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Effects of Cannabinoids and Postmortem Interval on Gene Expression: Considerations for the Forensic Genetic Analysis of Civil Aviation Accident Victims

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16. Abstract

Cannabis is the third most commonly used drug of abuse following alcohol and tobacco in the United States. Cannabis is federally classified as a Schedule I substance under the Controlled Substances Act of 1970 but is legal for medicinal and/or recreational purposes in 39 US states. However, cannabis use by safety-sensitive personnel, including certificated pilots, remains prohibited in the US. Despite the prohibition on cannabis use among pilots, a number of fatal accidents in which the deceased pilot tests positive for delta-9-tetrahydrocannabinol (THC) and/or metabolites in post-accident toxicological analyses still occur. No correlation is known to exist between blood or tissue THC concentration and degree of functional impairment, frustrating efforts to ascribe causality for this subset of aviation accidents. One possible solution for this lack of correlation is forensic transcriptome analysis, specifically postmortem analysis of the expression of cannabis-responsive genes whose expression can be correlated with measures of cognitive impairment. Cannabis consumption results in quantifiable changes in gene expression, from which biomarkers correlating with the timeline of use and impairment may be identified. Complicating matters is that the transcriptome is not static postmortem, with hundreds, if not thousands, of genes exhibiting differential expression throughout the postmortem interval.

This review surveyed recent studies that investigated the effects of cannabis and THC exposure on gene expression in multiple tissues of interest, as well as studies that have sought to characterize the "thanatotranscriptome," or genes whose expression changes significantly following organismal death. Additional studies will be necessary over the coming years to determine the effects of cannabis consumption on gene expression both ante- and postmortem.

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List of abbreviations

Abbreviation	Definition
FAA	Federal Aviation Administration
DOT	United States Department of Transportation
ToxDB	FAA toxicological accident records database
THC	Delta-9-tetrahydrocannabinol
THC-COOH	11-nor-9-carboxy-delta-9-tetrahydrocannabinol
11-OH-THC	11-hydroxy-delta-9-tetrahydrocannabinol
RNA	Ribonucleic acid
DNA	Deoxyribonucleic acid
CBD	Cannabidiol
PCR	Polymerase chain reaction
RT-qPCR	Quantitative reverse transcription PCR
RNA-seq	RNA sequencing
scRNA-seq	Single cell RNA sequencing
KEGG	Kyoto Encyclopedia of Genes and Genomes
GO	Gene Ontology
HP	Human Phenology
DEG	Differentially expressed gene
hiPSCs	Human-induced pluripotent stem cells
NPCs	Neural progenitor cells
PMBCs	Peripheral mononuclear blood cells
NKs	Natural killer cells
SEB	Staphylococcal enterotoxin B
CB2	Cannabinoid receptor type 2
LN	Lymph nodes
miRNA	Micro RNA
lncRNA	Long non-coding RNA
pri-miRNA	Micro RNA primary transcript
H3K27me3	Tri-methylation of lysine 27 on histone H3
H3K9Ac	Acetylation of lysine 9 residue on histone H3
PMI	Postmortem interval

Abstract

Cannabis is the third most commonly used drug of abuse following alcohol and tobacco in the United States. Cannabis is federally classified as a Schedule I substance under the Controlled Substances Act of 1970, but is legal for medicinal and/or recreational purposes in 39 U.S. states. However, cannabis use by safety-sensitive personnel, including certificated pilots, remains prohibited in the US. Despite the prohibition on cannabis use among pilots, a number of fatal accidents in which the deceased pilot tests positive for THC and/or metabolites in post-accident toxicological analyses still occur. No direct correlation is currently known to exist between blood or tissue THC concentration and degree of functional impairment, frustrating efforts to ascribe causality for this subset of aviation accidents. One possible solution for this lack of correlation is forensic transcriptome analysis, specifically postmortem analysis of the expression of cannabis-responsive genes whose expression can be correlated with measures of cognitive impairment. Cannabis consumption results in quantifiable changes in gene expression, from which biomarkers correlating with the timeline of use and impairment may be identified. Complicating matters is that the transcriptome is not static postmortem, with hundreds, if not thousands, of genes exhibiting differential expression throughout the postmortem interval.

This review surveyed recent studies that investigated the effects of cannabis and THC exposure on gene expression in multiple tissues of interest, as well as studies that have sought to characterize the “thanatotranscriptome,” or genes whose expression changes significantly following organismal death. Additional studies will be necessary over the coming years to determine the effects of cannabis consumption on gene expression both ante- and postmortem.

1. Introduction

The use of cannabis, cannabinoids, and products that contain cannabis or cannabinoids has increased as individual states have passed laws legalizing or decriminalizing their use for medicinal or recreational purposes [1, 2]. This increased use, and the potential for further increase in use, has led to a renewed interest in the effects of these substances on human health and performance. At the time of writing, 39 states have passed legislation allowing for the use of cannabis products for medicinal and/or recreational purposes [3]. Data from the National Survey on Drug Use and Health, conducted between 1979 and 2016, show that for US residents aged 12 to 25, current cannabis use (use reported in the previous 30 days) increased between 2006 and 2016; also, use is positively correlated with the proportion of the population covered by medical cannabis laws [4]. Further, 2016 survey data from the US Centers for Disease Control and Prevention's Behavioral Risk Factor Surveillance System, representing 12 states with variable cannabis legalization laws at the time of the survey, report a rate of current cannabis use among adults aged 18 years or older of 9.1%, with a rate of 12.0% among males and 6.3% among females [5]. Additionally, among respondents who indicated current cannabis use, 33.7% also reported using multiple consumption methods [5]. However, because of cannabis' continued illegality at the federal level for any purpose and its status as a Schedule I substance under the Controlled Substances Act of 1970, there has been a relative dearth of studies and research funding concerning cannabis and cannabinoid-containing products aside from those that have focused on its misuse and negative effects [6, 7].

Given its role in safeguarding the United States National Airspace System, specific Federal Aviation Administration (FAA) regulations forbid use of any impairing substances, including cannabis, among individuals engaged in safety-sensitive operations [8]. The FAA's Random Drug and Alcohol Testing program, as described in 14 CFR part 120, requires employers to conduct random annual drug testing of at least 50% of their eligible safety-sensitive employee populations each year; this rate may be administratively lowered to 25% if the proportion of employees with positive tests remains below 1% for two consecutive years [9]. Marijuana (cannabis) use remains federally illegal, and per the US Department of Transportation (DOT) guidance, "it remains unacceptable for any safety-sensitive employee subject to drug testing under the Department of Transportation's drug testing regulations to use marijuana" for either medicinal or recreational purposes [10, 11].

FAA guidance states "if you are a pilot, a verified positive drug test for marijuana on a required DOT/FAA drug test will make you unqualified to hold an FAA-issued medical certificate" [12]. FAA guidance to aviation medical examiners dictates that applicants who are using controlled substances, including marijuana (cannabis), should not be issued airmen medical certificates (a document which affirms that the applicant in question meets FAA airmen medical standards) [13, 14]. Additionally relevant is the FAA policy for pilots regarding "Do Not Fly" medications, i.e., those that "may cause and impair cognitive function, seriously degrading pilot performance." For such medications, policy dictates that pilots should not fly "following

the last dose…until a period of time has elapsed equal to 5-times the maximum pharmacological half-life of the medication" [13]. These policies clearly indicate the degree of concern with which the FAA regards the use of impairing substances.

