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RESEARCH ARTICLE

INVESTIGATING THE IMPACT OF PRENATAL CANNABIS EXPOSURE ON FETAL BRAIN DEVELOPMENT AND INTERGENERATIONAL OUTCOMES USING AGENT-BASED MODELING

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Abstract

Cannabis use has risen globally, driven in part by its legalization for medicinal and recreational purposes in various regions. Alarmingly, approximately 10% of women report cannabis use before pregnancy, and 4.9% during pregnancy. Despite its perceived safety, emerging evidence from clinical and preclinical studies highlights significant adverse effects associated with prenatal cannabis exposure, including reduced birth weight, heightened susceptibility to anxiety and depression, and increased drug-seeking behaviors later in life. Using the agent-based computer simulation program COBWEB, we modeled the long-term and intergenerational consequences of prenatal cannabis exposure. Our model begins with a typical healthy fetal brain. Cannabinoid exposure is simulated as the downregulation of critical gene networks, represented by diseased agents disrupting previously healthy gene interactions, thereby decreasing DRD2 mRNA expression. This approach allows us to visualize and predict the progressive effects of chronic cannabinoid exposure on neural function and behavior across successive generations. Our findings demonstrate the model's capacity to simulate and accurately predict the long-term outcomes of prenatal cannabis exposure, offering new insights into the intergenerational effects of chronic cannabis use and targets of new treatments. Future work will focus on validating the model with empirical data and leveraging it to design and test novel therapeutic interventions aimed at mitigating these long-term effects.

Introduction:-

The legalization of cannabis across the United States and Canada has significantly increased its use for both recreational and medicinal purposes. Among its medicinal applications, cannabis is often prescribed as an antiemetic and analgesic to manage nausea and pain during pregnancy, driven by a widespread perception that it poses minimal risk to the developing fetus[1]. In the United States, approximately 4% of pregnant women report drug use during pregnancy, with cannabis (Cannabis sativa) being the most frequently used substance[2]. However, this assumption of safety is increasingly questioned by accumulating evidence that highlights potential adverse outcomes of prenatal cannabis exposure.

The prenatal period represents a critical window of neurodevelopmental vulnerability, during which a multitude of intricate processes, such as neuronal proliferation, migration, and synaptic formation, establish the foundational

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structure and function of the nervous system[3]. Contrary to the perception of cannabis as benign during pregnancy, clinical and preclinical studies have shown that prenatal cannabis exposure is associated with significant developmental disruptions, including reduced birth weight, heightened susceptibility to anxiety and depression, and increased likelihood of substance use disorders later in life[4]. These findings raise pressing concerns about the biological mechanisms underlying these adverse effects, particularly the role of cannabinoids, the active compounds in cannabis, in fetal brain development.

Delta-9-tetrahydrocannabinol (THC), the primary psychoactive component of cannabis, readily crosses the placental barrier and accumulates in fetal tissues, including the brain[5]. Cannabinoid receptors, particularly CB1 and CB2, are highly expressed in the developing brain and play crucial roles in regulating synaptic plasticity and neurotransmitter signaling[6]. Prenatal exposure to THC has been linked to long-term cognitive and behavioral deficits, such as increased sensitivity to opiates and addiction vulnerability[7]. Central to these outcomes is the disruption of the striatal dopamine system within the ventral striatum, a region involved in reward processing and goal-directed behavior[8].

The medium spiny neurons of the striatum, which are enriched in cannabinoid receptors, are particularly susceptible to THC-induced epigenetic modifications[9]. For instance, THC has been shown to downregulate the expression of dopamine D2 receptors (D2R) via mechanisms involving histone lysine methylation, leading to reduced DRD2 mRNA levels, which is the gene that encodes D2R[9]. This reduction in D2R expression has been implicated in heightened addiction vulnerability, as individuals with lower striatal D2R levels exhibit increased sensitivity to the rewarding effects of drugs[10]. Preclinical studies have further corroborated this association, demonstrating that prenatal THC exposure in rodent models results in enhanced heroin self-administration behaviors[11].

To investigate these complex interactions, we employed the COBWEB (Complexity and Organized Behavior Within Environmental Bounds) simulation framework, an agent-based modeling platform originally developed to simulate dynamic biological and ecological systems[12]. COBWEB allows for the parameterization of agents to represent molecular entities[12], such as cannabinoids and dopamine receptors. By modeling these interactions, we sought to simulate the mechanistic impact of prenatal cannabis exposure on fetal neurodevelopment. The dynamics of the system were captured by agents navigating a grid environment, with time progression measured in computational units called "ticks."

This study aims to elucidate the biological pathways through which prenatal cannabis exposure disrupts neurodevelopment and contributes to long-term addiction vulnerability. By leveraging COBWEB, we hypothesize that prenatal cannabis exposure can be effectively modeled to predict both immediate and transgenerational outcomes, offering novel insights into the potential risks associated with cannabis use during pregnancy.

Materials and Methods:-

Model 1 (Control)

This study utilized COBWEB software to construct an agent-based model simulating the interactions between endocannabinoid receptors and epigenetic processes in a healthy brain. All models were adapted from previous research[9]. Model 1 was designed as a baseline for evaluating the effects of external perturbations, such as cannabis exposure, on neurobiological processes (Fig. 6).

The simulation was structured on an 80 × 80 grid, divided into two vertical sections to represent distinct neural environments. The left section simulated the external brain environment, while the right section represented the dorsal striatum (DS) output pathway, incorporating the endogenous epigenetic processes associated with healthy brain function. To accurately model these interactions, three distinct agents were implemented; Agent 1 represented endogenous endocannabinoids, Agent 2 represented CB1 and CB2 receptors, and Agent 3 represented unmodified epigenetic processes (Fig. 1).

