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Low dose deltamethrin exposure affects gene expression in rat frontal cortex

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Abstract

Pyrethroids are a class of commonly used synthetic insecticides, widely used in agricultural and residential settings due to their efficacy and relatively low environmental impact. Nonetheless, epidemiological studies have found that exposure to pyrethroids during developmental stages is linked to risk for neurodevelopmental disorders. However, the molecular mechanisms behind these neurotoxic effects remain unclear. Our study investigates the impact of oral exposure to deltamethrin, a widely used Type II pyrethroid pesticide, on gene expression in the frontal cortex of rats. We used differential gene expression data from frontal cortex dissections from male Long-Evans rats exposed to a 3 mg/kg oral dose of deltamethrin (or vehicle) to perform a 3Pod analysis in R Studio, which included GSEA, Enrichr, and iLINCS analyses. We found that rats who were exposed to deltamethrin had significant changes in gene expression in cortex in pathways related to inflammation, apoptosis, cellular energy metabolism, and synapses. Our study provides important insight on the effects of pesticide exposure on the brain and possible treatments and preventions. This study also emphasizes the need for further research on pyrethroid pesticides and their relationship to neurodevelopmental disorders.

Keywords: pyrethroids, pesticides, exposure science, neurotoxicology

1. Introduction

1.1. Pyrethroid Pesticides. Pyrethroid pesticides are synthetic chemicals widely used in agricultural, public, and residential settings, because of their low toxicity and rapid environmental degradation (1). These compounds are also found in household insecticides, pet sprays, shampoos, lice treatments, mosquito repellents, and scabies treatments. They have consistently held a 15%

share of the global market over several decades (2), with an average annual production of 7,000 tons worldwide. Pyrethroids are present in various environmental compartments, including crops, surface water, soil, and air (3, 4). Recent epidemiological data show that the primary metabolite of several pyrethroids (including deltamethrin), 3-phenoxybenzoic acid, is detected in urine at a rate of 78.1% among adults in the USA (5). Deltamethrin is one kind of Type II pyrethroid pesticide extensively used in agriculture, domestic settings, public spaces, and medical applications (6). Deltamethrin is also effective against mosquitoes (7), is recommended by the World Health Organization for this purpose (8) and is widely used for mosquito control in areas where mosquito-borne illnesses are a public health concern. Its presence is common worldwide, significantly boosting crop yields (9). Deltamethrin's impact on the world is substantially large, from increasing public health to supporting the agricultural economy.

1.2. Deltamethrin Risks. Despite its countless benefits, deltamethrin has also raised concerns due to its increasing usage and potential implications for neurodevelopmental disorders (10). Multiple recent epidemiology studies have shown that exposure to pyrethroid pesticides during pregnancy is a risk factor for autism and other neurodevelopmental disorders (11, 12). This finding is supported by laboratory research, including studies in which rats given 3 mg/kg of deltamethrin orally had behavioral and neurological changes relevant to neurodevelopmental disorders, including autism and ADHD (13-16). These studies point to the critical need for additional research into the molecular effects of deltamethrin exposure on the brain.

1.3. Toxicology. In this study, we will examine the effects of an orally administered dose (3 mg/kg) of deltamethrin on gene expression in the frontal cortex of rats, using data generated in an earlier study (17) and accessed through the Gene Expression Omnibus (GEO) database (18). The study used an oral route of exposure, modeling the most common way humans are chronically exposed to deltamethrin and other similar pyrethroids (19). The relevance of the 3 mg/kg dose of deltamethrin lies in its relation to preexisting and well-established benchmarks. The EPA-set benchmark dose (lower confidence limit) for oral exposure to deltamethrin used for regulatory guidance is 10.1 mg/kg, well above the dose used in this study. The "no observable adverse effect limit" (NOAEL) for oral exposure to deltamethrin relative to acute effects on mobility, as identified in some studies (20), is 1.0 mg/kg, just below the 3 mg/kg dose. These two boundaries make the study's 3 mg/kg dose within an area of uncertainty that has a potential for adverse effects but is still well below than the benchmark dose.

1.4. Hypothesis. To further investigate the effect of deltamethrin on brain function, we examined the effects of acute deltamethrin exposure on gene expression profiles from male rat frontal cortex using a previously published dataset. We used innovative bioinformatics approaches to assess gene pathway and network level changes to compare the gene expression in the frontal cortex of rats acutely exposed to deltamethrin versus vehicle controls. We hypothesize that there is a difference in the gene expression in the frontal cortex of deltamethrin exposed rats versus the control rats.

2. Materials and Methods

2.1. Data access. The workflow for this project is outlined in Figure 1. We accessed a dataset from the NCBI GEO database (GSE7955) containing data from a study that investigated the impact of the pyrethroid pesticide deltamethrin on gene transcription in rat frontal cortex (17). The data was accessed on June 24, 2024. The gene count data was downloaded from the GEO website using GEOquery (v 2.66.0) (21). All samples except for

the control and deltamethrin-treated (3 mg/kg dose) groups were excluded from the analysis.