Regardless of cannabis' prohibited status and that detection of its use is grounds for the revoking a pilot's airman medical certificate, a subset of pilots do consume cannabis and fly. Using data from the FAA toxicological accident records database (ToxDB) from 2007 to2016, FAA researchers observed that 3.4% of individuals involved in fatal accidents during this period tested positive for either delta-9-tetrahydrocannabinol (THC) or its main secondary metabolite 11-nor-9-carboxy-delta-9-tetrahydrocannabinol (THC-COOH) in at least one specimen type (either fluid or tissue). This positive test rate was consistent with the previous 10-year period (1996 to 2007) [15].

Blood THC concentrations have been positively correlated with driver culpability in fatal motor vehicle accidents, with drivers having a blood THC concentration of 5 ng/mL or higher being 6.6 times more likely to be culpable than drug-free drivers [16]. For comparison, in the same study, drivers with a blood alcohol concentration of 0.10% to 0.15% were 3.7 times more likely to be culpable than drug-free drivers. As such, the ability to correlate blood or tissue THC concentrations with objective measures of neurocognitive impairment may assist investigators in determining causality in fatal accidents. Although THC metabolites maybe detected for weeks following heavy cannabis use [17], there are unfortunately no current objective means of determining an individual's degree of impairment solely by cannabinoid concentrations.

While not a direct measure of impairment, THC-induced changes in gene expression may represent an initial point of investigation from which postmortem impairment biomarker panels could be identified, quantified as a function of time post-consumption, and eventually reliably assayed. While toxicology results may indicate presence or absence of THC and/or its metabolites, these results alone cannot currently be used to determine the level of impairment of individuals. Alternatively, gene expression biomarkers associated with cognitive changes during cannabis consumption could one day enable inferences of impairment levels. Such biomarkers would be useful in elucidating the circumstances leading up to fatal aviation accidents involving pilots who test positive for THC and/or THC metabolites. However, a complicating factor in performing such postmortem gene expression analyses is that the molecule assayed—ribonucleic acid (RNA)—is inherently chemically unstable. Under representative physiological conditions, RNA is approximately 100,000-fold less stable than deoxyribonucleic acid (DNA) under similar conditions [18].

A previous analysis performed by the FAA documented multiple complicating variables present in aviation accidents that can impede the collection and quantification of RNA from victims. These included the severity and degree of aviation accident-induced trauma sustained by the victim, bacterial contamination of tissue, postmortem interval from the time of the accident until the cadaver is placed under refrigeration, presence of fire or water, and the weather/precipitation/air temperature at the accident site [19]. However, this study also indicated

that gene expression was measurable in lower-quality RNA samples, and that gene expression in certain tissues correlated to that observed in corresponding live tissues. Finally, emerging insight into the 'thanatotranscriptome', or how gene expression changes during the postmortem period, represents another factor in forensic postmortem gene expression analyses [20]. In order to have a better understanding of the current state of THC transcriptome and postmortem expression research, this report reviewed and then summarized the available literature for both fields.

2. THC Pharmacology

The pharmacokinetics of THC is well known. The degree, duration, and timing of functional impairment varies widely depending on the route of administration (i.e., inhalation, oral, and sublingual). This is due to variations in the bioavailability of pharmacologically active cannabinoids, as well as the amount of THC in the consumed substance, which can itself have a high degree of variation among cannabis strains, edibles, or extracts. Further, detection of THC, the primary psychoactive compound in cannabis, its active metabolite, 11-hydroxy-delta-9-tetrahydrocannabinol (11-OH-THC), or the secondary inactive metabolite, THC-COOH in a biological specimen does not necessarily indicate impairment [21, 22, 23].

Complicating matters, the pharmacokinetic profiles of THC in blood and tissues vary dramatically over time depending on the route of administration and the presence of cannabidiol (CBD) [24]. In rats, Hložek et al. observed that subcutaneous THC administration resulted in THC serum concentration peaks within two hours, with THC remaining present in the serum between eight and 24 hours. However, in the presence of CBD, THC concentration peaked within approximately one hour but then peaked again at a lower concentration at eight hours, while remaining detectable at 24 hours. THC serum concentrations peaked sharply immediately following pulmonary administration and then decreased rapidly over two hours, irrespective of CBD co-administration, and then tailed off slowly after 24 hours. Following oral administration of THC alone, THC concentration peaked within one hour and tailed off quickly after eight hours, while when co-administered with CBD, THC concentration peaked after two hours and then slowly tailed off over 24 hours [24].

While multiple models exist that attempt to estimate the time of last ingestion of cannabis based on blood plasma THC and metabolite concentrations, no direct correlation currently exists between blood concentration and any *post hoc* determinations of the degree of impairment [25, 26, 27]. Absent any additional information regarding route of ingestion or time of ingestion, toxicological quantification of tissue THC or metabolite concentration can only establish that an individual has consumed cannabis at some point previously or a general state of impairment, but is unable to quantify the specific degree of impairment. Furthermore, cannabinoid blood and plasma concentrations can be significantly higher in chronic users due to tolerance versus acute users and remain detectable for longer periods of time [28].

3. THC-Induced Changes in Gene Expression

Gene expression profiling, i.e., transcriptomic analysis, represents a potential solution to the question of if, when, and for how long an individual is impaired following cannabis consumption in the absence of a significant correlation between cannabinoid concentration and measures of impairment. Technologies including quantitative reverse transcription polymerase chain reaction (RT-qPCR), microarray, and RNA-sequencing (RNA-seq) permit the quantification of the expression of a single transcript present within a tissue sample to the quantification of all transcripts in a given tissue sample or single cell (scRNA-seq) [29, 30, 31, 32, 33]. These technologies are powerful tools by which changes in gene expression in response to stimuli, in this case, cannabis consumption, can be quantified on a gene by gene or transcript by transcript basis. These transcriptomic changes could, in practice, be used as a proxy for estimating degree of impairment, which would be useful in determining the presence of impairment due to cannabis use when investigating the causes of civilian aviation accidents.

3.1 Organismal Development

Pandelides et al. investigated the transcriptomic effects of developmental THC and CBD exposure in larval zebrafish using RNA-seq and RT-qPCR [34]. Embryos were dosed with either THC or CBD, added to the embryo water starting at 6 hours postfertilization through 96 hours. Genes found to be differentially expressed were profiled based on Gene Ontology (GO) (molecular biology) term enrichment and then compared across the treatment groups (THC-treated, CBD-treated, and untreated-control); Kyoto Encyclopedia of Genes and Genomes (KEGG) and Human Phenology (HP) term analysis were also performed.

THC and CBD exposure to embryos resulted in significant differential expression of 904 and 1095 genes, respectively, relative to the control. Between the two data sets, 360 differentially expressed genes (DEGs) were shared in response to both THC and CBD. KEGG analysis of the THC exposure response reflected differential expression of genes involved in drug metabolism, various metabolic pathways, steroid hormone biosynthesis, retinol metabolism, and peroxisome proliferator-activated receptor (PPAR) signaling. Similar pathways were enriched in response to CBD exposure as well, with metabolic pathways, steroid hormone biosynthesis, retinol metabolism, and PPAR signaling again all reflecting involvement of THC and CBD in similar biological pathways.