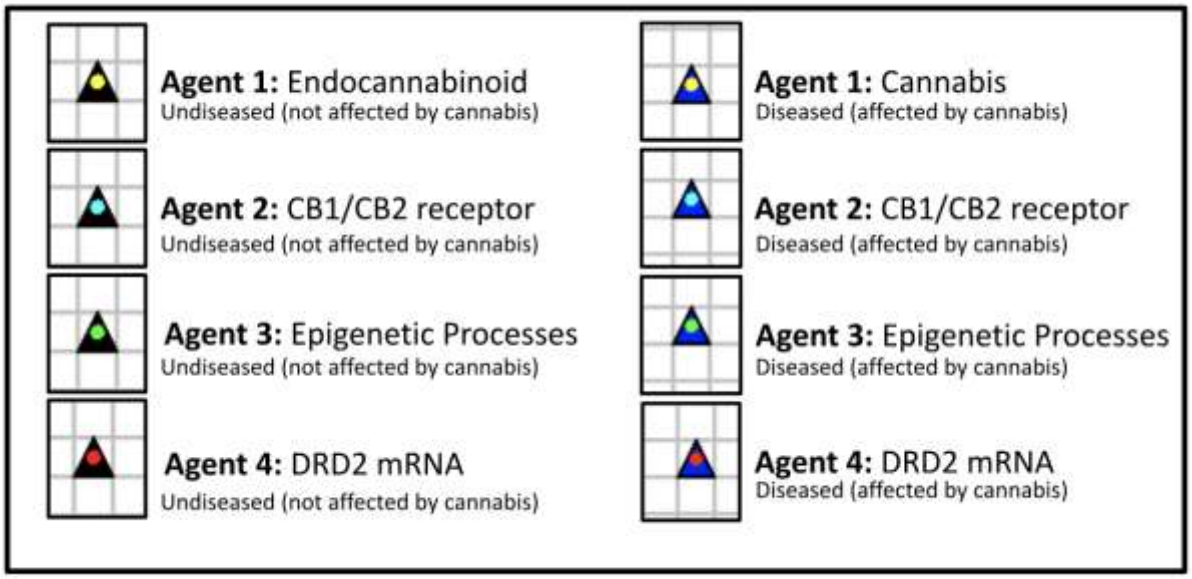


Figure 1:-Legend of COBWEB agents.

To enforce spatial constraints and accurately reflect biological conditions, environmental structuring was implemented through COBWEB's Abiotic Factors tab (Fig. 2). Vertical bands were added to control agent movement, with Band 1 set to 0 and Band 2 set to 1, while the punishment/barrier option was enabled. This configuration ensured that Agent 2 (CB1/CB2 receptors) and Agent 3 (epigenetic processes) remained localized within the DS pathway, preventing unnatural displacement. Agent 1 (endocannabinoids) was permitted to diffuse freely toward the DS pathway, simulating the physiological crossing of the blood-brain barrier (BBB) and endogenous cannabinoid signaling[9].

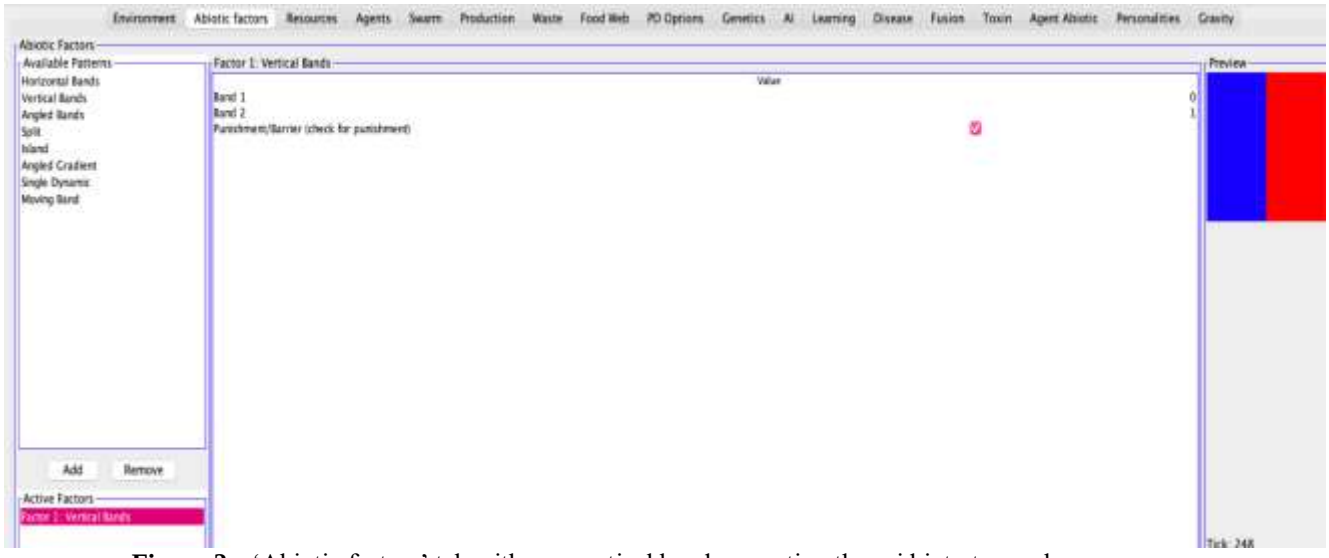


Figure 2:- 'Abiotic factors' tab with one vertical band separating the grid into two columns.

