In utero optical coherence tomography reveals changes in murine embryonic brain vasculature after prenatal cannabinoid exposure

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In utero optical coherence tomography reveals changes in murine embryonic brain vasculature after prenatal cannabinoid exposure

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ABSTRACT

Prenatal substance abuse is a major public health concern. Much research has been focused on alcohol and other drug use, but there is a lack of information about prenatal cannabinoid use. Nevertheless, marijuana use during pregnancy increases the risk of a stillbirth by approximately 2.3X. Synthetic cannabinoids (SCB) are a group of heterogeneous compounds which were developed to understand the endogenous cannabinoid system and as potential therapeutics. SCBs are legally available for purchase in several places, and the use of natural and synthetic cannabinoids is high among women of reproductive age. Combined with the prevalence of unplanned pregnancies, the high use of cannabinoids may lead to an increase in prenatal exposure to cannabinoids. Early studies have shown morphological and behavioral anomalies similar to fetal alcohol syndrome. Even though the mechanisms of Δ^9-tetrahydrocannabinol (Δ^9-THC), the major psychoactive component of marijuana, and SCB are similar, there are several important differences. Subsequently, some SCBs have a 40 to 600 fold higher potency than Δ^9-THC. However, there is paucity of research focused on the prenatal effects of SCBs. This study uses correlation mapping optical coherence tomography (cm-OCT) to evaluate acute changes in the murine fetal brain vasculature *in utero* after exposure to CP-55,940, a well-characterized and commonly used reference compound in cannabinoid research. Our results showed a rapid decrease in parameters quantifying vasculature, i.e., vessel area density, and vessel length fraction, as compared to the sham group, demonstrating a dramatic and rapid effect of cannabinoids on fetal brain vasculature. Our work shows the need for further research on the effects of cannabinoids on fetal development.

1. INTRODUCTION

Prenatal substance abuse is a major public health concern [1], and marijuana is one of the most commonly abused substances during pregnancy [2, 3]. Synthetic cannabinoids (SCB) [4], a group of heterogeneous compounds with a 40 to 600 fold higher potency than Δ^9-tetrahydrocannabinol (Δ^9-THC), are legally available for purchase in several places. SCB users have reported several dangerous health effects [5]. Due its toxic effects on users, it is expected to have similar effects due to prenatal exposures. Combined with the prevalence of unplanned pregnancies and high use during reproductive age, easy availability of natural and synthetic cannabinoids may lead to an increase in prenatal cannabinoid exposure, which emphasizes the need to study its toxic effects.

The second trimester is considered the peak period for fetal neurogenesis and angiogenesis. The microvasculature that develops during this period performs several functions including supporting nutritional needs [6], providing endocrine control of fetal development [7], and promoting neural development [8]. Although early studies to evaluate the effects prenatal cannabinoid exposure have shown morphological and behavioral anomalies similar to fetal alcohol syndrome

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(FAS) [9], further work is needed to develop a deeper understanding of the causes of the morphological and behavioral abnormalities.

Although histological sectioning and imaging has been the gold standard to evaluate embryonic development, its invasive and time-consuming nature and the need to fix tissue have made it unsuitable for longitudinal and live embryonic imaging. Optical coherence tomography (OCT) [10, 11] has rapidly become a preferred embryo imaging modality over other techniques such as confocal microscopy, ultrasound biomicroscopy, micromagnetic resonance imaging, and microcomputed tomography [12] because of its noninvasive nature, superior resolution, rapid imaging speed, nonionizing imaging, and intrinsic imaging capabilities that require no exogeneous contrast agents. Moreover, the superior spatial resolution of OCT means that it can blood vessels less than 50 μm in diameter, which other imaging modalities such as ultrasound biomicroscopy are incapable of resolving.

In this work, we use correlation mapping optical coherence tomography (cm-OCT) [13] to evaluate acute changes in fetal brain vasculature of murine embryos, *in utero*, after prenatal exposure to CP-55,940, a well-characterized and commonly used reference compound in cannabinoid research. Results showed a rapid decrease in the vasculature, similar to effects of prenatal alcohol exposure.

2. METHODS

A phase stabilized swept source OCT (PhS-SSOCT) system was used for structural and cm-OCT imaging. The PhS-SSOCT system consisted of a broadband swept source laser source (Santec Corporation, Japan) with a central wavelength of 1310 nm, scan range of 150 nm, scan rate of 30 kHz, and axial resolution of 11 μm in air. The interference pattern was recorded by a balanced photodetector and digitized by a high-speed analog-to-digital converter. After resampling the raw interference pattern into linear k-space, a fast Fourier transform was performed on the fringe to obtain the A-scan. Using a scanning galvanometer-mounted mirror, a B-scan was reconstructed. More details on the system can be found in our previous work [14].