3.2 Neurons and Neurological Development

Obiorah et al. cultured human excitatory neurons and then exposed them to THC to model *in utero* THC exposure on developing human neurons [35]. *NGN2*-hiPSC (human-induced pluripotent stem cell) and *NGN2*-NPC (neural progenitor cell) neurons were treated with 5 nM THC every 48 hours for seven days while forebrain neurons were treated with either 5 nM THC every 48 hours for seven days, 1 μM THC for 24 hours (to simulate acute exposure), or 50 nM THC every 24 hours for seven days (to simulate chronic THC exposure). Following THC treatment, expression of genes *GRIA1*, *GRIA2*, *GRIN2A*, and *GRIN2B* was reduced significantly

relative to vehicle-treated controls in both *NGN2*-hiPSC and -NPC neurons, while *CNR1* and *COX2* expression was increased significantly.

Following on from Obiorah et al., Guennewig et al. cultured hiPSC-derived neurons and then used them to model transcriptomic neurological effects of acute and chronic THC exposure *in vitro* [36]. NPCs were treated with THC concentrations meant to mimic either acute (1 µM THC for 24 hours) or chronic (50 nM THC from five treatments across seven days) exposure, after which the cells were immediately collected for study via RNA-seq. Following acute exposure, 497 DEGs were identified relative to vehicle-treated controls, while 810 genes showed significant differential expression in the chronic THC-exposed neurons, with 421 DEGs shared between the two sets. Genes involved in the glutamate receptor pathway and mitochondrial function were enriched at both levels of exposure [37]. Additional genes and clusters exhibiting differential expression included those involved in postsynaptic density (*GRID2*, *CAP2*, *GRIK1*, *SIPA1L1*, *HOMER1*) and multiple potassium voltage-gate channel genes (*KCNE4*, *KCNA4*, *KCNJ10*, *KCNN3*), with additional DEGs in these clusters affected following chronic exposure relative to acute. Furthermore, genes involved in critical epigenetic regulatory mechanisms, including dynamic methylation and demethylation (*DNMT1*, *GADD45B*, *APOBEC3C*), were differentially expressed in response to either exposure paradigm, while several histone modification (*SETD1A*, *SETD5*, *CBX6*, *KMT2A*, *KMT2C*, *NCOA6*) and methyl-binding protein (*MECP2*, *MBD5*) genes showed additional repression following chronic exposure. Enrichment and pathway analysis for GO, KEGG, and HUGO Gene Nomenclature Committee database additionally implicated developmental, chromatin regulation, and mitochondrial biology pathways.

Philippot et al. investigated the effect of THC exposure on brain development during the brain growth spurt period, using mice as a proxy for human development [38]. Male pups were injected subcutaneously with vehicle, 10 mg/kg, or 50 mg/kg THC at postnatal day 10 and then euthanized 24 hours post-administration. Expression levels of select genes of interest, including neurotrophic (*Bdnf* and *Trkb*), endocannabinoid (*Cb1r* and *Faah*), synaptic density (*Syp* and *Psd95*), and oxidative stress (*Nrf2* and *Keap1*) gene markers, in the frontal cortex, parietal cortex, and hippocampus were quantitated via RT-qPCR. *Trkb* expression was significantly depressed across all three tissues in the 50 mg/kg THC dosed animals relative to control while *Cb1r* was significantly elevated in the parietal cortex at both the 10 and 50 mg/kg THC doses relative to control. The synaptic density gene markers were unaffected at both doses in all tissues. Finally, *Keap1* expression was significantly decreased in the parietal cortex at both doses, and the *Nrf2*/*Keap1* ratio, an indicator of cellular oxidative stressors, was increased significantly at both doses in the parietal cortex and at the 50 mg/kg dose in hippocampus.

Orihuel et al. reported in a pre-print posting the effects of early adolescent THC exposure on gene expression in the rat nucleus accumbens using RNA-seq [39]. Between postnatal days 28 and 44, male and female animals were administered nine intraperitoneal injections of either THC (3 mg/kg) or vehicle and then euthanized at day 90 for analysis. Ninety-six DEGs were

identified in the THC-males relative to vehicle-treated males; in contrast, 87 DEGs were present in the THC-treated females. Of the DEG totals, only nine genes were shared across both THC-treated males and females. Of these nine genes, *Calb1* and *Slc17a6* were expressed more highly in both THC-treated males and females, *Dus2* and *RGD1310819* showed lower expression in the THC-treated animals, *Ttr*, *Nov*, and *Cck* showed high expression in the THC-treated males and lower expression in the THC-treated females, and *Zfhx3* and *Tenm4* were expressed more highly in the THC-treated females while showing lower expression in the THC-treated males.

The authors additionally used Metascape to determine GO term and pathway enrichment. Terms related to behavioral regulation, learning and memory, locomotory behavior, neuron project morphogenesis, axon development, positive regulation of neurogenesis, and transport of amino acids showed the greatest degree of enrichment in the THC-treated males [39]. A separate behavioral regulation term was enriched in the THC-treated females, in addition to terms related to regulation of neurotransmitter levels, hormone transport and secretion, microtubule reorganization, and extracellular matrix reorganization.

Miller et al. explored the transcriptomic effects of adolescent exposure of rats to THC on layer III pyramidal neurons within the prefrontal cortex using scRNA-seq [40]. Male adolescent rats were administered a total of eight intraperitoneal injections of either THC at 1.5 mg/kg or vehicle beginning at postnatal day 28 and continuing every third day and then euthanized either 24 hours after the last injection at postnatal day 50 ("adolescence") or after two weeks, at postnatal day 63 ("early adulthood"). Relative to control animals, the authors identified 698 DEGs in the layer III prelimbic pyramidal neurons isolated from the THC-treated animals euthanized in adolescence. GO term enrichment from this dataset identified terms related to the cellular response to organonitrogen compounds and the Cul3-RING ubiquitin ligase complex. In contrast, 608 DEGs were identified in the THC-treated animals euthanized during early adulthood relative to control, with terms related to microtubule organization and cytochrome complex assembly most affected.

The authors additionally followed the developmental trajectory of the prelimbic pyramidal transcriptome in the THC-treated animals relative to controls. Between adolescence and early adulthood, 797 DEGs were present in the vehicle-treated animals while 975 DEGs were identified in the THC-treated adult-euthanized animals relative to the THC-treated adolescent-euthanized animals, with only 83 genes shared between the two sets. The control animal dataset was enriched for genes associated with signal transduction, cytoskeletal protein actin projection protrusion, and cell morphogenesis. In contrast, the THC-treated dataset contained DEGs related to actin cytoskeleton, dendritic regulation, as well as epigenetic regulatory mechanisms including chromatin modification and histone methylation. *Dstn*, *Pacsin1*, and *Bap1* were among the most highly differentially expressed genes influenced by THC-exposure during development. Furthermore, the authors identified a strong functional association between the THC-treated DEGs and the chromatin methyltransferase *Kmt2a*. Pathway analysis using MetaCore identified significant enrichment of developmentally regulated

genes associated with organelle organization, cellular component organization, and histone modification.