Agent properties were calibrated to maintain biological accuracy. The initial count of Agent 1 (endocannabinoids) was set to 30 to keep consistency within the models. Agent 2 (CB1/CB2 receptors) and Agent 3 (epigenetic processes) were maintained at default levels to preserve normal receptor density and epigenetic activity. Agent interactions were programmed to reflect endogenous neurobiological processes. Using COBWEB's Food Web tab (Fig. 3), Agent 1 (endocannabinoids) was assigned an interaction pathway with Agent 2 (CB1/CB2 receptors) based

on established receptor-binding dynamics. Agent 3 (epigenetic processes) was programmed to modulate these interactions to maintain homeostatic equilibrium.

Figure 3:- Parameters under 'Food Web' tab to represent the conditions required by each agent to stay alive.

This baseline model represents a healthy neural environment, providing a fundamental reference point for evaluating the effects of cannabis-induced epigenetic modifications in subsequent experimental conditions.

Model 2

Model 2 simulates the interactions between medical cannabis and CB1/CB2 receptors and epigenetic processes in pregnant women. The grid was split vertically into two sections. The left section represented the external environment of the brain where cannabis was ingested, and the right section represented the dorsal striatum (DS) output pathway, incorporating the CB1 and CB2 receptors present in the pathway as well as the epigenetic mechanisms associated with cannabis ingestion[9].

In this model (Fig. 7), Agent 1 represented the cannabis compounds that were ingested (equivalent to 16 mg of THC, a typical low dose of cannabis cigarettes[9]), Agent 2 was designated as CB1 and CB2 receptors, and Agent 3 as epigenetic processes.

For this model, in the Abiotic factors tab, vertical bands were added to the model (Fig. 2). Band 1 was set to the value 0 and band 2 was set to the value 1 and the punishment/barrier was checked. This was used to make Agent 2 and 3 to be in place at their respective area on the right in the represented DS pathway, as well as prevent it from moving backwards to the external environment of the brain.

In the Agents tab (Fig. 4), the initial count for Agent 1 was set to 30 to show that Agent 1 represents a 16 mg dosage of cannabis, which needs to interact with CB1 and CB2 receptors (Agent 2) to mimic the binding of THC to these receptors in the simulation. By setting the initial count to 30, the likelihood of these interactions occurring early and frequently in the simulation rises. This adjustment mirrors the real-world scenario where cannabis is introduced into the system in sufficient quantities to disrupt normal receptor activity[9]. Additionally, the favourite food energy was set to 100 for Agent 1 (Fig. 4) because the favourite food energy determines how much energy Agent 1 (cannabis) gains when consuming its resource. By setting this low value, the model ensures that Agent 1 doesn't multiply or persist unnecessarily beyond its intended role of representing THC binding. This aligns with the biological reality where THC's presence is transient[9]. Lastly, in this tab, initial energy for Agent 1 was set to 300 and 1200 for all other agents (Fig. 4). This was done to ensure that Agent 1 doesn't dominate the system or continuously spawn after the critical "binding" events have occurred. This ensures that Agent 1 has a limited lifespan, reflecting how THC is metabolized and cleared over time. For Agents 2 and 3, the higher energy ensures their survival and functionality throughout the simulation, as they represent a stable biological system within the neural environment.

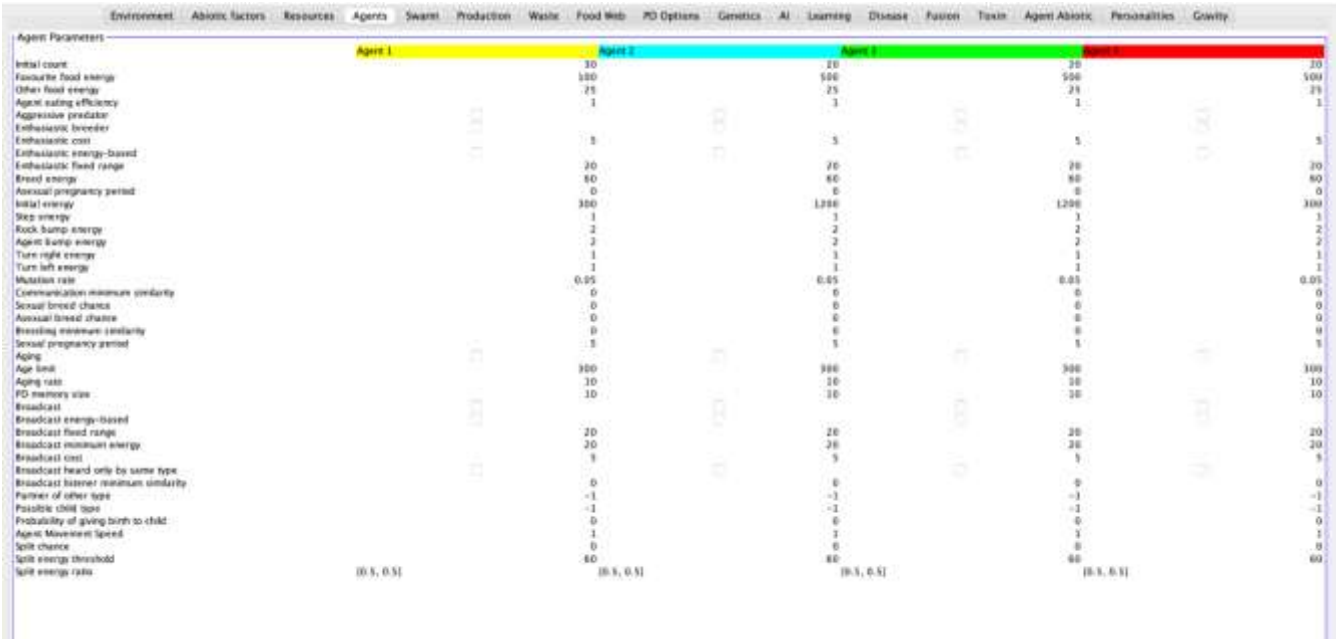


Figure 4:- Parameters under the 'Agents' tab to represent the different constraints for each agent, thereby simulating real neural conditions.

