of growth, maturation and development.

Keywords: Cocaine, behavior, exploratory activity, thigmotaxis, rat offspring. Prenatal exposure, epigenetics.

INTRODUCTION

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, and their combined embryonic manifestations often only discerned upon parturition when physical abnormalities may become better visualized. In addition, the incidence of birth defects in Western Civilization has recently been estimated to impact 1 in 33 live births, often where the causative agent is typically unknown with certainty.[1] 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 on the developing fetus.[2-5] 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, where the final growth of the

fetus and its organs occurs.[5-8] Gestation in the rat comprises a mere 21 days, compared to an average of approximately 280 days in humans.[3,4] 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.[2,5-9] In contrast to humans, rat pups have little capacity for memory based on past learning experiences at the time of weaning when 21 days of age, while in humans, prenatal and early postnatal experiences likely contribute to memory and learning processes.[3,4] Thus, the earlier in embryogenesis and early growth an insult may occur, the greater the potential impact on epigenetic expression of later postweaning learning and developmental processes.[6,7,10,11]

The traditional use of cocaine, typically obtained from the native coca leaves, has been in use for many centuries 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.[12-16] Unlike cocaine usage trends in current Western civilization, where purified cocaine preparations may be taken by inhaling microcrystalline or vaporized forms of the drug, 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 where once absorbed, cocaine can bypass the hepatic first pass phenomena to prolong the therapeutic effects.[13] 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.[12,13] Among accepted applications include therapeutic use as a topical upper respiratory anesthetic among 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.[13]

While recreational use of other, more potent opiates have 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 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.[12,13] Caulkins et al reported that cocaine had the capacity to bind to surface receptor domains of live spermatozoa during spermatogenesis, thus being able to transport the cocaine moieties into the ova during fertilization, where they could potentially impact of chemical embryogenesis from the origin of zygote formation with as yet unknown consequences.[17] Thus, 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 by assessment via both running wheel and open field exploratory activities.

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 22-24°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 rats, 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.[8,9,18]

Experimental

Groups of naive female rats of reproductive age (82±3 days of age). Males and females were separated at weaning. Upon adulthood and attaining sexual maturity, males were then subjected to daily administration of cocaine HCL (30 mg/kg BW, subcutaneously, 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.[19] 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,[20-22] 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. Data were 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.).[23] Each test group had equal number of male and female pups, of similar body weights (40±2 g BW)

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.[24]

RESULTS

The effects of male cocaine prefertilization 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.

URL: http://dx.doi.org/10.14738/bjhr.1203.18846.

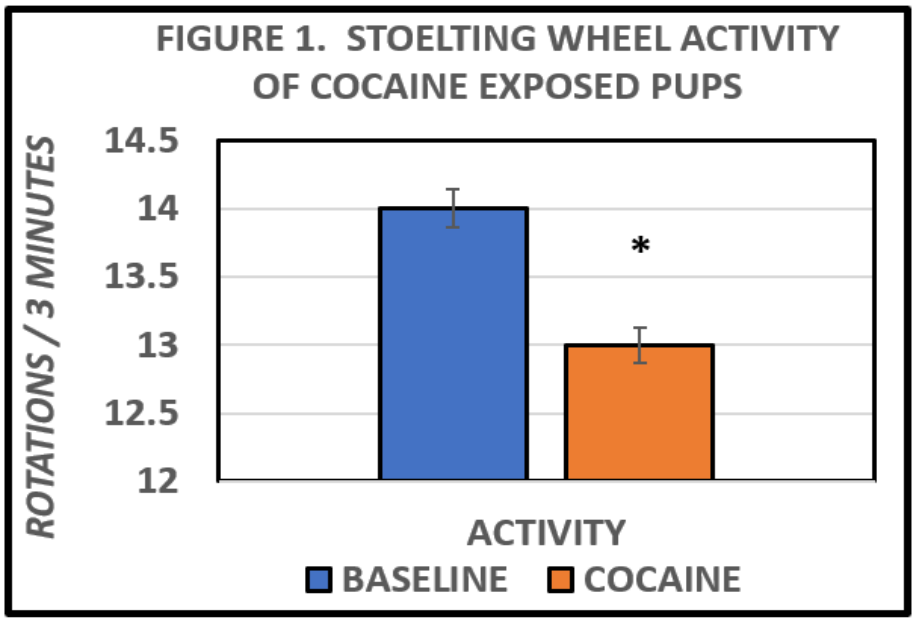

Figure 1: Activity of offspring of cocaine treated sires. Data are the mean ± 1 SEM, n= 6-8 rats/group. P=< 0.05 control vs treated. Male cocaine was administered at a dosage of 30 mg./kg BW for 90 days prior to mating with confirmed naive females of a similar age. P = < 0.05.