2.2. Animal subjects. All animal subjects were treated, the subsequent biological samples obtained, and microarray transcriptomics performed, as described in the referenced publication (17). Briefly, the researchers in the study exposed male Long-Evans rats to deltamethrin (3 mg/kg via oral gavage, N=8) or vehicle (N=12), euthanized the rats at a 6-hour time point, and dissected frontal cortex samples for use in transcriptomics.

2.3. Differential Gene Expression. Differential gene expression analysis was performed on the gene count data using the limma (v3.54.0) R package (22). As in the source study (17), no covariates were used.

2.4. 3Pod Report. The output of the differential gene expression analysis was used as the input for the 3Pod R script (version 3400184) (23). The 3Pod report was generated on June 25, 2024 (Supplemental File 1). The 3Pod script produces a 3Pod Report that includes the Gene Set Enrichment Analysis (GSEA) (24), Enrichr (25, 26), Library of Integrated Network-based Cellular Signatures (LINCS), and Pathway Analysis Visualization with Embedding Representations (PAVER) (27) analyses listed below. All figures and tables were derived from the 3Pod Report.

2.4.1. GSEA. We analyzed the differential gene expressions using Gene Set Enrichment Analysis (GSEA) (24). GSEA compares a list of genes to gene sets in a compatible database and generates normalized enrichment scores (NES), with positive NES corresponding to "upregulated" gene sets and negative NES corresponding to "downregulated" gene sets. 3Pod used the output of differential gene expression analysis as input to the GSEA and compared this input to the most recent version of the "Enrichment Map Gene Sets" combined database for rat (28) at the time of report generation. The subsequently identified gene sets were placed on the "Upregulated" list (positive NES) or "Downregulated" list (negative NES) and sorted by raw p-value.

2.4.2. Enrichr. Enrichr is a web application that compares a list of genes to a database of gene set libraries and generates Combined Scores (CS) identifying gene sets that are significantly enriched for the listed genes (25, 26). 3Pod used the top 90% and bottom 10% of differentially expressed genes by fold change as input into EnrichR and restricted EnrichR to the Gene Ontology libraries. The subsequently identified gene sets were placed on the "Upregulated" or "Downregulated" list and sorted by raw p-value.

2.4.3. iLINCS. The Integrative Library of Integrated Network-based Cellular Signatures (iLINCS) is a web platform that allows us to compare the differential gene expression signature of a dataset with a large LINCS database of transcriptomic signatures and to generate a similarity score (29). 3Pod used the output of differential gene expression analysis as input into iLINCS and restricted iLINCS to the "LINCS chemical perturbagen signatures" database to produce "concordant" perturbagens with similar transcriptomic profiles and "discordant" perturbagens with dissimilar transcriptomic profiles. iLINCS was then used to perform "Mechanism of Action" analysis to identify molecular mechanisms of action shared by concordant and discordant perturbagens, and "Known Targets" analysis to identify known gene targets of the concordant and discordant perturbagens.

2.4.4. PAVER Analysis. The results from GSEA, EnrichR, and iLINCS analyses were separately clustered using Pathway Analysis Visualization with Embedding Representations (PAVER) (27). PAVER organizes the gene sets into hierarchical clusters and assigns each cluster a name using pathway embeddings. 3Pod separately used the gene set lists from GSEA, EnrichR and iLINCS as

input to PAVER, which produced separate cluster plots and heatmap plots for each.

2.4.5. Venn diagram. 3Pod combined the results from GSEA and EnrichR to produce a list of gene sets common to both analyses. First, the GSEA and EnrichR lists of altered gene sets were each reduced to gene sets present in both databases. Then, the two lists were compared, and a Venn diagram was generated showing the total number of commonly altered gene sets. Finally, two tables were generated showing the subset of these commonly altered gene sets that were either upregulated on both lists ("Shared Upregulated") or downregulated on both lists ("Shared Downregulated").

3. Results

3.1. Differential Gene Expression. We performed differential gene expression analysis on a downloaded transcriptomics dataset from an experiment in which rats were exposed acutely to deltamethrin (Fig. 1). The differentially expressed genes in frontal cortex between control and deltamethrin-exposed rats are visualized in the volcano plot (Fig. 2), which include the upregulated genes Nr4a1 (nuclear receptor subfamily 4 group A member 1), Klf2 (KLF transcription factor 2), and Rnf6 (ring finger protein 6), and the downregulated genes Gpd1 (glycerol-3-phosphate dehydrogenase 1), Fkbp5 (FKBP prolyl isomerase 5), and Hspb1 (heat shock protein family B (small) member 1).

3.2. GSEA. GSEA identified 1055 altered gene sets (unadjusted $p<0.05$) in the exposed group as compared to vehicle controls, among which 155 were significant at adjusted $p<0.05$ (Table 1, Supplemental File 1). Of the 1055 identified gene sets, 598 were upregulated and 457 were downregulated. The highest NES for the upregulated pathways corresponded to the "positive regulation of t-cell proliferation" gene set, and the three lowest enrichment scores for the downregulated pathways all related to mitochondrial gene sets. A PAVER heatmap was generated to organize upregulated and downregulated gene sets into identifiable clusters (Fig. 3). The most significant primarily upregulated gene set cluster (in red) was "actin-based cell projections," while the most significant primarily downregulated gene set cluster was "intracellular protein transport."