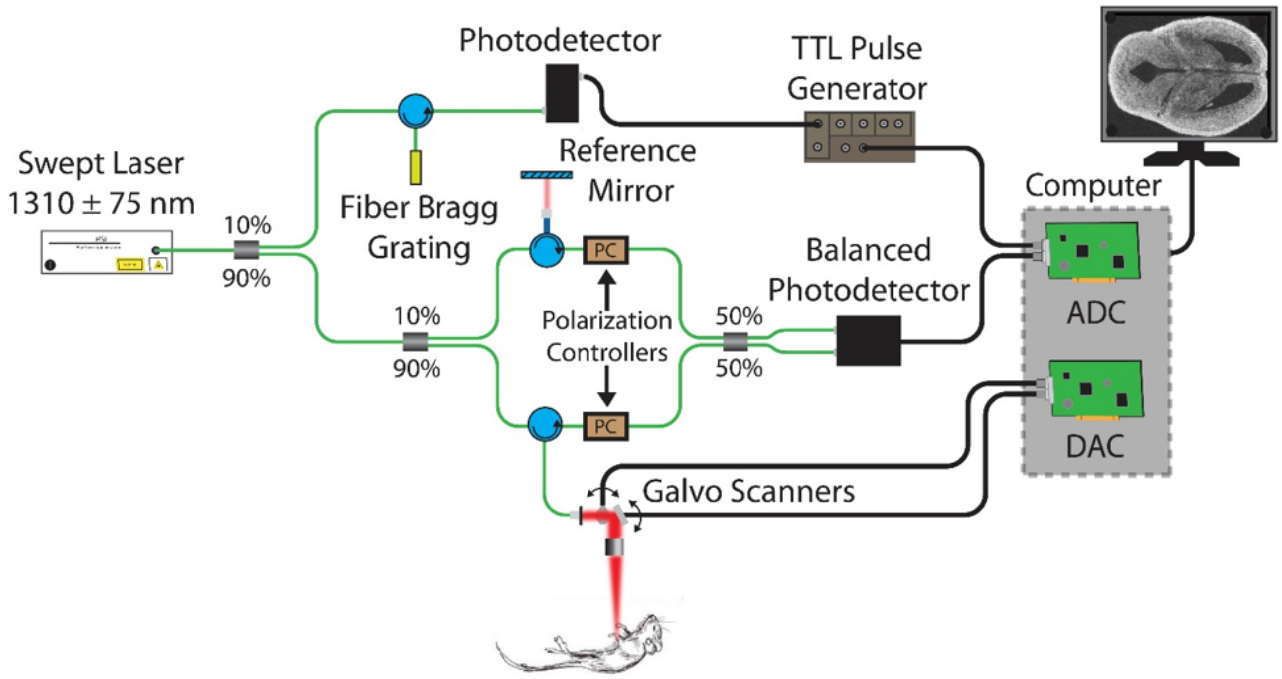

Figure 1. Schematic of the experimental setup. ADC: analog to digital converter; DAC: digital to analog converter

Timed overnight matings of CD-1 mice were set up, and the presence of a vaginal plug was considered 0.5 days post coitum (DPC). Pregnant female mice were anesthetized by inhalation of isoflurane at 14.5 DPC, corresponding to the

transition between the 1st and 2nd trimester period of brain development. The mice were weighed and placed on a heated platform at 37°C during imaging to maintain body temperature. The depth of maternal anesthesia was maintained with a continuous flow of isoflurane. Abdominal hair was removed, and a small incision was made to expose the uterine horn for imaging. The embryo selected for imaging was stabilized using forceps to minimize bulk motion, and the imaging was performed through the uterine wall. After initial measurements before cannabinoid exposure, the liver of the pregnant mother was sprayed with CP-55,940 at a dose of 2 mg/kg, which was suspended in a vehicle solution of DMSO: Alkamuls El620 (Rhodia, NJ): lactated Ringer's solution at a ratio of 1:1:18. Control animals at the same stage were sprayed with only the vehicle solution. Subsequent measurements were taken every 5 minutes for a 45 minute period after the exposure, but only the results at 45 minutes are presented here. The animal was euthanized following imaging by an overdose of isoflurane followed by cervical dislocation.