Lastly, the Disease tab was modified to make Agent 1 infectious to represent the displacement of endocannabinoids that were present in Model 1 with cannabis in Model 2 (Fig. 5). This then demonstrates the act of cannabis binding to and affecting the CB1 and CB2 receptors, which thereby results in THC-induced epigenetic modifications in the brain[9]. The contact transmission rate was set to 0.95 so we can effectively model this "binding" action, which then affects the epigenetic processes of the brain. Additionally, the agents were modified in a way that Agent 1 affects Agent 2 only, and the then-infected Agent 2 affects Agent 3 only to directly translate the process of cannabis binding to cannabinoid receptors which then affects epigenetic processes, specifically downregulating histone lysine methylation[9]. Additionally, the initially infected fraction for Agent 1 was set to 1, ensuring that the population begins in a susceptible state and can be infected by Agent 1, allowing the disease to spread to other agents.

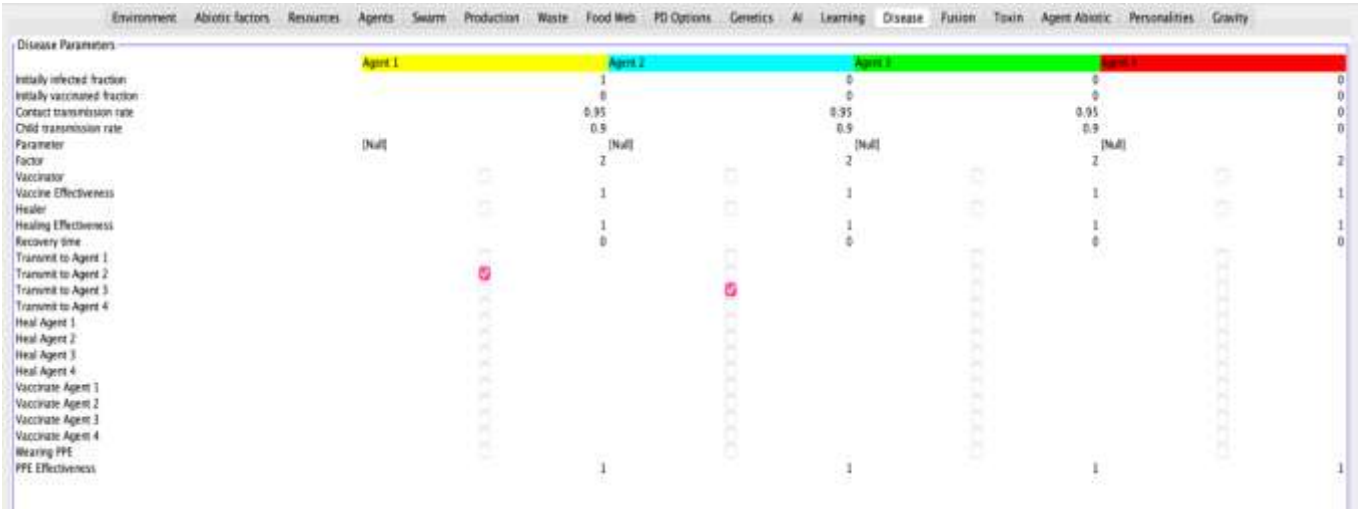


Figure 5:-Parameters under 'Disease' tab.

Model 3

Model 3 was designed to represent cannabis-induced epigenetic modifications within the dorsal striatum (DS) output pathway and their subsequent impact on gene expression (Fig. 8).

The grid was split vertically into two sections. The left section represented the DS output pathway, where cannabis exposure was modeled to induce epigenetic modifications (Agent 3). The right section corresponded to the NAc, where DRD2 mRNA expression (Agent 4) was affected due to the altered epigenetic landscape influenced by Agent 3[9]. This spatial distinction ensured that the modeled interactions adhered to neuroanatomical specificity.

To maintain spatial fidelity, vertical bands were applied within the Abiotic factors tab, preventing Agent 4 (DRD2 mRNA) from migrating into the left section of the grid, thereby ensuring that gene expression changes occurred exclusively within the NAc (Fig. 2).

Within the Agents tab, the favorite food energy value was set to 500 for both Agent 3 and Agent 4, while their initial energy was configured at 1200 (Fig. 4). These values were selected to ensure that the agents possessed sufficient lifespan and activity duration to model epigenetic transmission effects beyond initial fetal birth and over time into maturation.

The Disease tab was manipulated to render Agent 3 infectious, allowing it to model THC-induced epigenetic modifications. This infection mechanism was employed to simulate the downstream effect of cannabis exposure on DRD2 mRNA expression. Specifically, upon interaction, Agent 3's epigenetic modifications have fully affected Agent 4, resulting in a downregulation of DRD2 mRNA expression. The contact transmission rate for both Agent 3 and Agent 4 was set to 0.01, with an initially infected fraction of Agent 3 fixed at 1. These values were calibrated to reflect the gradual and cumulative impact of cannabis-induced epigenetic changes over time[9].

By structuring the model in this manner, the simulation was able to capture the progressive decline in DRD2 mRNA expression following cannabis exposure, mirroring observed biological trends.

Time
We let 1 day (24 hours) be equivalent to 29.5 ticks in COBWEB (Fig. 10).

Food
Agents in COBWEB can only stay alive through the consumption of food, (the yellow, blue, green, and red squares across the grid). In the food web tab (Fig. 3), each agent was made to eat the food that corresponds to their agent to make sure that the agents themselves stay alive throughout the simulation so that they can properly mimic neural interactions.

