PROTEOMIC PROFILE OF EXTRACELLULAR VESICLES IN THE BRAIN AFTER THC EXPOSURE

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Abstract

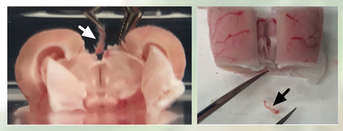
Background: With legalization of cannabis in the US, there is an urgent need to more clearly understand the drug's effects on central signaling mechanisms. Extracellular (EVs) vesicles have been identified as intercellular signaling mediators, which contain a variety of cargo, including proteins, enzymes, and RNA transcripts. The focus of these studies was to examine whether the main psychoactive component in cannabis, Δ9-tetrahydrocannabinol (THC), alters EV cargo in the brain.

Methods: In vitro studies were first conducted to determine whether THC can act on primary epithelial cells derived from the dorsal third ventricle of rats. Next, to examine the impact of THC in vivo, male and female rats were exposed to aerosolized THC or vehicle in vapor chambers. The first cohort of rats (n=12/group/sex) received a single session of exposure, and the second cohort (n=12/group/sex) received 14 consecutive daily sessions of exposure. CSF was collected from the cisterna magna, and EVs were extracted with SBI SmartSEC and then processed for label free quantitative proteomics analyses via high resolution tandem mass spectrometry. Quantitative LFQ mass spectral data were analyzed using Progensis QI Proteomics software.

Results: Cannabinoid receptor (CB1R) expression was localized in the choroid plexus, and THC upregulated the expression of c-fos, CB1R mRNA, and mir-204, a transcript localized in EVs. In the THC vape exposed rats, multiple EV proteins were identified as being differentially expressed following either acute or chronic exposure. Interestingly, exponential effects were found with some proteins between acute exposure and chronic exposure expression.

Conclusions: Our findings reveal that cannabinoids can modulate intercellular signaling mechanisms in the brain, with differential effects following single or chronic exposure. Our data further support the contention that THC can act on CB1 receptors in the choroid plexus to mediate EV signaling, which may then integrate into different brain regions to modulate cellular function.

Dissection of choroid plexus from the dorsal third ventricle



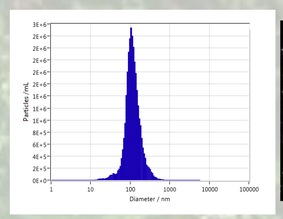
Timeline of vapor exposure experiment

Acute: 1 h exposure

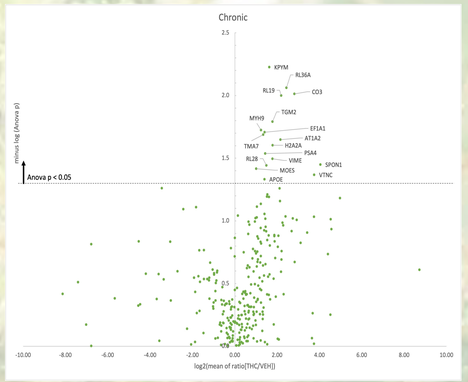
Chronic: 1 h exposure x 14 days

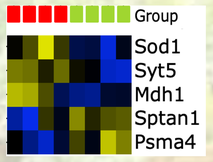
CSF extraction after the exposure

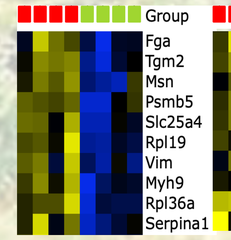
Quantification and visualization of EVs from CSF of rats

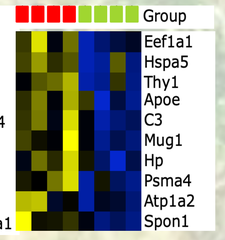


Change in Extracellular protein content of CSF after Acute and Chronic 50 mg/ml THC vape in Male subject