Leishman et al. performed transcriptomic analysis of the effects of THC consumption in the adolescent and adult female mouse hippocampus using RNA-seq [41]. Postnatal day 35 ("adolescent") and adult mice (approximately four months old) were administered a single injection of 3 mg/kg THC or vehicle control and then euthanized two hours post-injection. Relative to vehicle control, 89 DEGs were identified in the THC-treated adolescent hippocampus, with 48 downregulated DEGs and 41 upregulated. Amongst these DEGs, *Plekhf1* displayed the largest degree of upregulation, while *Kdr* was the most downregulated gene. In contrast, 189 DEGs were identified in the THC-treated adult hippocampus, with 109 upregulated DEGs and 80 downregulated. As in the adolescents, the most upregulated gene was again *Plfekhf1*, however, the fold change was higher in the adult hippocampus (1.79 versus 2.57); the most downregulated gene in adults was *Cldn5*. Thirty-one DEGs were shared across the two time points, with 20 upregulated and 11 downregulated. Similar to *Plfekhf1*, the fold change values were larger for the adult hippocampus relative to the adolescent.

The authors additionally performed pathway analysis using Ingenuity Pathway Analysis. In general, a larger percent of DEGs identified from the THC-treated adult hippocampus were enriched in relation to the adolescent. A subset of diseases, including cancer, gastrointestinal disease, neurological disease, and organismal injury and abnormalities, were associated with DEGs from both age groups. Similarly, pathways involved in physiological system development and various molecular and cellular functions, including cell death and survival, cellular function and maintenance, and cellular growth and proliferation, were identified.

3.3 Immune Response

Hu et al. investigated the acute effects of THC exposure on gene expression in human peripheral mononuclear blood cells (PMBCs) using scRNA-seq [42]. Two healthy individuals with a history of cannabis exposure were administered THC at 0.03 mg/kg intravenously, a dosage known to induce effects consistent with cannabis consumption. PMBCs were isolated pre-THC administration and then again 70 minutes post-administration. Ultimately, 15,973 cells were profiled, representing eight PMBC subtypes, and the expression of 21,430 genes assayed. The authors identified 294 DEGs across the eight cell types, with 69 DEGs observed in at least two cell types and 225 in only one, with an overall trend towards upregulation of gene expression following THC administration. Of the 28 DEGs that were shared across at least three cell types, the authors identified upregulated genes involved in cell death (*BTG1*, *DDIT4*, *GZMB*) and genes that were downregulated that contribute to differentiation and cell growth (*TMBS10*, *RPS21*, *RPL41*). Also common among the 28 shared genes whose expression was altered by THC exposure were genes that play prominent roles in the adaptive immune response (*S100A9*, *S100A8*, *CCL4*, *GNLY*, *IGLC2*, *IGKC*).

DEGs unique to specific cell types were also identified, including genes involved in cytotoxic T-cell activation (*IL7R*), histone modification (*H3F3B*), and transcriptional regulation (*MYC*) in CD4+ T-cells, genes contributing to immune response and inflammation (*IL32*, *SOCs1*, *IRF1*) in CD8+ T-cells, and in B cells, genes involved in B cell maturation (*VPREB3*), MHC function (*HLA-DQA1*, *HLA-DQA2*), calcium signaling (*CALM2*), toll-like receptors (*CD180*), and MAP kinase MAPK1/ERK2-activated environmental stress response (*DUSP1*). In natural killer (NK) cells, genes that play roles in immune response and cell proliferation (*DDIT4*, *CCL4*, *BTG1 ID2*, *CD53*) displayed differential expression in response to THC infusion, and finally, in CD14+ monocytes, genes involved in regulating cell fate (*MCL1*, *FOSB*, *MYADM*) were observed.

For each of these five cell types, the authors additionally performed KEGG-based gene network analysis and GO term enrichment. In CD4+ T cells, KEGG-network analysis enriched GO terms included immune response, cell surface receptor signaling, and cellular response to stimulus. Similar enriched GO terms were identified in CD8+ T cells. MHC protein complex, immune response, and peptide antigen binding were among those pathways enriched in B cells, while in NK cells, enriched pathways included chemokine-mediated signaling, inflammatory response, and chemokine receptor activity. Finally, enriched pathways in CD14+ monocytes included MHC class II protein complex, antigen processing, immune response, and cellular response to interferon-gamma. Thirty-nine significant KEGG pathways were identified in at least two cell types and included immune response, inflammation, and cell survival and apoptosis. Further, autoimmune disease pathways were affected in multiple cell types and the ribosomal pathway was significantly enriched in all five cell types.

Yang et al. generated an active immune response in mice using Staphylococcal enterotoxin B (SEB) and then investigated the effect of THC on gene expression in activated lymphocytes using RNA-seq and RT-qPCR [43]. Female mice received two 20 µg/kg doses of THC or vehicle control, separated by 24 hours, by intraperitoneal injection followed two hours later by injection of 10 µg SEB in each hind footpad. To determine the role of cannabinoid receptor type 2 (CB2) in the immune response, select mice also received an intraperitoneal injection of SR144528, a CB2 antagonist, concomitant with THC administration. Seventy-two hours post-SEB immunization, the authors collected the popliteal draining lymph nodes (LN) from the vehicle and THC-treated mice and then single cells were isolated and pooled according to treatment group, from which the CD4+ T cells were further isolated.

THC administration in mice immunized with SEB significantly reduced the total numbers of draining LN cells isolated relative to vehicle controls. Further, animals that received SR144528 concomitant with THC did not display such immunosuppressive effects, positively implicating CB2 in facilitating the observed THC-induced effects. In analyzing their RNA-seq data, the authors identified 310 and 260 DEGs that were significantly downregulated (greater than 2-fold difference) in the SEB plus THC total LN and CD4+ cells, respectively, relative to the SEB plus vehicle cells, with 37 genes overlapping between the two cell populations. In

contrast, 328 and 333 DEGs were upregulated in the SEB plus THC total LN and CD4+ cells, respectively, relative to the SEB plus vehicle cells, with 56 genes present in both populations. A large proportion of the identified genes displaying differential expression following SEB plus THC treatment encode microRNA (miRNA) precursors and long non-coding RNAs (lncRNAs): 122 and 128 downregulated transcripts, with 17 overlapping, and 111 and 100 upregulated transcripts, with 17 overlapping, in total LN and CD4+ cells, respectively.

RNA-seq identified the expression of miRNA primary transcripts (pri-miRNA) from miRNA clusters miR-17/92 and miR-374b/421 as downregulated following THC-treatment. Another miRNA, miR-146a, along with its precursor, were upregulated in LN cells. The authors further identified that the promoter of cluster miR-374b/421 was enriched for the histone mark H3K27me3, a mark that is associated with transcriptional repression, only in SEB plus THC-treated cells. The promoter of miR-146a was also enriched for the mark H3K9Ac, a mark associated with active promoters, again only in the SEB plus THC cells. The authors speculate that these histone marks may mediate the THC-responsive changes in pri-miRNA expression.