Results:-

Healthy Fetal Brain
The baseline model (Fig. 6) represents a healthy fetal brain. In the left side of the grid, endogenous endocannabinoids are depicted migrating into the dorsal striatum on the right side of the grid, where they bind to CB1 and CB2 receptors within the dorsal striatum output pathway. This interaction facilitates normal epigenetic processes, including typical histone lysine methylation, which ensures the maintenance of typical behavioral and motor function9.

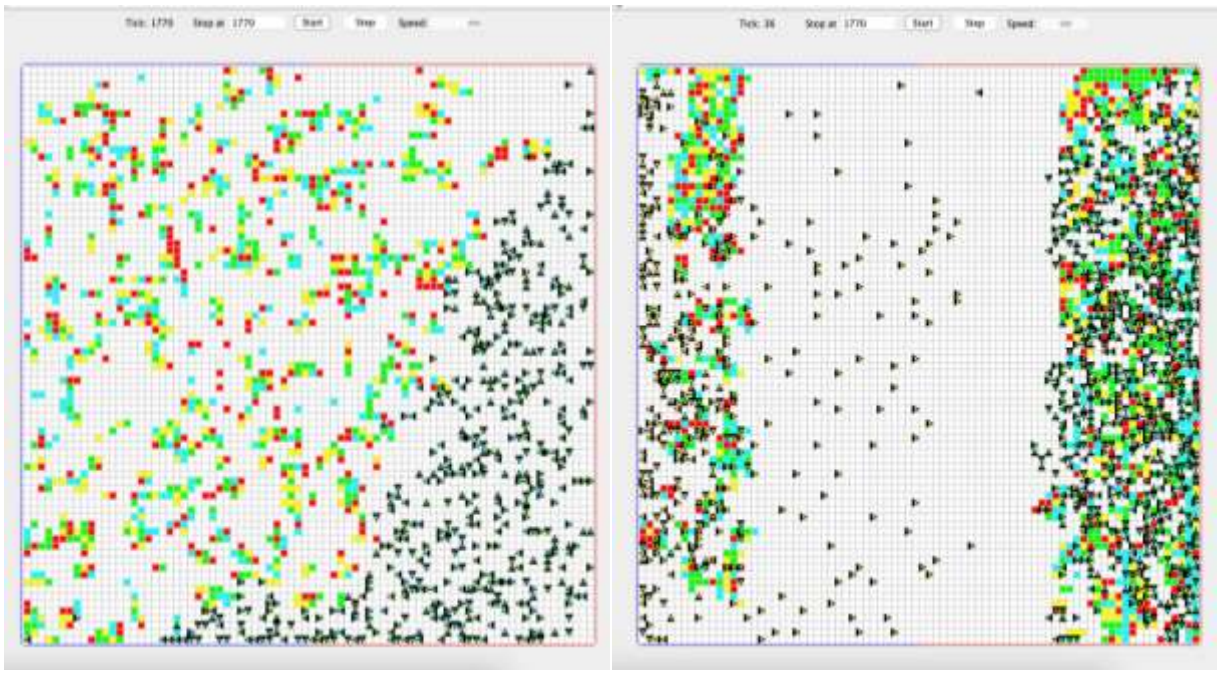


Figure 6:- Model 1 (control model) depicting the typical movement of endocannabinoids binding to CB1/CB2 receptors, thereby inducing typical epigenetic processes.

Prenatal THC Exposure and Disruption of the Endocannabinoid System

Model 2 (Fig. 7) demonstrates the effects of chronic prenatal exposure to THC during gestation. Using an experimental rat model as the basis, Model 2 depicts the fetal brain alterations as a result of pregnant subjects who were administered a 16 mg dose of THC daily for 20 gestation days, which is representative of typical cannabis ingestion for pregnant women[9]. Following ingestion, THC enters the dorsal striatum, competitively binding to CB1 and CB2 receptors, displacing endogenous endocannabinoids. This competitive binding disrupts the epigenetic regulatory mechanisms governing histone lysine methylation, visualized in the dorsal striatum output pathway (Fig. 11). These disruptions are hypothesized to induce molecular changes that alter neurodevelopmental trajectories[9].