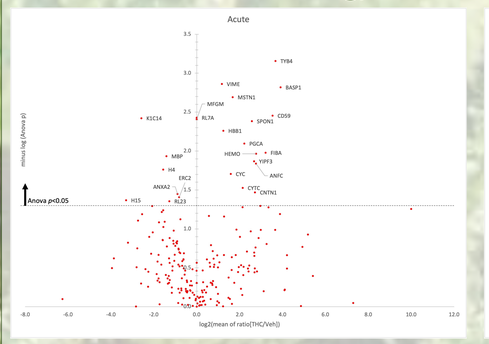


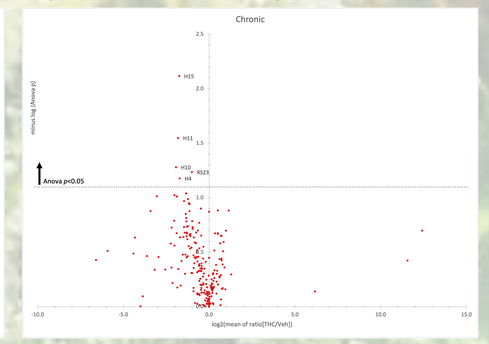


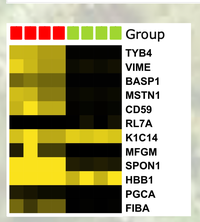


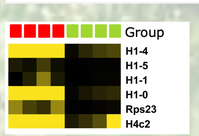


Change in Extracellular protein content of CSF after Acute and Chronic 50 mg/ml THC vape in Female subject









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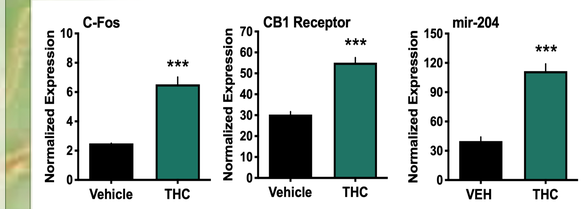
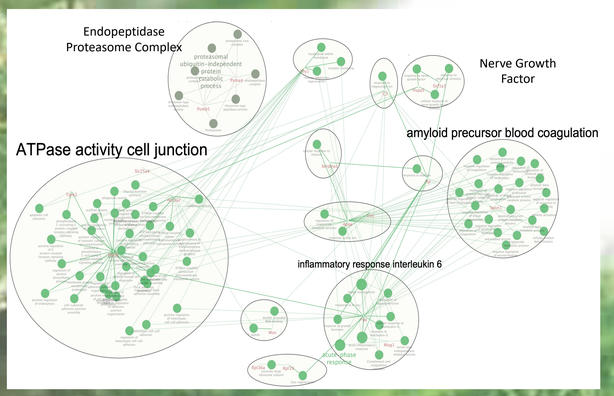
Effect of THC on Primary Epithelial Cell Culture from the Rat Choroid Plexus



C-fos and CB1 mRNA expression in choroid plexus epithelial cells, and mir-204 in EV from the medium after acute vehicle or THC (50 µM) treatment in vitro.



Acute		
Networks	FDR	Network Objects from Active Data
Cell adhesion_Synaptic contact	3.944E-03	Alpha-fodrin, Synaptotagmin V, Synaptotagmin
Transport_Synaptic vesicle exocytosis	4.078E-02	Synaptotagmin V, Synaptotagmin
Development_Neurogenesis_Synaptogenesis	4.078E-02	Synaptotagmin V, Synaptotagmin

Chronic		
Networks	FDR	Network Objects from Active Data
Immune response_Phagocytosis	2.483E-06	ERM proteins, C3, C3b, iC3b, MyHC, C3dg, HDL proteins, MSN (moesin), APOE
Immune response_Phagosome in antigen presentation	2.483E-06	ERM proteins, C3, HSP70, iC3b, PSMB5, PSMA4, C3dg, GRP78, MSN (moesin)
Inflammation_Complement system	1.091E-04	C3, C3b, iC3b, C3dg, C3a
Inflammation_IL-6 signaling	7.766E-04	Fibrinogen alpha, C3, Alpha 1-antitrypsin, HDL proteins, HP
Cytoskeleton_Actin filaments	3.194E-02	ERM proteins, MYH9, MyHC, MSN (moesin)
Cytoskeleton_Regulation of cytoskeleton rearrangement	3.194E-02	Vimentin, ERM proteins, MyHC, MSN (moesin)
Inflammation_Kallikrein-kinin system	3.194E-02	Fibrinogen alpha, C3, Alpha 1-antitrypsin, C3a
Immune response_Antigen presentation	3.194E-02	HSP70, PSMB5, PSMA4, GRP78
Cell adhesion_Integrin-mediated cell-matrix adhesion	4.086E-02	ERM proteins, TGM2, MyHC, MSN (moesin)
Development_Neurogenesis_Axonal guidance	4.655E-02	ERM proteins, MYH9, MyHC, APOE

Conclusion

THC triggers cellular activation mechanisms:

→Lead to variations in the release of EVs.

→EVs cargo are a fundamental messengers of physiological processes, such as ionic exchange, cell development and immunological states.

→ In our model, sex and length of exposure modulate these mechanisms.