Pathway analysis confirmed that the majority of the known miRNAs were implicated in cell proliferation. Analysis of the differentially expressed protein-coding genes showed that the inflammatory response pathway was most affected. Expression of several lncRNAs was also affected following THC exposure, the function of which is unknown. The authors queried several of the lncRNAs further and determined that they are transcribed from the opposite strand of known protein-coding genes, suggesting that these lncRNAs may negatively affect the transcription of these genes. To confirm that expression of the lncRNAs was induced by THC by means of the CB2 receptor, their expression was compared between cells treated with and without the CB2 antagonist SR144528; expression of the lncRNAs was suppressed in cells treated with SR144528. Finally, the authors also identified approximately 600 transcripts following SEB and THC treatment that used different promoters than those used in SEB and vehicle-treated total LN and CD4+ T cells, with about 300 of these transcripts shared in both cell types. Additionally, approximately 2500 transcripts showed alternative splicing, with about half of the transcripts shared between the LN and CD4+ T cells. While many pathways incorporated these alternatively spliced transcripts or those that employed different promoters, cell death and survival was the top network identified.

3.4 Non-Specific

He et al. compared genome-wide gene expression in whole blood using microarray between heavy cannabis users (N=90) and individuals with no history of cannabis consumption (N=100) [37]. The authors identified two significant DE transcripts following false discovery rate correction (< 0.05): Protein Tyrosine Phosphatase Receptor Type F Polypeptide-Interacting Protein Alpha-2 (*PPFIA2*) was upregulated in users when compared to non-users, while C-X3-C Motif Chemokine Receptor 1 (*CX3CR1*) was seen to be downregulated in heavy cannabis users. Validation with RT-qPCR confirmed the upregulation of one *PPFIA2* transcript but not the downregulation of *CX3CR1*. Additionally, changes in *PPFIA2* expression *in vitro* were assayed

in primary monocytes and the neuroblastoma cell line SH-SY5Y at 6 hours post-THC or CBD exposure (10 μM) by RT-qPCR. Expression in the primary myocytes was variable, with only two-thirds of the lines showing non-significant increases in *PPFIA2* expression. Similar non-significant *PPFIA2* upregulation was observed in the SH-SY5Y lines.

4. The Thanatotranscriptome

A long-standing concern in the use of RNA in forensic genetics is the inherent instability associated with RNA as a molecule [44]. Multiple studies over the past twenty years have shown that both postmortem RNA stability and integrity are intrinsically dependent on tissue of origin, the genes being assayed, genotype, the length of the postmortem interval (PMI), environmental conditions at the site of death, as well as individual biological parameters (such as age, sex, and body mass index for specific tissues) [19, 45, 46, 47, 48, 49, 50, 51]. With these factors in mind, the study of the thanatotranscriptome (coined by Javan et al. [52]), or RNA expression following organismal death, has emerged as a field of interest in the last seven years. Both the variability in RNA integrity and stability and the discovery that the cellular transcriptional machinery remains active for some time postmortem, have the potential to complicate the assay of gene expression biomarkers related to cannabis consumption in the uncontrolled conditions of aviation accidents.

4.1 Whole Blood

Antiga et al. collected whole blood samples from seven human donors over a PMI of two hours 21 minutes to 37 hours 50 minutes at room temperature, which were then separated into one of six groups based on the PMI at collection [53]. Following statistical (ANOVA-Dunnett and linear regression) and power analysis of the significant transcripts following RNA-seq, the authors identified 99 upregulated and 89 downregulated transcripts in a comparison between the first and earliest group and the sixth and latest group. The most enriched GO terms among the upregulated genes were the establishment of mitochondrion localization, positive regulation of insulin receptor signaling, and nucleotide excision repair, while the most enriched terms within the downregulated genes were death-inducing signaling complex assembly, positive regulation of macrophage differentiation, toll-like receptor 3 signaling, and the regulation of necrotic/necroptotic cell death.

4.2 Prostate

Tolbert et al. assayed the postmortem expression of apoptosis-related genes and pathways in human prostate tissue between PMIs of 24 and 120 hours using a PCR array [54]. Postmortem prostate mRNA remained detectable and quantifiable even at 120 hours, and a number of differentially expressed anti-apoptotic, pro-apoptotic, and negative regulators of apoptosis genes were identified. The anti-apoptotic gene *BCL2* was significantly upregulated at all PMIs (38, 77, 96, and 120 hours) relative to the 24-hour control, with additional Bcl-2 family genes showing significant upregulation at 96 and 120 hours. Comparatively, the pro-apoptotic gene *CASP2* and additional caspase family genes were significantly upregulated at 96 and 120 hours relative to control along with several caspase recruitment domain family genes. Inhibitor of apoptosis (IAP)

motif-containing protein-coding genes, including *XIAP* and *BIRC3*, were additionally upregulated at 96 and 120 hours relative to control.

4.3 Liver

Javan et al. similarly assayed postmortem expression of apoptosis-related genes and pathways in human liver tissue at PMIs between six and 48 hours using PCR arrays [55]. Expression of genes involved in apoptosis induction, anti-apoptosis, apoptosis regulation, death domain proteins, and caspases and regulators were quantified; furthermore, RNA remained stable at 48 hours postmortem. Multiple anti-apoptotic, negative regulators of apoptosis, and death domain protein coding genes were significantly downregulated at 16 and 48 hours postmortem relative to PMI six hour control, including *BCL2*, *BAX*, and *BIRC5* as well as several pro-apoptotic genes including *CASP2* and *CASP8*, while the IAP-containing anti-apoptotic gene *XIAP* was significantly overexpressed. Other overexpressed genes and gene families included several other pro-apoptotic caspase family members (*CASP3*, *CASP4*, and *CASP9*) as well as the anti-apoptotic *BIRC3*.

Halawa et al. investigated the effect of postmortem heat stress on the expression of select genes involved in inflammation (*Il1b* and *Tnf*), apoptosis (*Bcl2* and *Casp3*), or neuronal activation and stress (*c-fos*) in rat liver by RT-qPCR [56]. Animals were left at room temperature or exposed to heat conditions of 41°C for one, three, and six hours postmortem prior to tissue collection. Expression of *Tnf* and *Il1b* were both reduced significantly relative to PMI 0 hour controls in both room temperature and heat-stressed samples. *Bcl2* expression was significantly reduced at both temperatures at PMI of 1 hour and in heat-stressed samples at six hours; however, expression increased significantly after three and six hours at room temperature. *Casp3* expression was significantly elevated at one hour in heat stressed tissue and at both temperatures at three and six hours. Finally, expression of *c-fos* increased significantly after three and six hours in both room temperature and heat stressed samples, with no significant difference observed after one hour relative to the control.

4.4 Brain

Expanding on their previous efforts, Halawa et al. investigated the effect of postmortem heat stress on the expression of the same panel of genes in rat brains by RT-qPCR [57]. Under the same conditions as their earlier study, the authors saw that *Tnf* expression was elevated significantly in the heat-stressed samples relative to PMI 0-hour controls after PMIs of one and six hours while *Il1b* was significantly upregulated at three and six hours; no significant difference for either gene was observed between room temperature samples and controls. In contrast, expression of the apoptotic genes was somewhat more variable: *Bcl2* expression was significantly reduced in room temperature samples at PMIs of one and three hours but significantly increased in the heat-stressed samples at six hours while *Casp3* was significantly reduced at all PMIs at both temperatures. Expression of *c-fos* was significantly increased at all

intervals in the heat-stressed animals relative to control, with no significant change observed in the room-temperature animals.