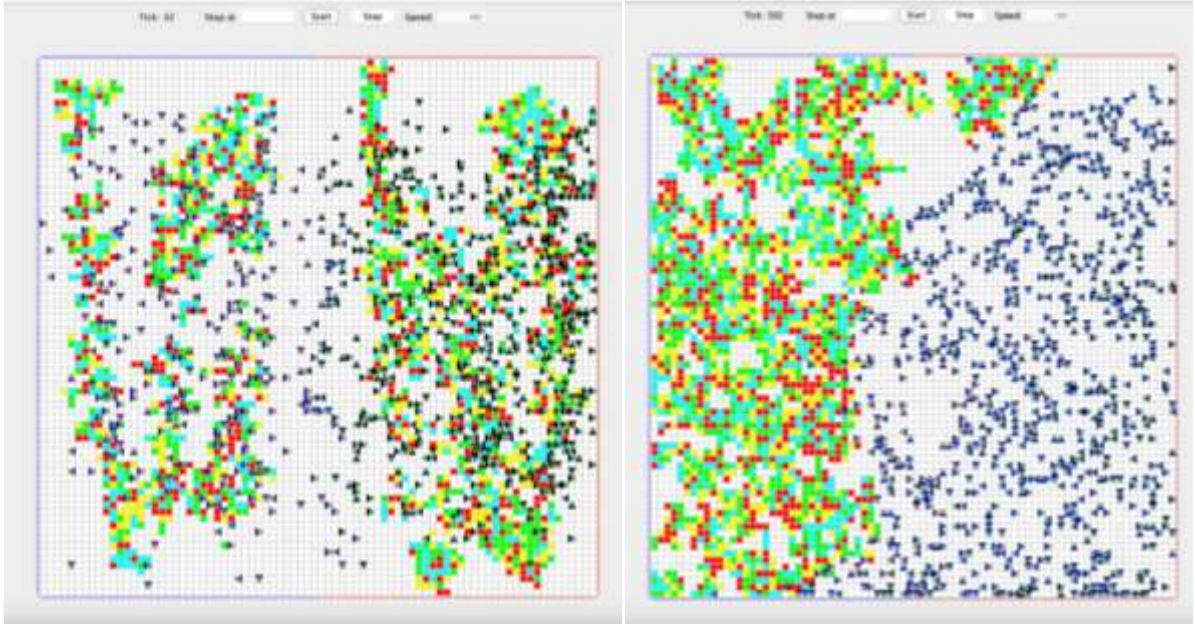


Figure 7: THC entering the DS, leading to competitive binding to the CB1 and CB2 receptors, causing the displacement of endogenous endocannabinoids and illustrating the consequence of exposure to THC during gestation.

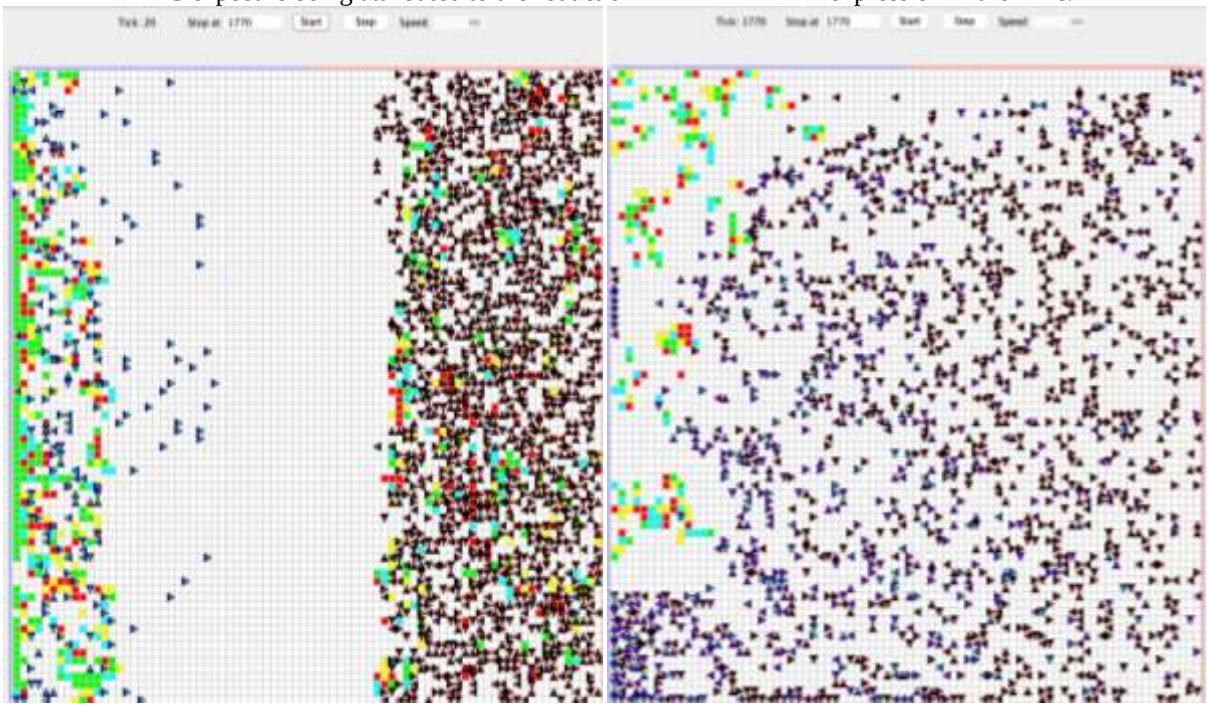
As the simulation progresses, the endocannabinoid system's regulatory capacity is observed to degrade over time. Starting with 327 agents of endogenous endocannabinoids and 359 CB1 and CB2 receptors at gestation day 1, by gestation day 20 (represented by 590 ticks), the system is significantly disrupted, as THC replaces endogenous endocannabinoids (Fig. 7). This modifies epigenetic processes governing histone lysine methylation, and this altered post-translational modification of nucleosomal histones in the locus impairs normal gene regulation. These changes are consistent with observed reductions in DRD2 gene expression and corresponding decreases in DRD2 mRNA transcripts, as shown in Model 3.

Long-Term Effects on Mesolimbic Dopaminergic Regulation

The final model (Fig. 8) explores the long-term impact of prenatal THC exposure on DRD2 expression in the mesolimbic pathway. This model focuses on two regions: the DS in the left side and the NAc in the right side. Persistent alterations in epigenetic processes in the DS, driven by constant prenatal THC exposure, result in a 30% reduction in DRD2 mRNA expression within the NAc (Fig. 9). This reduction was measured from postnatal day 2 to postnatal day 62, corresponding to approximately 1770 ticks in the simulation (Fig. 10). This thus models the effects of prenatal cannabis exposure persisting into later life, not just during the days of initial fetal development.

DRD2 mRNA distribution patterns across brain regions, derived from human fetal striatum samples, corroborate these findings (Fig. 11). The sustained decrease in DRD2 transcripts leads to fewer D2R binding sites in the NAc[13]. This reduction has significant implications, as lower D2 receptor availability is strongly associated with increased vulnerability to addiction and neuropsychiatric disorders[14]. These findings align with existing literature, which links prenatal cannabis exposure during midgestation to long-term neurodevelopmental deficits and heightened addiction susceptibility in adulthood[7].

