Prenatal Nicotine and THC Exposure via E-Cigarettes in Rats Alters Select Maternal Factors

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Background

Nicotine and cannabis are two of the most commonly consumed drugs among pregnant women, with prevalence rates of 16% and 10% in the United States, respectively. These numbers are consistently increasing, partially due to the rise in popularity of electronic cigarettes (e-cigarettes). Consumption of drugs via e-cigarettes is assumed to be safer than traditional smoking routes, including among pregnant women. However, the longitudinal effects of prenatal e-cigarette use with either nicotine or cannabis constituents are not well understood. Moreover, the effects of combined use of these drugs has not yet been examined, particularly when consumed via e-cigarettes. This is the case even though nicotine and cannabis are more often consumed together than separately, a practice made easier with the tanks used for e-cigarettes. Unfortunately, data from prospective longitudinal studies examining this public health concern will not be completed for years to come.

Purpose and Objectives

- **To develop a clinically relevant co-exposure model of prenatal nicotine and THC exposure in pregnant rats via e-cigarette vapor inhalation.**
- **Confirm physiological effects of each drug in pregnant rats while avoiding potential nutritional confounds.**

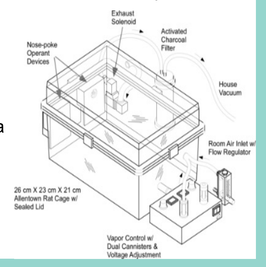
This paradigm was designed for use in future studies examining the long-term effects of prenatal nicotine and THC exposure on offspring brain and behavioral development.

Methodology

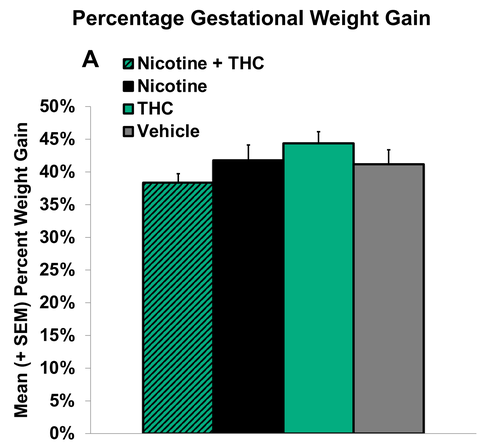
In rats, gestational days (GD) 5-20 mimics the first and second trimesters in humans. Beginning on GD 5, pregnant Sprague-Dawley rats were exposed to either nicotine (36 mg/mL), THC (100 mg/mL), the combination, or the vehicle (propylene glycol) via commercially available e-cigarettes (SMOK V8 X-Baby Q2). Dams were placed in the vapor inhalation chamber (La Jolla Alcohol Research Inc) for 30 min daily; e-cigarette drug administration was delivered through airflow (2 L/min) in individual 6-sec puffs every 5 min during the 30 min session (7 puffs total). Pregnant dams remained in the chamber for an additional 10 min with only airflow in order to clear any residual vapor before removal.

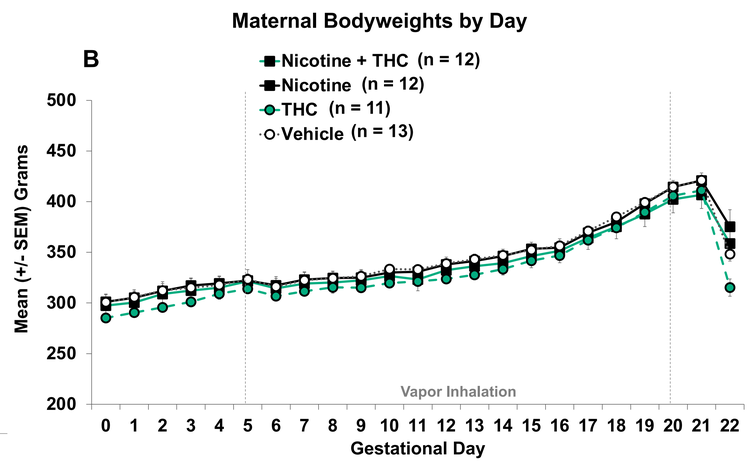
Throughout pregnancy, subjects' body weights, food intake, and water intake were measured daily. Core body temperatures were recorded before and after each exposure session, as THC via e-cigarettes is known to decrease temperature.

Plasma drug levels and litter outcomes were also recorded and are presented in a separate poster.