Imaging involved 5 B-scans per position, and cm-OCT was used to obtain the temporal correlation between frames at the same spatial position. The maximum intensity projection (MIP) was used to calculate vessel area density (VAD) and vessel length fraction (VLF). VAD is defined as the area of the vessels divided by the total area of the image. VLF is defined as the length of the vessels divided by the total image area. Only a portion of the image that included the main vessel under investigation was selected for quantification. ImageJ was used for the quantifications.

3. RESULTS

Our results showed a rapid decrease in all parameters used to quantify vasculature. MIPs of fetal brain vasculature of one embryo from each group are shown in Figures 2 and 3. Figures 2 (a) and (b) show the MIP of the cm-OCT image before and 45 minutes after maternal cannabinoid exposure respectively. A significant decrease in vasculature is seen between these two images. Figures 3 (a) and (b) show the MIP of the cm-OCT image before and 45 minutes after maternal exposure to the vehicle (without the cannabinoid), and there is a no noticeable decrease in brain vasculature. The appearance of smaller vessels is most likely due to the decrease in bulk motion, which will be corrected in future data processing steps.

In the case of cannabinoid exposure (N=2), the VAD decreased by almost 50% and the VLF decreased by almost 60%. In contrast, there was only a slight increase in both the parameters for the samples that were exposed to only the vehicle (N=2). Our future work is focused on evaluating more samples in order to obtain statistically significant results.

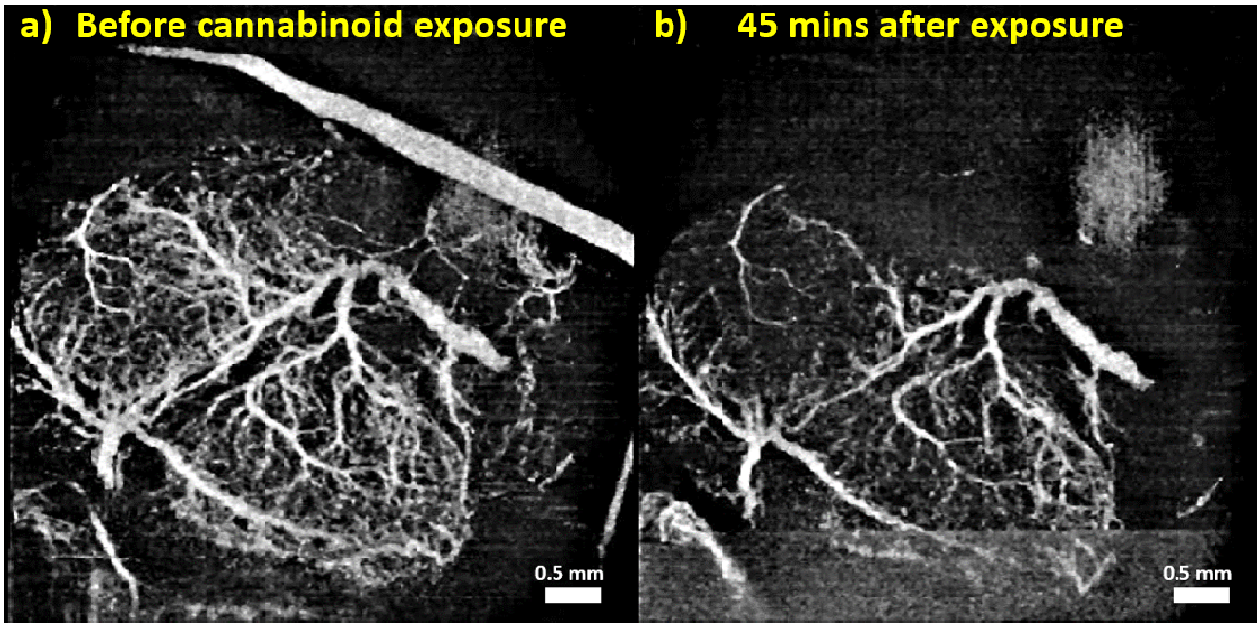

Figure 2. cm-OCT images of fetal brain (a) MIP of the cm-OCT image before cannabinoid exposure. (b) MIP of the cm-OCT image 45 minutes after cannabinoid exposure