3.3. EnrichR. EnrichR identified 375 significantly enriched gene sets at an unadjusted $p<0.05$. EnrichR was unable to identify any significant gene sets with adjusted $p<0.05$.

3.4. iLINCS. iLINCS identified 567 transcriptomic signatures in the chemical perturbagen database that were positively correlated with the deltamethrin exposure signature ("concordant perturbagens") and 918 that were negatively correlated ("discordant perturbagens") (Table 2, Supplemental File 1). The top concordant perturbagen that was an identifiable pharmacological agent was Lanacordin, an inhibitor of sodium-potassium ATPase; while the top discordant perturbagen was the pharmacological agent Tanespimycin, an antibiotic that acts to inhibit Heat Shock Protein 90 (HSP90). Mechanism of Action (MOA) analysis identified 246 concordant MOAs and 292 discordant MOAs. The most common concordant MOA was "Cyclin-dependent kinase (CDK) inhibitor," while the most common discordant MOA was "acetylcholine receptor antagonist." iLINCS Known Targets analysis produced no concordant or discordant gene targets that were significant at $p<0.05$.

3.5. GSEA and Enrichr Venn Diagram. The 3Pod report combined the GSEA and Enrichr results, creating a Venn diagram (Fig. 4, Supplemental File 1). The 1055 altered gene sets from GSEA and 375 enriched gene sets from EnrichR were first reduced to 1030 and 369 gene sets, respectively, which appeared in both databases. Among the altered gene sets contained in both databases, the GSEA and EnrichR results shared 44 gene sets in common, of which 26 were upregulated in both

analyses and 12 were downregulated in both analyses. The top 2 shared upregulated pathways were “neuron projection cytoplasm” and “dendrite cytoplasm,” and the top two shared downregulated pathways were “negative regulation of intrinsic apoptotic signaling” and “positive regulation of membrane potential.”

4. Discussion

4.1. To investigate the effects of acute deltamethrin exposure on the brain, we studied a previously existing dataset (17) containing gene expression data from frontal cortex tissue of acutely exposed rats, using advanced bioinformatics methods. Differential gene expression was analyzed and then further examined using 3Pod, which produced the GSEA, Enrichr, iLINCS, and PAVER analyses. We found that frontal cortex from rats who were exposed to deltamethrin had significant changes in gene expression compared to control, especially in gene sets related to inflammation, apoptosis, energy metabolism/mitochondria, and synaptic structure/function.

4.2. Inflammation & Apoptosis. 3Pod analysis revealed a pattern in gene expression changes in the brain caused by exposure to deltamethrin that corresponds to changes in gene sets for inflammation and apoptosis. Apoptosis, also known as programmed cell death, ensures both the removal of damaged neurons and overgrowth of glial cells (30) and is essential for developmental plasticity and organismal health, playing key roles in brain development by aiding cell differentiation, localization, and population control (31). Among the differentially expressed genes, three of the most upregulated by fold change were Nr4a1, Klf2, and Rnf6; and one of the most downregulated was Fkbp5. Nr4a1 is a transcription factor involved in cellular processes like cell cycle mediation, inflammation, and apoptosis, and plays an important role in both cell survival and death (32). An increase in the gene expression of Nr4a1 also signifies more cellular stress and inflammation (33), both contributing to neurodevelopmental conditions like autism spectrum disorder and ADHD (34). Klf2 is also a transcription factor involved in vascular and immune functions in the body (35). An increased expression of Klf2 significantly affects the brain and leads to neuroinflammation, which can lead to an increased risk of neurodevelopmental disorders like autism (36). Rnf6 is a protein-coding involved in protein degradation (37) that also helps maintain protein homeostasis and create signaling pathways. An increase in Rnf6 expression means that the cell has detected and is responding to inflammation (38). GSEA showed the “positive regulation of t-cell proliferation” as the most upregulated gene set. As a part of the immune system, T-cells are a type of white blood cell that helps protect the body from infection by directly killing virus-infected cells or sending signals to trigger a larger immune response. Positive regulation of T-cell proliferation indicates an increase in T-cell production and activity, which could be indicative of an immune response to an inflammatory condition. Fkbp5 codes for a protein in the immunophilin protein family which plays an important role in immunoregulation. It is thought to control calcineurin inhibition, which itself activates T-cells (39). Finally, the Venn diagram of GSEA and EnrichR results shows the “negative regulation of intrinsic apoptotic signaling pathway” was downregulated in both analyses, indicating a disinhibition of apoptotic signaling.