Bonadio et al. subjected mouse corpses to multiple simulated microenvironments (exposed, buried, and submerged) for a PMI of 192 hours to determine the extent of RNA degradation over time in the microenvironments and then quantified expression in the brain by microarray after 48 hours (48 hours being the highest PMI that still retained RNA of sufficient quality for microarray analysis) to better understand the effect different environmental conditions may have on the postmortem transcriptome [58]. As part of their analysis, the authors observed that both RNA concentration and RNA integrity decreased consistently over a PMI of 192 hours in all three simulated microenvironments, with no statistical difference in rate between the three. Across all three conditions, a total of 658 upregulated and 1099 downregulated DEGs were identified relative to controls collected immediately postmortem, with 111 upregulated and 497 downregulated DEGs present in all groups. The majority of the commonly upregulated DEGs were non-coding RNAs while genes contributing to metabolism regulation were amongst the shared downregulated DEGs.

5. Conclusions

Cannabis and cannabinoid-containing products are becoming increasingly prevalent in the US as medical and recreational access in many states has increased, beginning with the legalization of medical cannabis in California in 1996. In the National Survey Results on Drug Use (1975-2020), amongst male and female respondents aged 19-30 in 2020, 63.9% of respondents reported using cannabis in their lifetime, with 42.0% using annually, 26.8% within the past 30 days, and 9.8% on a daily basis [59]. Between 1996 and 2020, annual, 30-day, and daily use prevalence has either remained constant or steadily increased amongst all respondents, aged 18 to 60, with most trends at their highest reported rate for a given age group between 2018 and 2020 [59].

The increasing prevalence of cannabis use thus has important implications for the FAA and the active civil aviation population; namely, no methodology currently exists to definitively correlate blood or tissue THC concentration with degree of functional impairment. Absent such a correlation, assaying for THC-induced gene expression biomarkers that correlate with objective measures of ante-mortem neurocognitive impairment may be useful as a proxy measure for determining impairment *post hoc*, with hundreds or thousands of genes showing differential expression across multiple tissue types following THC or cannabinoid exposure. Further, allowance must be made in any gene expression-based assay to account for thanatotranscriptome-related changes in gene abundance in any sample, requiring knowledge of time elapsed from accident to sample collection, stabilization, or extraction.

In order to truly correlate cannabis-induced gene expression with impairment, volunteer subject studies will need to be performed to measure the relative impairment of cannabis-exposed and objectively impaired individuals compared with cannabis-exposed but objectively

not impaired individuals (such as acute versus chronic users), while assessing global gene expression from one or several sample sources from each subject. From such studies, gene expression profiles that correlate with THC-induced impairment may be determined, thereby providing a basis to objectively assess impairment using specific gene expression panels. Such panels could then, after validation, be used to screen postmortem samples for evidence of impairment due to cannabis consumption. Such a panel might also find applications in determining the presence of impairment in living individuals, providing a more direct and objective measure of impairment than THC presence or behavioral observation alone. In summary, more work remains to better identify THC-induced and repressed genes, to what extent they may interact or overlap with postmortem differentially expressed genes, and how they correlate with cognitive and psychomotor performance.

6. References

1. National Institute on Drug Abuse. (2019, December 24). *Cannabis (Marijuana) DrugFacts*. https://www.drugabuse.gov/publications/drugfacts/marijuana
2. Gabay M. (2013). The federal controlled substances act: schedules and pharmacy registration. *Hospital Pharmacy*, *48*(6), 473–474. https://doi.org/10.1310/hpj4806-473
3. National Conference of State Legislatures. (2021, November 29). *State Medical Marijuana Laws*. https://www.ncsl.org/research/health/state-medical-marijuana-laws.aspx
4. Yu, B., Chen, X., Chen, X., & Yan, H. (2020). Marijuana legalization and historical trends in marijuana use among US residents aged 12-25: results from the 1979-2016 National Survey on drug use and health. *BMC Public Health*, *20*(1), 156. https://doi.org/10.1186/s12889-020-8253-4
5. Schauer, G. L., Njai, R., & Grant-Lenzy, A. M. (2020). Modes of marijuana use - smoking, vaping, eating, and dabbing: Results from the 2016 BRFSS in 12 States. *Drug and Alcohol Dependence*, *209*, 107900. https://doi.org/10.1016/j.drugalcdep.2020.107900
6. Svrakic, D. M., Lustman, P. J., Mallya, A., Lynn, T. A., Finney, R., & Svrakic, N. M. (2012). Legalization, decriminalization & medicinal use of cannabis: a scientific and public health perspective. *Missouri Medicine*, *109*(2), 90–98.
7. O'Grady, C. (2020). Cannabis research database shows how U.S. funding focuses on harms of the drug. *Science*. https://www.science.org/content/article/cannabis-research-database-shows-how-us-funding-focuses-harms-drug
8. Federal Aviation Administration. (2018). FAA Strategic Plan FY 2019-2022. U.S. Department of Transportation.
9. Drug Abatement Division. (2021). FAA's Random Drug and Alcohol Testing Program. Federal Aviation Administration.
10. Swart, J. L. (2009). DOT "Medical Marijuana" Notice. U.S. Department of Transportation.
11. Swart, J. L. (2012). DOT "Recreational Marijuana" Notice. U.S. Department of Transportation.
12. Federal Aviation Administration. (2016). *I tested positive for marijuana on a DOT/FAA drug test, but recreational use of marijuana is legal in my state. Will my test be cancelled?* Q&As for Safety-Sensitive Employees. https://www.faa.gov/about/office_org/headquarters_offices/avs/offices/aam/drug_alcohol/policy/qa_sse/a3/
13. Federal Aviation Administration. (2021). *Pharmaceuticals (Therapeutic Medications).* Guide for Aviation Medical Examiners. https://www.faa.gov/about/office_org/headquarters_offices/avs/offices/aam/ame/guide/pharm/
14. Federal Aviation Administration. (2013). *What is a medical certificate?* Pilot Medical Certification Questions and Answers. https://www.faa.gov/licenses_certificates/medical_certification/faq/response1/
15. Norris, A., Cliburn, K., Kemp, P., & Skaggs V. (2018). *Assessing Trends in Cannabinoid Concentrations Found in Specimens from Aviation Fatalities between 2007 and 2016.* (Report No. DOT/FAA/AM-18/2). U.S. Department of Transportation.
16. Drummer, O. H., Gerostamoulos, J., Batziris, H., Chu, M., Caplehorn, J., Robertson, M. D., & Swann, P. (2004). The involvement of drugs in drivers of motor vehicles killed in

Australian road traffic crashes. *Accident, Analysis, and Prevention*, *36*(2), 239–248. https://doi.org/10.1016/s0001-4575(02)00153-7