Figure 8:- Progression of changes in epigenetic processes starting in DS and moving NAc to represent prenatal THC exposure being attributed to the reduction in DRD2 mRNA expression in the NAc.



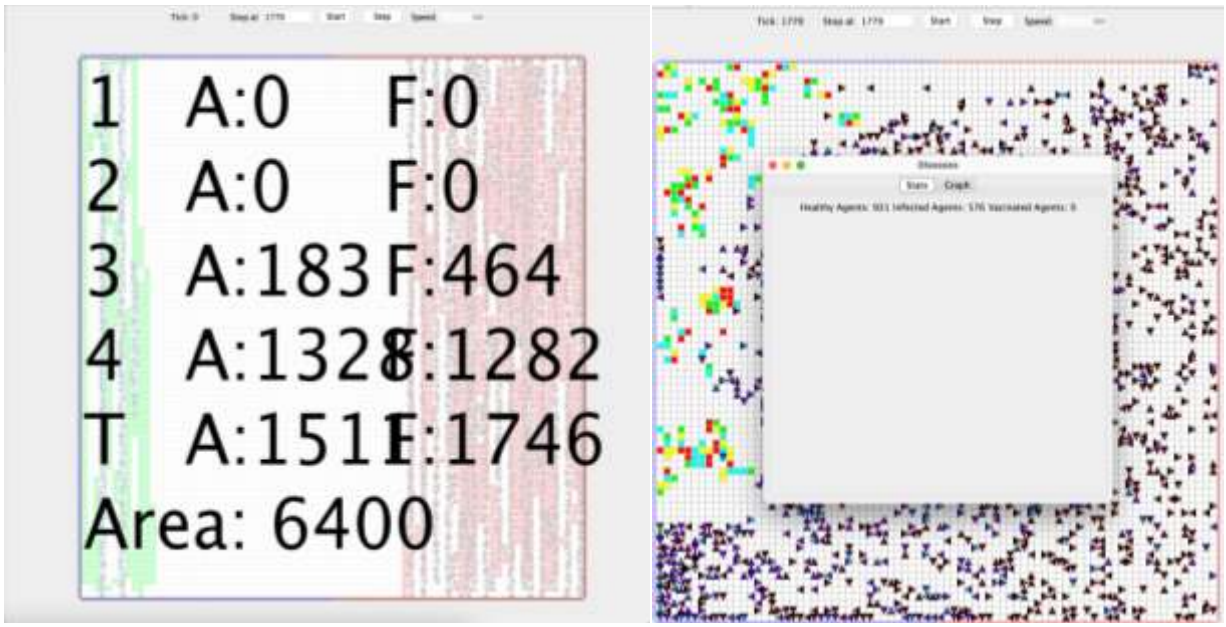


Figure 9:- Initial expression of DRD2 mRNA is set at 1328 agents. After running the simulation, the number of healthy agents (unaffected DRD2 mRNA) is 921 agents, which demonstrates an approximate 30% decrease in DRD2 mRNA expression in the NAc.

$$\frac{29.5 \text{ ticks}}{1 \text{ day}} \times 20 \text{ days} = 590 \text{ ticks}$$

$$\frac{29.5 \text{ ticks}}{1 \text{ day}} \times 60 \text{ days} = 1770 \text{ ticks}$$

Figure 10: Calculations showing the relationship between tick marks and real-time. 20 days is equivalent to 590 ticks and 60 days is equivalent to 1770 ticks.

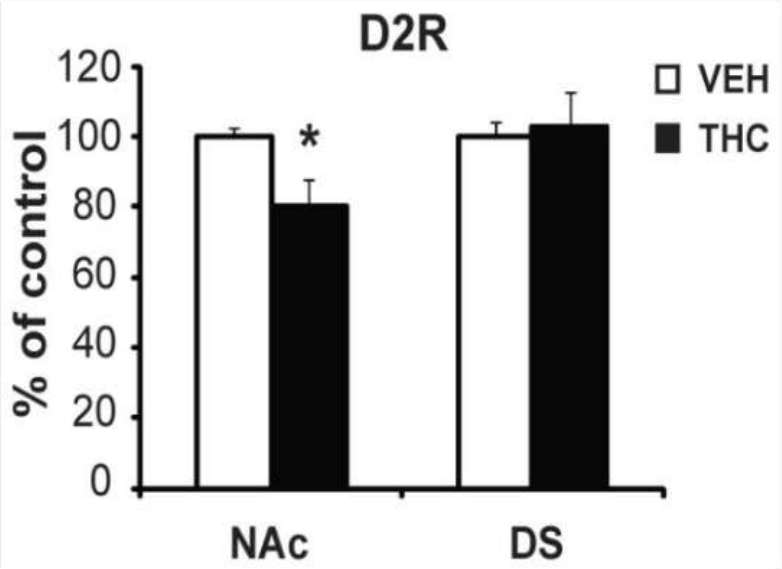


Figure 11:- Modeling D2R function with a model organism after prenatal THC exposure. Comparing D2R binding sites in NAc and DS showcasing a decrease in D2R binding to NAc upon THC exposure. Source: DiNieri, J. et al. Maternal cannabis use alters ventral striatal dopamine D2 gene regulation in the offspring. Biological Psychiatry **70**, 763-769 (2011).