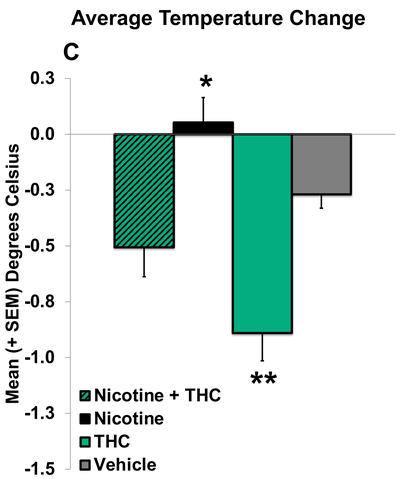


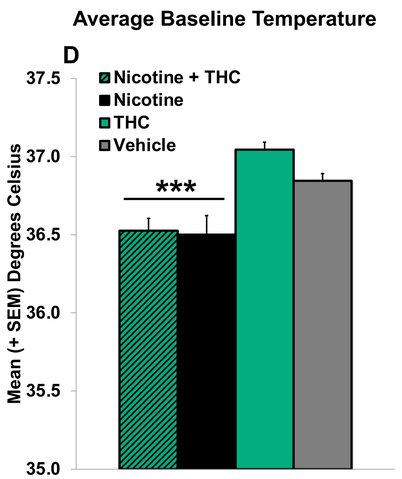
Results

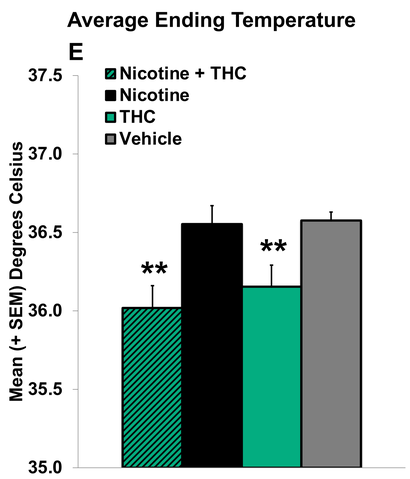




Exposure to nicotine, THC, or the combination via e-cigarettes did not alter the overall percentage of maternal weight gain during pregnancy (A), nor did it alter body weights on or across individual days (B). Similarly, prenatal exposure to either drug did not change the daily food or water intake (data not shown). Thus, this co-exposure model does not produce nutritional confounds in pregnant rats.





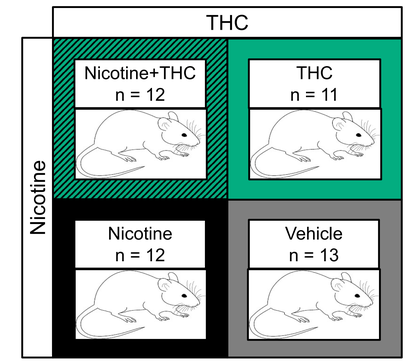


Pregnant rats exposed to THC alone showed significantly greater temperature changes than dams exposed to the Vehicle following intoxication. In contrast, dams exposed to Nicotine alone had significantly smaller temperature changes during intoxication compared to the Vehicle controls, while dams exposed to combined Nicotine+THC had an intermediate effect and did not differ from controls ($F[3,44] = 12.83$, $p < 0.001$, SNK p's < 0.05; C).

To better understand the magnitude of these temperature changes during intoxication, we also examined subjects' body temperatures before (baseline) and after (ending) vapor inhalation sessions. Overall, chronic nicotine exposure via e-cigarettes to pregnant rats decreased baseline core body temperatures ($F[1,44] = 29.06$, $p < 0.001$; D); this effect took place during the latter half of pregnancy (data not shown). Following drug exposure, dams exposed to THC had lower body temperatures, alone or in combination with nicotine ($F[1,44] = 17.19$, $p < 0.001$; E). Thus, the smaller temperature change in the combined exposure group may have been due to a lower baseline temperature.

* = Nicotine > all other groups, $p < 0.05$. ** = THC different from all other groups, p's < 0.05. *** = any Nicotine < no Nicotine, $p < 0.001$.