4.3. Cellular Energy & Metabolism. Our results show changes in gene expression corresponding to cellular energy and metabolism. Among the differentially expressed genes, one of the most downregulated by fold change was Gpd1. Gpd1 codes for an enzyme crucial in carbohydrate and lipid metabolism (40), which is crucial for energy. A decreased expression could mean less energy available to neurons of the frontal cortex. The animals exposed to deltamethrin were found to move 30% less and have decreased neuronal firing in the frontal cortex (17). GSEA also revealed

numerous downregulated gene sets related to mitochondrial function, including the three most significantly downregulated gene sets, "mitochondrial protein-containing complex," "mitochondrial inner membrane," and "mitochondrial ribosome." Mitochondria are organelles responsible for generating cellular energy in the form of adenosine triphosphate (ATP). The mitochondrial protein-containing complex can refer to components of the electron transport chain (ETC) and ATP synthase. Downregulation of mitochondrial protein-containing complexes leads to impaired energy production because of reduced ATP levels (41). Similarly, the inner mitochondrial member separates mitochondria into two regions and is the working space of the ETC, which is responsible for oxidative phosphorylation. Mitochondrial ribosomes are also active in the ETC (42). Neurons are very demanding in ATP, so a downregulation of three important functions in the mitochondria can severely impact brain function.

4.4. Brain Development. PAVER analysis of the GSEA gene sets showed multiple dysregulated gene set clusters related to synaptic function, including "cluster of actin-based cell projections," "postsynaptic membrane," "monoatomic anion channel activity," and "cell surface receptor signaling pathway." Actin-based cell projections are structures that extend from the surface of a cell rich in cytoskeleton protein actin. Actin-based cell projections are important for the development of both dendrites and their dendritic spines (43), which are essential structural components of neuronal transmission (44). The combined GSEA and Enrichr gene sets also support our hypothesis that deltamethrin impacts pathways relevant to brain development, as there is a shared upregulation of the "neuron projection cytoplasm" and "dendrite cytoplasm" gene sets. Dynamic changes in dendrites and dendritic spines are often significant in brain development and a disruption in those dynamics can lead to neurodevelopmental disorders, including autism spectrum disorder (45). GSEA analysis also showed gene expression changes related to intracellular protein transport. Proteins are synthesized in ribosomes, which are then transported in vesicles between different organelles (46). A downregulation in intracellular protein transport can impact brain development as well. Certain proteins participate in neurotransmission and synapse function. Disruption in protein transport in these areas can harm synapses and their function, which are important for memory and learning (47). There are also proteins that assist in the development of neurons, therefore a downregulation in transport could adversely affect brain development.

4.5. Lanacordin & Tanespimycin. Perturbagen analysis identified specific chemicals that have both similar and dissimilar effects to deltamethrin exposure in frontal cortex. In our study, we found that Lanacordin treated cells showed a gene expression pattern highly similar to deltamethrin exposure. This means that the drug mimics the effect of deltamethrin on cells. Lancordin, commonly referred to as digoxin, is a cardiac glycoside prescribed to treat heart problems and known for its cardiotoxic effects (48). Lanacordin's mechanism of action involves inhibiting the sodium potassium pump (49). Lanacordin impacts neuronal function by disrupting cellular ion homeostasis, which is critical for neuronal membrane potentials (50) . This can lead to changes in excitability and action potentials, leading to neurological side effects such as confusion, memory impairment, hallucinations, and delirium (51). Tanespimycin, whose transcriptional profile had a high dissimilarity with deltamethrin exposure, serves as an antineoplastic agent and apoptosis inducer (52). Tanespimycin's mechanism of action is the inhibition of heat shock protein 90 (Hsp90), which is crucial in function and stability of proteins involved in cell growth by coupling with the ubiquitination pathway (53, 54).

4.6. iLINCS CDK inhibitor/iLINCS acetylcholine receptor antagonist. The mechanism of action shared by the most drugs on the concordant perturbagen list was “cyclin-dependent kinase (CDK) inhibitor.” The function of CDK inhibitors is to prevent the activities of CDKs during the cell cycle (55, 56). Exposure to the pyrethroid β-cypermethrin can downregulate both the protein expression levels of CDK4, CDK6, and mRNA expression levels of p21 and cyclin3, all of which contribute to the cell cycle (57). CDK4 is particularly involved in controlling the progression of cells from the G1 phase to S phase. Downregulation and inhibitors of this may halt the cell cycle, leading to a delay in cell division and cell growth (56). Disruptions in CDK4 functioning have also been associated with neurodevelopmental disorders (58). Although more research needs to be done, it can be inferred that deltamethrin may directly act as an inhibitor or may facilitate the downregulation of CDK4. The mechanism of action shared by the most drugs on the discordant perturbagen list was “acetylcholine receptor antagonist.” Acetylcholine receptors are critical cholinergic signaling molecules important for neuronal signaling, particularly in learning and memory (59). This disruption in acetylcholine signaling is particularly relevant to neurodevelopmental disorders, as proper functioning of acetylcholine receptors is essential for normal brain development and function (60).