Discussion:-

The findings of this study provide compelling insights into the biological mechanisms through which prenatal cannabis exposure may impact fetal brain development and contribute to long-term addiction vulnerability. By utilizing the COBWEB simulation framework, we captured the dynamic effects of THC on critical neurobiological pathways. Our model demonstrated that prenatal cannabis exposure could disrupt the normal function of cannabinoid receptors (CB1 and CB2) and induce epigenetic changes that reduce DRD2 mRNA expression, ultimately leading to decreased D2R protein levels (Fig. 9). This finding aligns with previous studies indicating that THC exposure during development alters histone lysine methylation, reducing D2R expression and increasing the risk of addiction[15]. Furthermore, the spatial configuration of agents within the simulation effectively illustrated how THC, upon crossing the BBB, interacts with cannabinoid receptors in the striatal output pathways, disrupting dopamine signaling[16]. Agents representing epigenetic processes retained the effects of THC exposure, which could be passed on to subsequent generations through alterations in the germline epigenome[17]. This supports emerging evidence that prenatal environmental exposures, including cannabis, may have long-lasting and heritable effects on offspring THC, the primary psychoactive component of cannabis, readily crosses the BBB due to its lipophilic nature[18]. Once in the brain, THC binds to CB1 cannabinoid receptors[9]. This binding disrupts normal endocannabinoid signaling, leading to altered dopamine transmission—a key factor in addiction-related neurocircuitry[19].

Our findings also align with previous in vivo and clinical studies that have investigated the impact of prenatal cannabis exposure on fetal neurodevelopment. Empirical research has demonstrated that THC readily crosses the placenta and accumulates in fetal tissues, particularly in the developing brain[20]. Studies on rodent models have shown that prenatal exposure to cannabis compounds leads to altered neurodevelopmental trajectories[21] and increased vulnerability to substance use later in life[20].

Our model also challenges the notion that the endocannabinoid system can fully compensate for THC-induced disruptions. Some researchers suggest that homeostatic mechanisms within the developing brain may buffer the effects of external cannabinoids, minimizing long-term damage[22]. However, our simulation demonstrates that THC's displacement of endogenous endocannabinoids leads to persistent epigenetic alterations, which are not reversed within the timeframe modeled. This suggests that while compensatory mechanisms may exist, they may be insufficient to counteract the epigenetic changes induced by prenatal THC exposure.

However, while the COBWEB simulation framework effectively models neurobiological interactions, it has inherent limitations, particularly in its inability to account for genetic variability, compensatory mechanisms, and individual genetic differences. Refining the model to incorporate these factors, as well as simulating different cannabis dosages and exposure windows, could help establish a more precise dose-response relationship. One significant limitation of our study is the lack of environmental and social factors in the model, which may influence the neurodevelopmental effects of prenatal cannabis exposure. Real-world influences such as maternal stress, nutrition, concurrent substance use, and environments play crucial roles in shaping neurodevelopmental outcomes and should be integrated into future studies.

Another key limitation is the exclusion of other neurotransmitter systems beyond dopamine. While our study focuses on the dopaminergic system, prenatal cannabis exposure may also affect serotonin, glutamate, and GABAergic systems, which are critical for mood regulation, cognition, and neural excitability. Investigating these additional pathways could provide a more comprehensive understanding of cannabis-induced neurodevelopmental changes.

Additionally, sex differences in neurodevelopmental outcomes were not explicitly modeled in our study. Given that males and females exhibit differences in endocannabinoid signaling, dopamine receptor expression, and susceptibility to neuropsychiatric disorders, future research should investigate whether prenatal THC exposure differentially affects male and female offspring.

Addressing these limitations will provide a comprehensive understanding of the long-term consequences of prenatal cannabis exposure, ultimately informing clinical guidelines, public health policies, and potential therapeutic strategies to mitigate its effects.

While cannabis is often prescribed as an antiemetic or analgesic for pregnant individuals, our study suggests that its use could have significant consequences for fetal neurodevelopment. As cannabis use continues to rise in the wake of legalization, it is critical to inform pregnant individuals and healthcare providers about the potential risks associated with prenatal exposure.

Conclusion:-

This study provides compelling evidence that prenatal cannabis exposure disrupts fetal neurodevelopment by altering key epigenetic and neurochemical pathways. Through agent-based modeling using the COBWEB framework, we demonstrated that THC exposure during pregnancy competitively binds to CB1 and CB2 receptors, displacing endogenous endocannabinoids and disrupting normal epigenetic regulation. These disruptions result in decreased histone lysine methylation and subsequent downregulation of DRD2 mRNA expression, leading to long-term impairments in dopamine signaling. Given that dopamine transmission plays a critical role in reward processing, motivation, and behavioral regulation, these findings suggest a mechanistic link between prenatal THC exposure and increased susceptibility to addiction and neuropsychiatric disorders later in life.

By providing a mechanistic simulation of the long-term consequences of THC exposure on fetal brain development, this study reinforces concerns about the safety of cannabis use during pregnancy and its potential impact on future generations.

Conflict Of Interest Disclosure:

The authors declare that there are no financial, personal, or professional conflicts of interest that could have influenced the conduct or findings of this study. This research was conducted independently under the guidance of Dr. Brad Bass and was not funded, directly or indirectly, by any organizations, institutions, or corporations with a vested interest in cannabis production, regulation, or pharmaceutical applications.

The simulation framework (COBWEB) utilized in this study was selected based on its computational modeling capabilities and was not influenced by affiliations with its developers or associated research institutions. The interpretation of the results and conclusions drawn are solely based on empirical evidence from preexisting studies, expanding upon the work of DiNieri et al. (2011) using artificial intelligence and computational modeling.

All data sources, citations, and methodologies have been transparently disclosed, and the authors affirm their commitment to academic integrity, unbiased scientific inquiry, and adherence to ethical research standards.

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