The effect of exploration activity in a Calvin Hall Maze is depicted in Figure 2 below and indicates that pups exposed to preconception 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 = < 005 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.

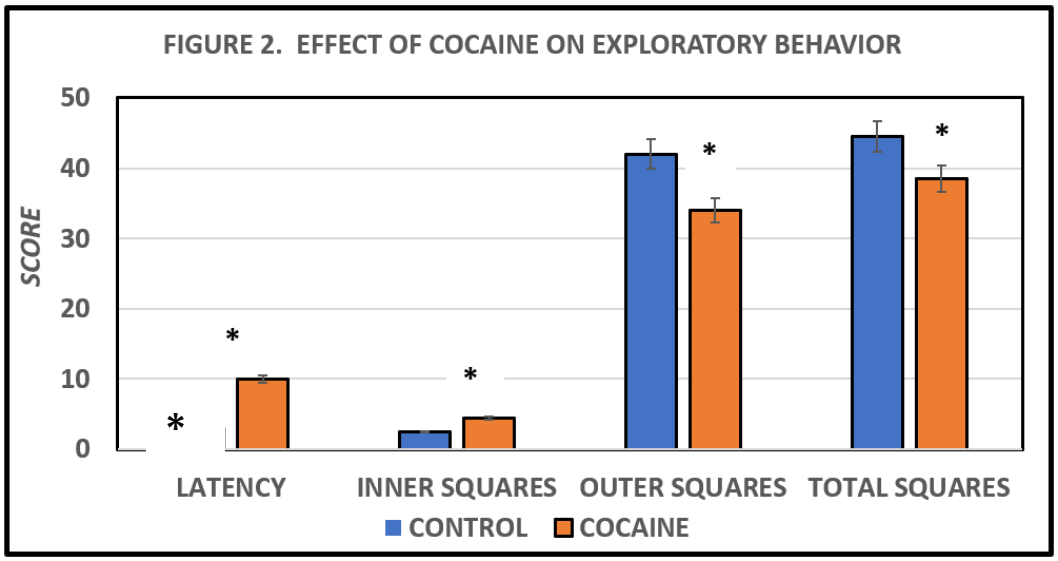

Figure 2: Exploratory open field activity parameters of offspring of preconception cocaine treated sires. Data are mean ±1 SEM. N= 6-8 ats/Group. P = < 0.05 for latency, initial squares, outer squares, and final total squares explored.

DISCUSSION

The epigenetic control of neuronal gene expression patterns have been reported to have emerged as contributors to underlying regulatory mechanisms for neuronal function, neuronal identity, neuronal plasticity, neurocellular 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 mail 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. While the molecular mechanism 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 may occur due to a variety of nutritional, strain, age, and other factors.[19,25] Thus, the duration of mail cocaine exposure exceeded the anticipated duration of spermatogenesis. Rats have been observed to attain reproductive age soon after puberty, from approximately 42 days of age in our experience in this strain. 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.[17] In a clinical study, however, opiate users were found to have decreased sperm counts, and significant DNA fragmentation compared to sperm obtained from health volunteers.[26] Thus the possibility of preconception DNA damage from the male gamete may also have occurred. In another study, Telese et al noted that cocaine addicted rats had different neuronal levels of GABA signaling, an inhibitory neurochemical in the amygdala.[10] 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 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 rt occurs after 4 week of age.[10,11,27] Since only variable deemed important in the current study was the opiate exposure throughout the duration of spermatogenesis, the likelihood of 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 would have normally occurred.

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, 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

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exploratory and activity patterns as determined by exposure to a Calvin Hall Maze and a Stoelting activity wheel. 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 zygote, 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 protected from maternal chemical insults prior to implantation, potential DNA damage prior to implantation would 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 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. Regardless of the neurochemical mechanism, the effects of cocaine exposure on the male gamete prior to conception may bring about behavioral and activity changes in the offspring.

ACKNOWLEDGEMENT

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

Disclaimer (Artificial Intelligence)

Author hereby declares 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.

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