5. Limitations

Our study involves several limitations that impact the depth and interpretation of our findings. The primary limitation on our findings is the use of an extensive range of exploratory bioinformatics techniques. These methods are very robust in generating new hypotheses, but the ability to conclusively determine statistical significance is limited by the number of analyses run and the number of hypotheses tested by each. This is amplified by the use of unadjusted p-values by some analyses in 3Pod, as either the input, the output, or both. Additionally, the selection of a previously analyzed dataset from the GEO database makes this study subject both to selection bias and to the authors’ foreknowledge of some existing differences. Another major limitation is the reliance on a single time point to assess gene expression dynamics following exposure to deltamethrin. Gene expression can vary significantly over time following acute exposure to a toxicant, influencing the observed outcomes (61). Incorporating multiple time points throughout the exposure and recovery phases could have helped in capturing more dynamic changes in gene expression patterns. Finally, the original study which was the source of the data analyzed here had several key limitations. First, the use of an animal model, and also the use of only male subjects, limits the generalizability of any results derived from the experiment. A second limitation of the original study, published in 2008, is that the gene count data was collected using a slightly outdated microarray technique instead of RNA-Seq. While microarrays offer a broad survey of gene expression patterns, RNA-Seq offers more differentially expressed protein coding genes, more toxicological and biological insight, and overall, it identifies more differentially expressed genes (62).

6. Conclusion

Using the 3Pod report containing Enrichr, LINCS, GSEA, and PAVER analyses, we were able to support the idea that there is a difference in the gene expression in the frontal cortex of deltamethrin exposed rats compared to the control rats. Deltamethrin exposure produced a wide range of changes in gene expression in genes and gene sets related to inflammation, apoptosis, cellular energy, and brain development. This leads to a wide range of effects on neuronal function that may explain, in part, the known effects of acute deltamethrin exposure on movement (17) and cognitive function (63) and the known effects of chronic developmental exposure on

neurodevelopmental disorder related neurobehavioral phenotypes (13-16). Our findings suggest new hypotheses for molecular mechanisms, new directions for research, and new potential avenues for preventing and reversing the effects of pyrethroid exposure.

7. Data Availability

All sequencing data were downloaded from NCBI GEO (GSE7955) on June 24, 2024. All code used to analyze the data is on GitHub repository CogDisResLab/galaxy1_3podr.

8. Disclosures

The authors declare no conflicts of interest.

9. Author Contributions

JPB and ASI selected the data set, designed the study, and designed the analytic strategy. ASI downloaded the data from the GEO database. JW, AS, RR, and ZR performed background research and participated in data analysis, interpretation of results, and assembly of figures under the guidance of NS, ASI, and JPB. All authors participated in writing the manuscript and approved the final version.

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Figures

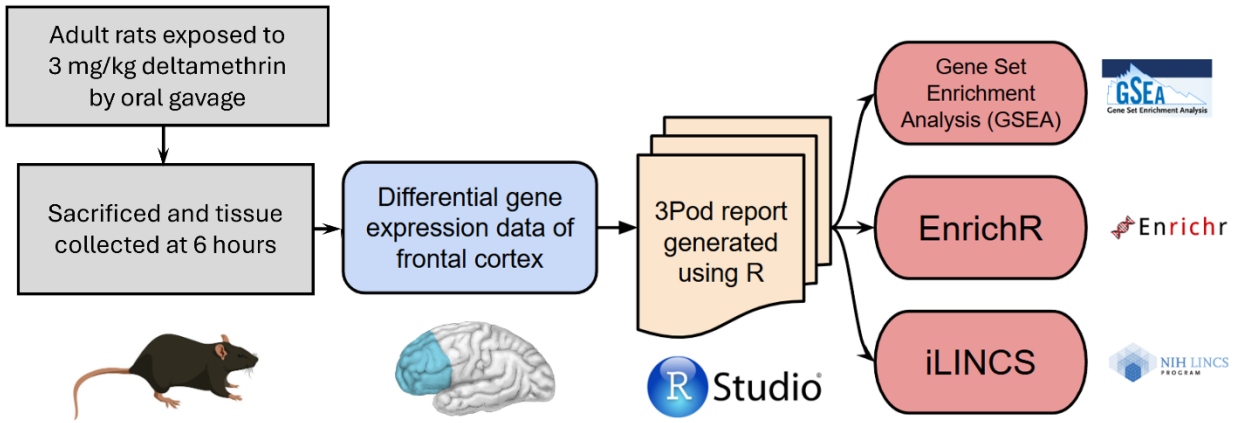


Figure 1. Workflow diagram showing the steps used to perform the exposure, generate data, and analyze the results.