Subject Information



Conclusions

These data suggest that this prenatal co-exposure paradigm to nicotine and THC via e-cigarettes among pregnant rats:

- Avoids potential nutritional confounds
- Replicates expected physiological effects of THC intoxication
- Induces clear physiological effects of repeated nicotine intoxication

Taken together, use of this paradigm will:

- Provide a clinically relevant model of co-exposure to nicotine and THC via e-cigarettes for preclinical research
- Help inform both the public and public policy on e-cigarette use during pregnancy

Acknowledgements

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All data were collected at the Center for Behavioral Teratology (CBT) at San Diego State University. Thank you to members of the CBT for assisting in study design and data collection, including a special thanks to Cristina Rodriguez, Samirah Hussain, Brandonn Zamudio, and Karen Thomas.

Data were analyzed and interpreted at WCUPA by the authors.

Identification of mitochondrial transfer sequences in homologs of a folic acid metabolism gene

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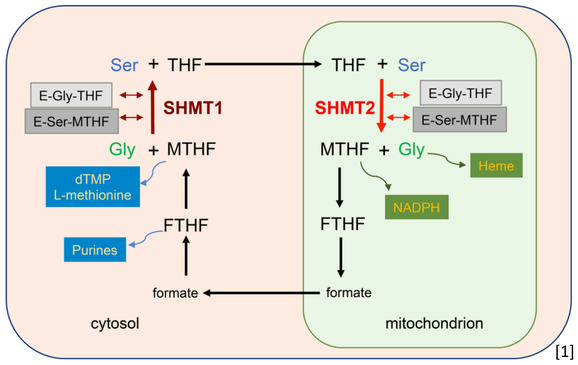
Abstract

Neural tube defects (NTDs) are common malformities resulting in exposed spinal cord or brain tissue caused by the inability to close the neural tube in embryogenesis. Previous research has shown folate deficiency increases the risk of NTDs. A folic acid metabolism gene, *serine hydroxymethyltransferase* (*Shmt)* is responsible for the synthesis of thymidylates, purines, and methionine which are important for DNA replication especially during embryogenesis. Folic acid metabolism has two main pathways, one in the cytosol and one in the mitochondria which enforces eukaryotes to have two forms of SHMT. The different localizations are a result of mitochondrial target sequences in the N-terminus. Interestingly, the model system Caenorhabditis elegans only have one homolog of *Shmt* called *mel-32* and it was unclear if this gene's product was cytosolic, mitochondrial, or both. To address this question, a bioinformatics approach was taken to identify if *mel-32/Shmt* has a mitochondrial transfer sequence. We identified putative mitochondrial transfer sequences that are present in specific isoforms. Molecular phylogenies of different organisms were then generated to show prominent cytosolic SHMT and mitochondrial SHMT clustering especially around the phyla Nematoda, Arthropoda, and Tardigrada. By comparing isoforms with different SHMT localizations, potential mitochondrial target sequences were identified for organisms that could later be experimentally assessed.

Mitochondrial target sequences

The mitochondrial target sequence is based on the physiochemical properties needed to bind to translocase of the outer mitochondrial membrane (TOM). Previous experiments demonstrate the mitochondrial target sequences have the motif, ΦXXΦΦ where Φ represents a bulky hydrophobic amino acid and X represents any amino acid, but there are exceptions. [2,3]

Cytosolic and mitochondrial SHMT pathways



[1]

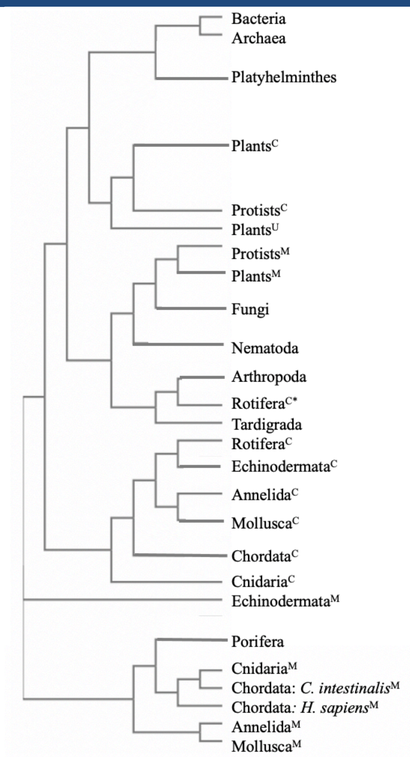
Determined SHMT localizations and percent identify within species

Table 1: Determined SHMT Localizations and Percent Identity within Species[1]