17. Bergamaschi, M. M., Karschner, E. L., Goodwin, R. S., Scheidweiler, K. B., Hirvonen, J., Queiroz, R. H., & Huestis, M. A. (2013). Impact of prolonged cannabinoid excretion in chronic daily cannabis smokers' blood on per se drugged driving laws. *Clinical Chemistry*, *59*(3), 519–526. https://doi.org/10.1373/clinchem.2012.195503
18. Li, Y., & Breaker, R. R. (1999). Kinetics of RNA degradation by specific base catalysis of transesterification involving the 2 ‘-hydroxyl group. *Journal of the American Chemical Society*, *121*(23), 5364-5372. https://doi.org/10.1021/ja990592p
19. Burian, D., Hutchings, D., Uyhelji, H., McCauley, A., Williams, D., Kupfer, D., White, V. L., White, C., Jung, R., & Smith, S. W. (2017). *Postmortem Samples from Aviation Accident Victims Maintain Tissue-Specific mRNA Expression Profiles*. (Report No. DOT/FAA/AM-17/16). US Department of Transportation.
20. Pozhitkov, A. E., Neme, R., Domazet-Lošo, T., Leroux, B. G., Soni, S., Tautz, D., & Noble, P. A. (2017). Tracing the dynamics of gene transcripts after organismal death. *Open Biology*, *7*(1), 160267. https://doi.org/10.1098/rsob.160267
21. Banta-Green, C. & Williams, J. (2016). *Overview of Major Issues Regarding the Impacts of Alcohol and Marijuana on Driving (Research Brief)*. AAA Foundation for Traffic Safety. https://aaafoundation.org/overview-major-issues-regarding-impacts-alcohol-marijuana-driving/
22. Drug Policy Alliance. (2021). *How Marijuana is Consumed*. https://drugpolicy.org/drug-facts/10-facts-about-marijuana/how-marijuana-consumed
23. Cash, M. C., Cunnane, K., Fan, C., & Romero-Sandoval, E. A. (2020). Mapping cannabis potency in medical and recreational programs in the United States. *PloS One*, *15*(3), e0230167. https://doi.org/10.1371/journal.pone.0230167
24. Hložek, T., Uttl, L., Kadeřábek, L., Balíková, M., Lhotková, E., Horsley, R. R., Nováková, P., Šíchová, K., Štefková, K., Tylš, F., Kuchař, M., & Páleníček, T. (2017). Pharmacokinetic and behavioural profile of THC, CBD, and THC+CBD combination after pulmonary, oral, and subcutaneous administration in rats and confirmation of conversion in vivo of CBD to THC. *European Neuropsychopharmacology : The Journal of the European College of Neuropsychopharmacology, 27*(12), 1223–1237. https://doi.org/10.1016/j.euroneuro.2017.10.037
25. Huestis, M. A., Barnes, A., & Smith, M. L. (2005). Estimating the time of last cannabis use from plasma delta9-tetrahydrocannabinol and 11-nor-9-carboxy-delta9-tetrahydrocannabinol concentrations. *Clinical Chemistry*, *51*(12), 2289–2295. https://doi.org/10.1373/clinchem.2005.056838
26. Huestis, M. A., Elsohly, M., Nebro, W., Barnes, A., Gustafson, R. A., & Smith, M. L. (2006). Estimating time of last oral ingestion of cannabis from plasma THC and THCCOOH concentrations. *Therapeutic Drug Monitoring*, *28*(4), 540–544. https://doi.org/10.1097/00007691-200608000-00009
27. Kunsman, G. W. (2013). Human Performance Toxicology. In B. Levine, Ed.), *Principles of Forensic Toxicology*. AACC Press.
28. Desrosiers, N. A., Himes, S. K., Scheidweiler, K. B., Concheiro-Guisan, M., Gorelick, D. A., & Huestis, M. A. (2014). Phase I and II cannabinoid disposition in blood and plasma of occasional and frequent smokers following controlled smoked cannabis. *Clinical Chemistry*, *60*(4), 631–643. https://doi.org/10.1373/clinchem.2013.216507

29. Bustin S. A. (2000). Absolute quantification of mRNA using real-time reverse transcription polymerase chain reaction assays. *Journal of Molecular Endocrinology*, *25*(2), 169–193. https://doi.org/10.1677/jme.0.0250169
30. Simpson, D. A., Feeney, S., Boyle, C., & Stitt, A. W. (2000). Retinal VEGF mRNA measured by SYBR green I fluorescence: A versatile approach to quantitative PCR. *Molecular Vision*, *6*, 178–183.
31. Bainbridge, M. N., Warren, R. L., Hirst, M., Romanuik, T., Zeng, T., Go, A., Delaney, A., Griffith, M., Hickenbotham, M., Magrini, V., Mardis, E. R., Sadar, M. D., Siddiqui, A. S., Marra, M. A., & Jones, S. J. (2006). Analysis of the prostate cancer cell line LNCaP transcriptome using a sequencing-by-synthesis approach. *BMC Genomics*, *7*, 246. https://doi.org/10.1186/1471-2164-7-246
32. Tang, F., Barbacioru, C., Wang, Y., Nordman, E., Lee, C., Xu, N., Wang, X., Bodeau, J., Tuch, B. B., Siddiqui, A., Lao, K., & Surani, M. A. (2009). mRNA-Seq whole-transcriptome analysis of a single cell. *Nature Methods*, *6*(5), 377–382. https://doi.org/10.1038/nmeth.1315
33. McGettigan P. A. (2013). Transcriptomics in the RNA-seq era. *Current Opinion in Chemical Biology*, *17*(1), 4–11. https://doi.org/10.1016/j.cbpa.2012.12.008
34. Pandelides, Z., Aluru, N., Thornton, C., Watts, H. E., & Willett, K. L. (2021). Transcriptomic Changes and the Roles of Cannabinoid Receptors and PPARγ in Developmental Toxicities Following Exposure to Δ9-Tetrahydrocannabinol and Cannabidiol. *Toxicological Sciences: An Official Journal of the* Society of Toxicology, *182*(1), 44–59. https://doi.org/10.1093/toxsci/kfab046
35. Obiorah, I. V., Muhammad, H., Stafford, K., Flaherty, E. K., & Brennand, K. J. (2017). THC Treatment Alters Glutamate Receptor Gene Expression in Human Stem Cell-Derived Neurons. *Molecular Neuropsychiatry*, 3(2), 73–84. https://doi.org/10.1159/000477762
36. Guennewig, B., Bitar, M., Obiorah, I., Hanks, J., O'Brien, E. A., Kaczorowski, D. C., Hurd, Y. L., Roussos, P., Brennand, K. J., & Barry, G. (2018). THC exposure of human iPSC neurons impacts genes associated with neuropsychiatric disorders. *Translational Psychiatry*, *8*(1), 89. https://doi.org/10.1038/s41398-018-0137-3
37. He, Y., de Witte, L. D., Schubart, C. D., Van Gastel, W. A., Koeleman, B. P. C., de Jong, S., Ophoff, R. A., Hol, E. M., & Boks, M. P. (2019). Liprin alfa 2 gene expression is increased by cannabis use and associated with neuropsychological function. *European Neuropsychopharmacology: The Journal of the European College of Neuropsychopharmacology*, *29*(5), 643–652. https://doi.org/10.1016/j.euroneuro.2019.03.004
38. Philippot, G., Forsberg, E., Tahan, C., Viberg, H., & Fredriksson, R. (2019). A Single δ9-Tetrahydrocannabinol (THC) Dose During Brain Development Affects Markers of Neurotrophy, Oxidative Stress, and Apoptosis. *Frontiers in Pharmacology*, *10*, 1156. https://doi.org/10.3389/fphar.2019.01156
39. Orihuel, J., Capellán, R., Roura-Martínez, D., Ucha, M., Gómez-Rubio, L., Valverde, C., Casquero-Veiga, M., Soto-Montenegro, M. L., Desco, M., Oteo Vives, M., Ibáñez Moragues, M., Magro Calvo, N., Morcillo, M. Á., Ambrosio, E., & Higuera-Matas, A. (2020). *bioRxiv* 2020.10.19.345322; doi: https://doi.org/10.1101/2020.10.19.345322
40. Miller, M. L., Chadwick, B., Dickstein, D. L., Purushothaman, I., Egervari, G., Rahman, T., Tessereau, C., Hof, P. R., Roussos, P., Shen, L., Baxter, M. G., & Hurd, Y. L. (2019). Adolescent exposure to Δ9-tetrahydrocannabinol alters the transcriptional trajectory and dendritic architecture of prefrontal pyramidal neurons. *Molecular Psychiatry*, 24(4), 588-600. https://doi.org/10.1038/s41380-018-0243-x