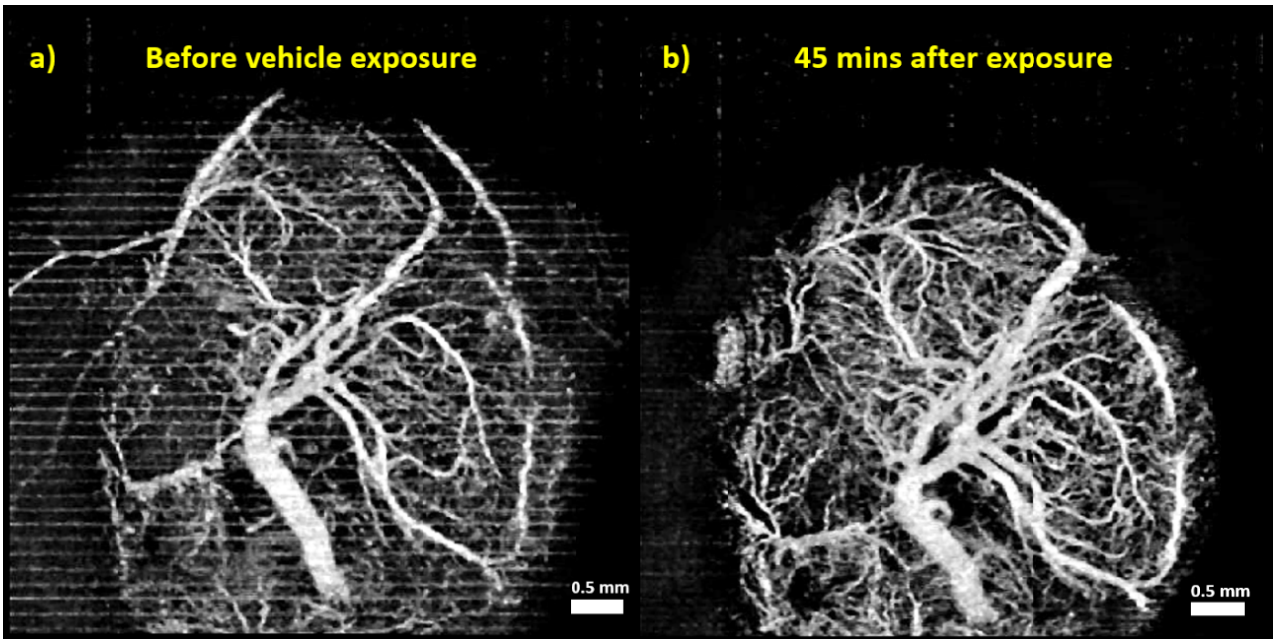

Figure 3. cm-OCT images of fetal brain (a) MIP of the cm-OCT image before vehicle exposure. (b) MIP of the cm-OCT image 45 minutes after vehicle exposure

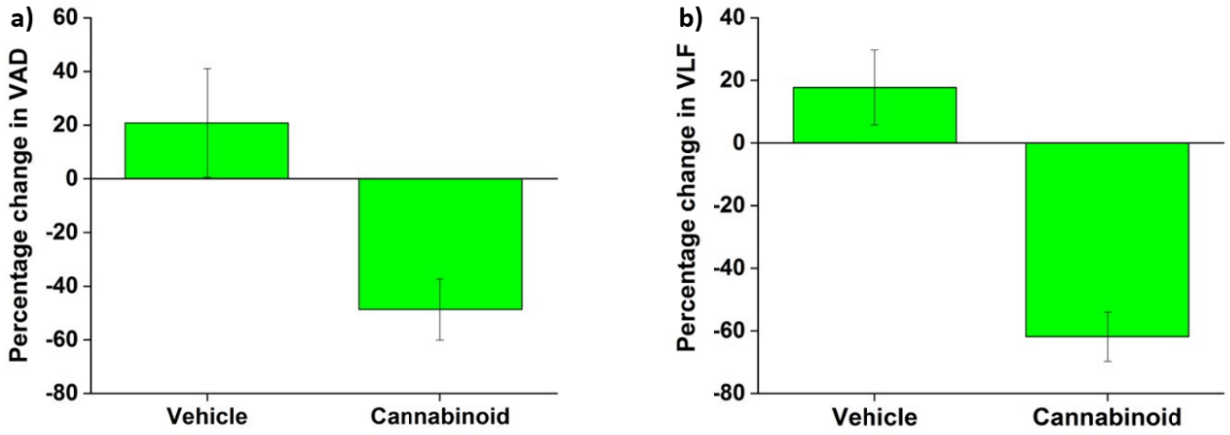

Figure 4. Percentage change in (a) VAD and (b) VLF after cannabinoid and vehicle exposure

4. CONCLUSION

This preliminary work has evaluated acute vasculature changes in the murine fetal brain caused by prenatal cannabinoid exposure using cm-OCT. The results show a dramatic decrease in vasculature 45 minutes after maternal cannabinoid exposure, similar to effects of prenatal alcohol exposure.

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