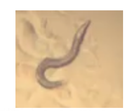
Kingdom/ Phylum Representation	Organism	Mitochondrial SHMT	Cytosolic SHMT	Undeclared SHMT[2]	Percent Identity
Stramenopiles (Protists)	*Thalassiosira pseudonana*	XP_002295557	XP_002289669 XP_002293993		54.58-58.54
Plant	*Arabidopsis thaliana*	NP_195506 NP_001331385	NP_193129 NP_193125 NP_001323098 NP_564473	NP_001119098	47.59-85.24
Fungi	*Saccharomyces cerevisiae*	AAA21024	AAA21023		59.10
Porifera	*Amphimedon queenslandica*	XP_019854079	XP_019854080	XP_003387864	99.20-100.00
Cnidaria	*Actinia tenebrosa*	XP_031559133	XP_031558549		63.00
Platyhelminthes	*Opisthorchis viverrini*	OON24063 XP_009166916	OON23958 XP_009166918		54.60-100.00
Annelida	*Capitella teleta*	ELU01860	ELU03449		64.39
Mollusca	*Crassostrea gigas*	XP_011420488	XP_011435353 XP_034311075		59.79-100.00
Rotifera	*Brachionus plicatilis*		RMZ93562 RNA14241		24.17
Nematoda	*Caenorhabditis elegans*	NP_741197	NP_001367440		100.00
Arthropoda	*Drosophila melanogaster*	NP_572278	NP_001138162		100.00
Echinodermata	*Strongylocentrotus purpuratus*	XP_030829045	XP_798074 XP_011661053		54.29-100.00
Chordata	*Ciona intestinalis*	XP_002126094	XP_002127233		60.50
Chordata	*Homo sapiens*	NP_005403	NP_004160		63.45

[1] *M. jannaschii* (Archaea), *E. coli* (Bacteria), and *H. dujardini* (Tardigrada) are not included since only one gene exists for each species so a comparison cannot be made.
2 Undeclared SHMT is determined by not having either a prominent mitochondrial or cytosolic localization determined by the localization for these being under 0.50.

Phylogeny shows clustering of cytosolic SHMT and mitochondrial SHMT



Isoforms with different SHMT localizations identify potential mitochondrial target sequences



A. *Amphimedon queenslandica* Isoforms

```
XP_019854079.1 ----------MLITVFRKAAKVTFRALDRRFQQVMA
XP_003387864.2 MSCKLFWIMSITCMGATIVGVFLKKAESLRRTRERCITVITKAAKVTFRALDRRFQQVMA
XP_019854080.1 ----------MA
                           **
```