41. Leishman, E., Murphy, M., Mackie, K., & Bradshaw, H. B. (2018). Δ(9)-Tetrahydrocannabinol changes the brain lipidome and transcriptome differentially in the adolescent and the adult. *Biochimica et Biophysica Acta. Molecular and Cell Biology of Lipids*, *1863*(5), 479-492. https://doi.org/10.1016/j.bbalip.2018.02.001
42. Hu, Y., Ranganathan, M., Shu, C., Liang, X., Ganesh, S., Osafo-Addo, A., Yan, C., Zhang, X., Aouizerat, B. E., Krystal, J. H., D'Souza, D. C., & Xu, K. (2020). Single-cell transcriptome mapping identifies common and cell-type specific genes affected by acute delta9-tetrahydrocannabinol in humans. *Scientific Reports*, *10*(1), 3450. https://doi.org/10.1038/s41598-020-59827-1
43. Yang, X., Bam, M., Nagarkatti, P. S., & Nagarkatti, M. (2016). RNA-seq analysis of δ9-tetrahydrocannabinol-treated T cells reveals altered gene expression profiles that regulate immune response and cell proliferation. *Journal of Biological Chemistry*, *291*(30), 15460-15472. https://doi.org/10.1038/s41598-020-59827-1
44. Vennemann, M., & Koppelkamm, A. (2010). mRNA profiling in forensic genetics I: Possibilities and limitations. *Forensic Science International*. *203*, 1-3, 71-75. https://doi.org/10.1016/j.forsciint.2010.07.006
45. Zhu, Y., Wang, L., Yin, Y., & Yang, E. (2017). Systematic analysis of gene expression patterns associated with postmortem interval in human tissues. *Scientific Reports*, *7*(1), 5435. https://doi.org/10.1038/s41598-017-05882-0
46. Heinrich, M., Matt, K., Lutz-Bonengel, S., & Schmidt, U. (2007). Successful RNA extraction from various human postmortem tissues. *International Journal of Legal Medicine*, *121*(2), 136-142. https://doi.org/10.1007/s00414-006-0131-9
47. Inoue, H., Kimura, A., & Tuji, T. (2002). Degradation profile of mRNA in a dead rat body: basic semi-quantification study. *Forensic Science International*, *130*(2), 127-132. https://doi.org/10.1016/s0379-0738(02)00352-3
48. Bahar, B., Monahan, F. J., Moloney, A. P., Schmidt, O., MacHugh, D. E., & Sweeney, T. (2007). Long-term stability of RNA in postmortem bovine skeletal muscle, liver and subcutaneous adipose tissues. *BMC Molecular Biology*, *8*(1), 108. https://doi.org/10.1186/1471-2199-8-108
49. Koppelkamm, A., Vennemann, B., Fracasso, T., Lutz-Bonengel, S., Schmidt, U., & Heinrich, M. (2010). Validation of adequate endogenous reference genes for the normalization of qPCR gene expression data in human post mortem tissue. International *Journal of Legal Medicine*, *124*(5), 371-380. https://doi.org/10.1007/s00414-010-0433-9
50. van der Linden, A., Blokker, B. M., Kap, M., Weustink, A. C., Riegman, P. H. J., & Oosterhuis, J. W. (2014). Post-Mortem Tissue Biopsies Obtained at Minimally Invasive Autopsy: An RNA-Quality Analysis. *PLOS One, 9*(12), e115675. https://doi.org/10.1371/journal.pone.0115675
51. Scrivano, S., Sanavio, M., Tozzo, P., & Caenazzo, L. (2019). Analysis of RNA in the estimation of postmortem interval: a review of current evidence. *International Journal of Legal Medicine*, *133*(6), 1629-1640. https://doi.org/10.1007/s00414-019-02125-x
52. Javan, G. T., Kwon, I., Finley, S. J., & Lee, Y. (2016). Progression of thanatophagy in cadaver brain and heart tissues. *Biochemistry and Biophysics Reports*, *5*, 152-159. https://doi.org/10.1016/j.bbrep.2015.11.013
53. Antiga, L. G., Sibbens, L., Abakkouy, Y., Decorte, R., Van Den Bogaert, W., Van de Voorde, W., & Bekaert, B. (2021). Cell survival and DNA damage repair are promoted in the

human blood thanatotranscriptome shortly after death. *Scientific Reports*, *11*(1), 16585. https://doi.org/10.1038/s41598-021-96095-z

54. Tolbert, M., Finley, S. J., Visonà, S. D., Soni, S., Osculati, A., & Javan, G. T. (2018). The thanatotranscriptome: Gene expression of male reproductive organs after death. *Gene*, *675*, 191-196. https://doi.org/10.1016/j.gene.2018.06.090
55. Javan, G. T., Can, I., Finley, S. J., & Soni, S. (2015). The apoptotic thanatotranscriptome associated with the liver of cadavers. *Forensic Science, Medicine, and Pathology*, *11*(4), 509-516. https://doi.org/10.1007/s12024-015-9704-6
56. Halawa, A. A., El-Adl, M. A., & Marghani, B. H. (2018). Thanatotranscriptome study on particular hepatic genes and their correlation with postmortem interval in the presence or absence of postmortem heat stress. *Alexandria Journal of Veterinary Sciences*, *57*(2), 13-20. https://doi.org/10.5455/ajvs.297063
57. Halawa, A. A., El-Adl, M. A., & Marghani, B. H. (2019). Postmortem heat stress upregulates thanatotranscriptome of genes encode inflammation, apoptosis and neuronal stress in brain of rats at short postmortem intervals. *Australian Journal of Forensic Sciences*, 53(3), 271-282. https://doi.org/10.1080/00450618.2019.1682669
58. Bonadio, R. S., Nunes, L. B., Moretti, P. N. S., Mazzeu, J. F., Cagnin, S., Pic-Taylor, A., & de Oliveira, S. F. (2021). Insights into how environment shapes postmortem RNA transcription in mouse brain. *Scientific Reports*, *11*(1), 13008. https://doi.org/10.1038/s41598-021-92268-y
59. Johnston, L. D., Miech, R. A., O'Malley, P. M., Bachman, J. G., Schulenberg, J. E., & Patrick, M. E. (2021). *Monitoring the Future National Survey Results on Drug Use, 1975-2020: Overview, Key Findings on Adolescent Drug Use*. Monitoring the Future Monograph Series. University of Michigan Institute for Social Research: Ann Arbor, MI.