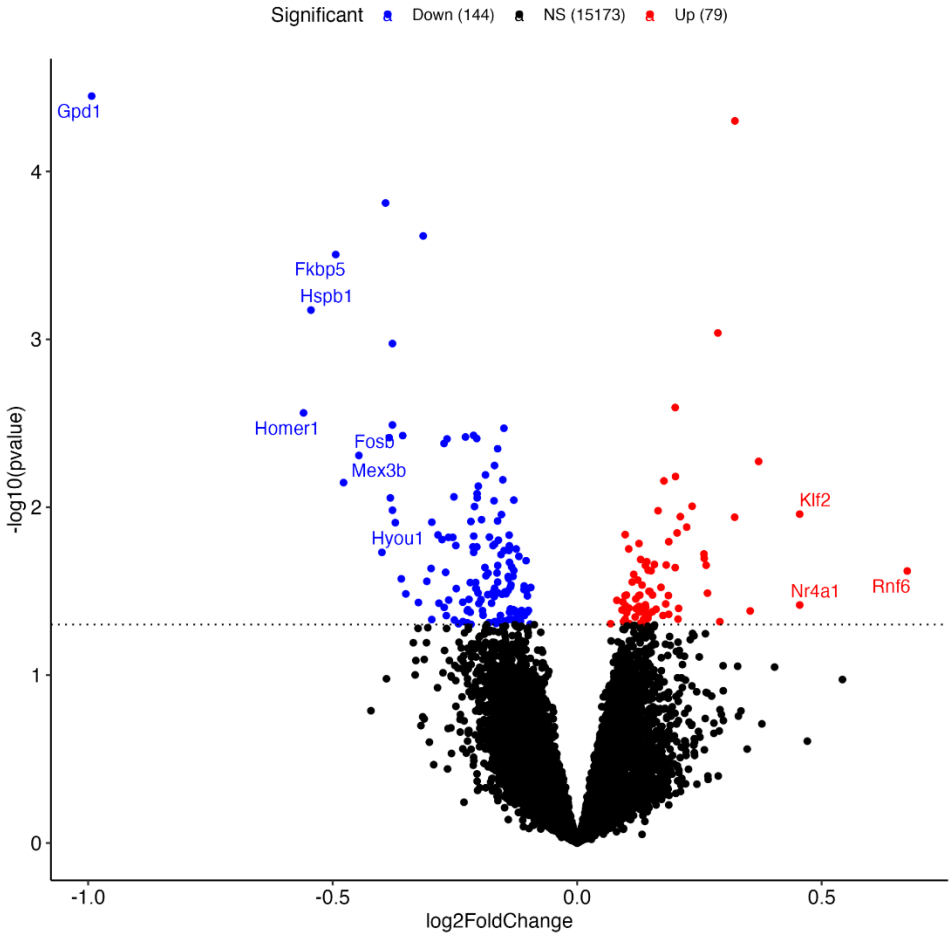


Figure 2. Volcano plot representing the differential gene expression of rats exposed to deltamethrin vs. vehicle controls. The x-axis is the $\log_2$ of the fold change in gene expression for each gene in the dataset, while the y-axis is the inverse $\log_{10}$ of the p-value. Upregulated genes are represented by red circles, and downregulated genes are represented with blue circles. The dotted line is the unadjusted threshold for significance ($p < 0.05$). The annotated genes represent the top differentially expressed genes by fold change.