B. *Caenorhabditis elegans* Isoforms

```
NP_741197.1    MFARIVSRRAATGLFAGASSQCKMADRQVHTPLAKVQRHKYTNNENILVDHVEKVDPEVF
NP_001367440.1 ----------MADRQVHTPLAKVQRHKYTNNENILVDHVEKVDPEVF
                          ************************************
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C. *Drosophila melanogaster* Isoforms

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NP_572278.1    MQRARSTLTQKLRFCLSRDLNTKVGNPVNFETGKLSGALTRIAAKKQPSPTPFLPAIRRY
NP_001138162.1 ----------------------------------------------------------

NP_572278.1    SDSKQSTLKNMADQKLLQTPLAQGDPELAELIKKEKERQREGLEMIASENFTSVAVLESL
NP_001138162.1 --------MADQKLLQTPLAQGDPELAELIKKEKERQREGLEMIASENFTSVAVLESL
                       ************************************************
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Conclusions and Future Studies

- Our sampling suggests eukaryotes generally have one cytosolic *Shmt* and one mitochondrial *Shmt*, but isoforms are prevalent in the cluster of Nematoda and Arthropoda.
- We were successful in identifying potential sequences responsible for targeting the protein to the mitochondrial by utilizing the isoforms of Nematoda, Arthropoda, and Porifera.
- Future experiments can be done to confirm mitochondrial localizations and determine which amino acids are essential for mitochondrial targeting.
- When all SHMT homologs were analyzed in the phylogeny, cytosolic SHMT clustered together and mitochondrial SHMT clustered together showing similarities in the N-terminus.
- The molecular SHMT phylogenies showed clusters of species that agreed with the widely accepted phylogeny, but major differences include Platyhelminthes being distant, and Chordata and Echinodermata not being as closely related.
- Previous research has shown that the *Shmt* homolog, *mel-32* in *C. elegans* is important in embryogenesis. Future experiments could be conducted to see if both isoforms are vital for development in *C. elegans*.

Acknowledgements

I would like to give a big thanks to Dr. Sullivan-Brown for guiding and assisting me through this research project!

References

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Comparing Structures of Nucleosomes and Tetrasomes Using DNase I Footprinting

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FOX CHASE CANCER CENTER
TEMPLE HEALTH

BACKGROUND

Nucleosomes are dynamic

In the eukaryotic genome, DNA is organized into compact repeating units called nucleosomes, these chromatin subunits consist of a histone octamer with DNA wrapped around it. These nucleosomal structures are dynamic and become less compact during replication and transcription (1). Histone chaperones play an important role in these events.

Histone interactions with hFACT

hFACT, a histone chaperone, facilitates transcription and aids in post-transcriptional nucleosomal recovery. The function of hFACT is up-regulated in cancer cells and it can be used as a target for cancer treatment (2). The intermediate complex investigated in this study is the tetrasome which consists of DNA wrapped around the histone H3-H4 tetramer. Interactions of hFACT with the H3-H4 components of the nucleosome are not fully understood.

Investigation of the tetrasome

This study investigated the intermediate structure of the H3-H4 tetrasome using a DNase I footprinting approach. The results of this project will be used to determine the mechanism of interaction of hFACT with H3-H4 tetrasomes and nucleosomes.

METHODS

- Nucleosomes and tetrasomes were assembled using Fam -labeled DNA containing 603 nucleosome positioning sequence.
- Three DNase I concentrations were tested to visualize where the DNA was left unprotected or protected compared to free DNA control.
- The samples were purified by phenol-chloroform extraction.
- Deoxyribonuclease (DNase) I degrades DNA that is not protected by proteins via binding (3).
- This protein footprinting allows for visualization of where such DNA exists in different conformational states via acrylamide gel electrophoresis.

FIGURES

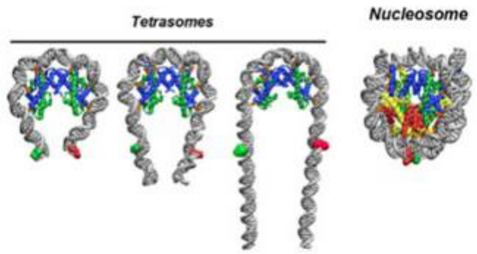

FIGURE 1: Tetrasome model
The relaxed nature of the H3/H4 tetrasome compared to the compact nucleosome structure. This relaxed nature leaves DNA less protected in the presence of DNase.

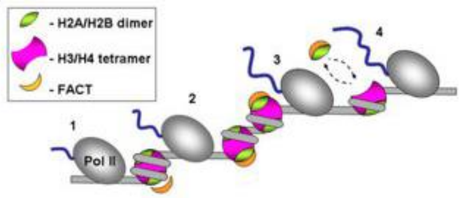

FIGURE 2: hFACT proposed mechanism
A theorized mechanism of how hFACT facilitates transcription via interactions with the histone core.

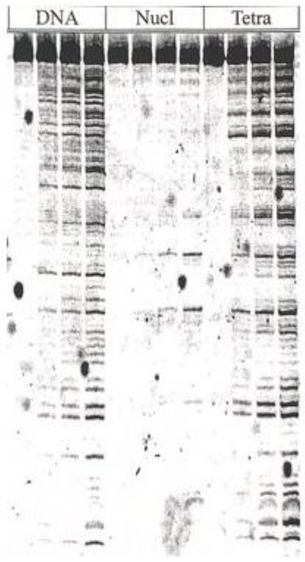

FIGURE 3: DNase I Footprint
In the presence of DNase, free DNA and H3-H4 tetrasomes displayed greater amounts of degradation when compared to the nucleosome.

RESULTS

- Nucleosomes remained almost completely protected from DNase I with minimal degradation seen at all 3 DNase I concentrations.
- DNA displayed more degradation when compared to the nucleosome at all concentrations.
- Tetrasomal DNA displayed more degradation when compared to the free DNA and displayed different patterns of degradation in certain regions.
- This suggests that the structures and DNA binding patterns of tetrasomes and nucleosomes are considerably different.

FUTURE DIRECTIONS

The results of this project will be used to determine the interaction of hFACT with the H3/H4 tetramers during transcription.

ACKNOWLEDGEMENTS

Thank you to the Studitsky lab at Fox Chase Cancer Center and Sarah Stamis.

LITERATURE

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