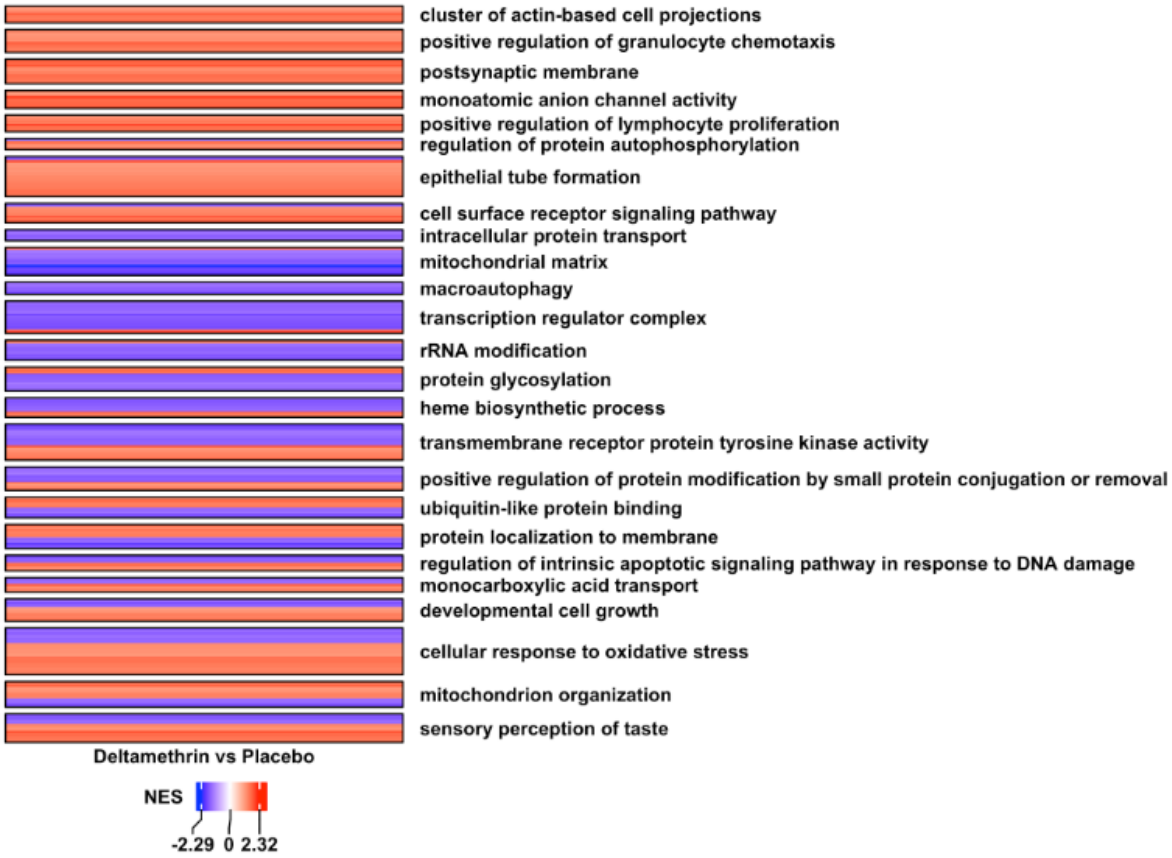
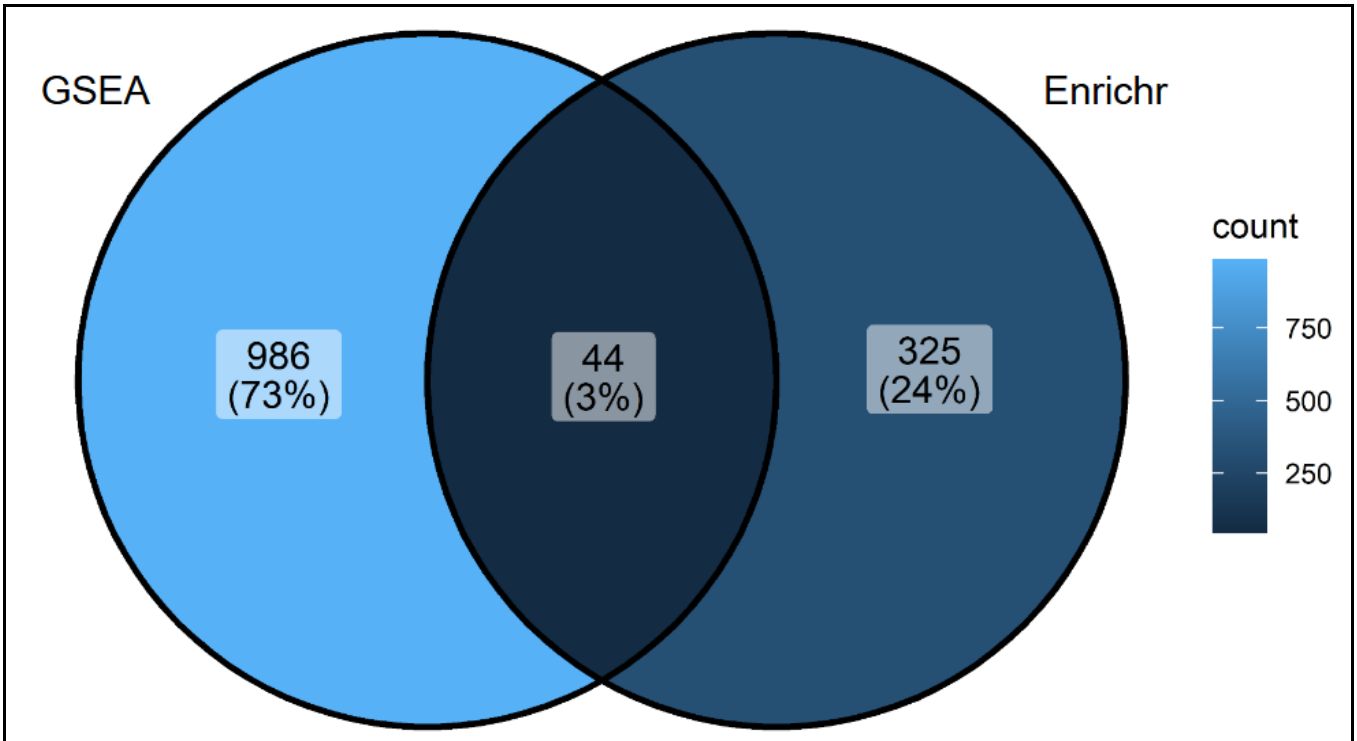


Figure 3. PAVER heatmap showing significant clusters of gene sets from GSEA. Clusters are organized from most significant at the top to least significant at the bottom. Gene sets within the clusters are sorted by normalized enrichment score, with positive scores (upregulated gene sets) shaded in red and negative scores (downregulated gene sets) shaded in blue.



Pathway	GSEA	Enrichr	Pathway	GSEA	Enrichr
Shared Upregulated			**Shared Downregulated**		
neuron projection cytoplasm	1.985	95.32	negative regulation of intrinsic apoptotic signaling pathway	-1.792	-18.58
dendrite cytoplasm	1.862	130.7	positive regulation of membrane potential	-1.538	-18.60
phenol-containing compound biosynthetic process	1.846	95.32	glutamate receptor binding	-1.530	-23.04
catecholamine biosynthetic process	1.805	83.34	response to unfolded protein	-1.495	-5.784
monoatomic anion homeostasis	1.734	110.6	COPI-coated vesicle	-1.483	-23.42
negative regulation of leukocyte chemotaxis	1.624	83.34	G protein-coupled receptor binding	-1.480	-4.524

biogenic amine biosynthetic process	1.623	83.34	phosphoric ester hydrolase activity	-1.474	-4.413
ventricular septum development	1.621	71.49	protein glycosylation	-1.454	-4.134
glucan metabolic process	1.621	130.7	regulation of cell growth	-1.444	-3.271
chloride ion homeostasis	1.580	110.6	negative regulation of protein kinase activity	-1.407	-4.934

Figure 4. Venn diagram illustrating the overlap between results obtained from the GSEA and Enrichr analyses. Color indicates the count of gene sets, with lighter shades representing higher counts. The top 10 upregulated and top 10 regulated pathways are listed and sorted using a combination of GSEA and Enrichr scores.

Pathway	P-value	NES	Pathway	P-value	NES
Upregulated			**Downregulated**		
Positive regulation of t cell proliferation	<0.001	2.321	Mitochondrial protein-containing complex	<0.001	-2.223
Cell surface receptor signaling pathway	<0.001	1.845	Mitochondrial inner membrane	<0.001	-1.938
External side of plasma membrane	<0.001	1.785	Mitochondrial ribosome	<0.001	-2.291
Receptor complex	<0.001	1.743	Organellar ribosome	<0.001	-2.291
Protein complex involved in cell adhesion	0.003	2.207	Organelle inner membrane	<0.001	-1.905
Multicellular organismal process	0.003	1.928	Ribosomal subunit	<0.001	-2.096
Side of membrane	0.003	1.508	Endoplasmic reticulum protein-containing complex	0.001	-2.053
Chloride transport	0.004	1.933	Mitochondrial envelope	0.001	-1.667
Response to ketone	0.007	1.538	Mitochondrial matrix	0.001	-1.821
Cell adhesion mediated by integrin	0.008	2.199	Mitochondrial large ribosomal subunit	0.001	-2.209

Table 1. Gene Set Enrichment Analysis showing the top up-regulated and down-regulated gene sets in exposed samples as compared to vehicle control. NES: Normalized Enrichment Score.

Perturbagen	Similarity	Perturbagen	Similarity
Concordant Signatures		**Discordant Signatures**	
BRD-K30126976	0.417	13-Hydroxy-8,14,19-T**	-0.471
6,10,10B-T*	0.366	Tanespimycin	-0.463
BRD-K86048057	0.365	BRD-K61341215-004-01-2	-0.455
Lanacordin	0.364	NVP-AUY922	-0.441
179324-69-7	0.361	MLS000718723	-0.434
Gossypol	0.358	BRD-K10010115	-0.419
Sepantronium	0.358	Mirin	-0.398
Niclosamide	0.358	PX 12	-0.393
Trichostatin A, Streptomyces Sp.	0.352	CHEMBL2139070	-0.392
BRD-A24396574-001-01-5	0.343	BIIB 021	-0.385

Table 2. iLINCS chemical perturbagen analysis of transcriptomic signatures. Perturbagens with the highest similarity scores relative to the deltamethrin transcriptomic signature are “concordant” while those with the lowest similarity scores are “discordant.”

*6,10,10B-Trihydroxy-3,4a,7,7,10a-pentamethyl-1-oxo-3-vinyldodecahydro-1H-benzo[f]chromen-5-yl acetate

**13-Hydroxy-8,14,19-Trimethoxy-4,10,12,16-tetramethyl-3,20,22-trioxo-2-azabicyclo[16.3.1]docosa-1(21),4,6,10,18-pentaen-9-yl carbamate

Mechanism of Action	**N**	**Mechanism of Action**	**N**
Concordant		**Discordant**	
CDK inhibitor	54	Acetylcholine receptor antagonist	51
NFkB pathway inhibitor	47	HDAC inhibitor	41
Proteasome inhibitor	38	26S proteasome inhibitor	38
D2-like dopamine receptor antagonist	30	CDK inhibitor	34
Serotonin 2a (5-HT2a) receptor antagonist (Serotonin 2a)	30	PI3K inhibitor	30
Epidermal growth factor receptor inhibitor	29	PDGFR tyrosine kinase receptor inhibitor	28
HDAC inhibitor	28	3’,5’-cyclic phosphodiesterase inhibitor	25
Tyrosine-protein kinase receptor RET inhibitor	27	Equilibrative nucleoside transporter 1 inhibitor	25
GABA receptor antagonist	26	Tyrosine kinase inhibitor	25
Dopamine receptor antagonist	25	VEGFR inhibitor	25

Table 3. iLINCS analysis of concordant and discordant mechanisms of action following deltamethrin exposure. Mechanisms of action were ranked by number of occurrences among the significantly concordant and discordant chemical perturbagens.

