

Psychoactive pharmaceuticals at environmental concentrations induce in vitro gene expressions associated with neurological disorders

A differential gene expression analysis

Daniel Bautista

2025-02-10

Contents

Introduction	2
Objectives	2
General objective	2
Specific objectives	2
Methods	2
Data Processing	2
Libraries	2
Collect data	2
Data formatting	3
Data cleaning	4
Data normalization	4
Expression analysis	5
Visualization	9
Results:	9
C7 (Complement C7)	9
Conclusions	10
References	10

Introduction

It was tested if psychoactive pharmaceuticals (fluoxetine, carbamazepine, venlafaxine) at lower concentrations (in ppb) could induce gene expressions linked with neurological disorders. Overall design: We used human neuronal cells as a model to study gene expressions induced by psychoactive pharmaceuticals at lower concentrations.

Objectives

General objective

Determine the gene expressions induced by psychoactive pharmaceuticals at lower concentrations.

Specific objectives

- To identify the gene expressions induced by psychoactive pharmaceuticals at lower concentrations.

Methods

Data Processing

Used the data from the study of Kaushik et al. (2016) uploaded to the recount3 database. The data was processed using the recount3 package in R.

Libraries

```
library(recount3)

## Warning: package 'matrixStats' was built under R version 4.4.2

library(limma)
library(edgeR)
library(ggplot2)
library(pheatmap)

## Warning: package 'pheatmap' was built under R version 4.4.2
```

Collect data

```
raw_data <- recount3::create_rse_manual(
  project = "SRP073802",
  project_home = "data_sources/sra",
  organism = "human",
  annotation = "gencode_v26",
  type = "gene"
)
```

```

## Warning in grep(pattern, bfr, value = TRUE): unable to translate ' El n<a3>mero
## de serie del volumen es: 8853-B02B' to a wide string

## Warning in grep(pattern, bfr, value = TRUE): input string 2 is invalid

## Warning in grep(pattern, bfr, value = TRUE): unable to translate ' El n<a3>mero
## de serie del volumen es: 8853-B02B' to a wide string

## Warning in grep(pattern, bfr, value = TRUE): input string 2 is invalid

## Warning in grep(pattern, bfr, value = TRUE): unable to translate ' El n<a3>mero
## de serie del volumen es: 8853-B02B' to a wide string

## Warning in grep(pattern, bfr, value = TRUE): input string 2 is invalid

```

Data formatting

```

assay(raw_data, "counts") <- compute_read_counts(raw_data)

raw_data <- expand_sra_attributes(raw_data)
colData(raw_data)[
  ,
  grepl("^sra_attribute", colnames(colData(raw_data)))
]

## DataFrame with 9 rows and 2 columns
##           sra_attribute.source_name sra_attribute.treatment
##           <character>             <character>
## SRR3438015   SK-N-SH neuronal cells  FLX 10 g/l; VNX 50 g..
## SRR3438018   SK-N-SH neuronal cells          VPA: 0.035mM
## SRR3438019   SK-N-SH neuronal cells          VPA: 0.035mM
## SRR3438011   SK-N-SH neuronal cells            none
## SRR3438012   SK-N-SH neuronal cells            none
## SRR3438013   SK-N-SH neuronal cells            none
## SRR3438014   SK-N-SH neuronal cells  FLX 10 g/l; VNX 50 g..
## SRR3438016   SK-N-SH neuronal cells  FLX 10 g/l; VNX 50 g..
## SRR3438017   SK-N-SH neuronal cells          VPA: 0.035mM

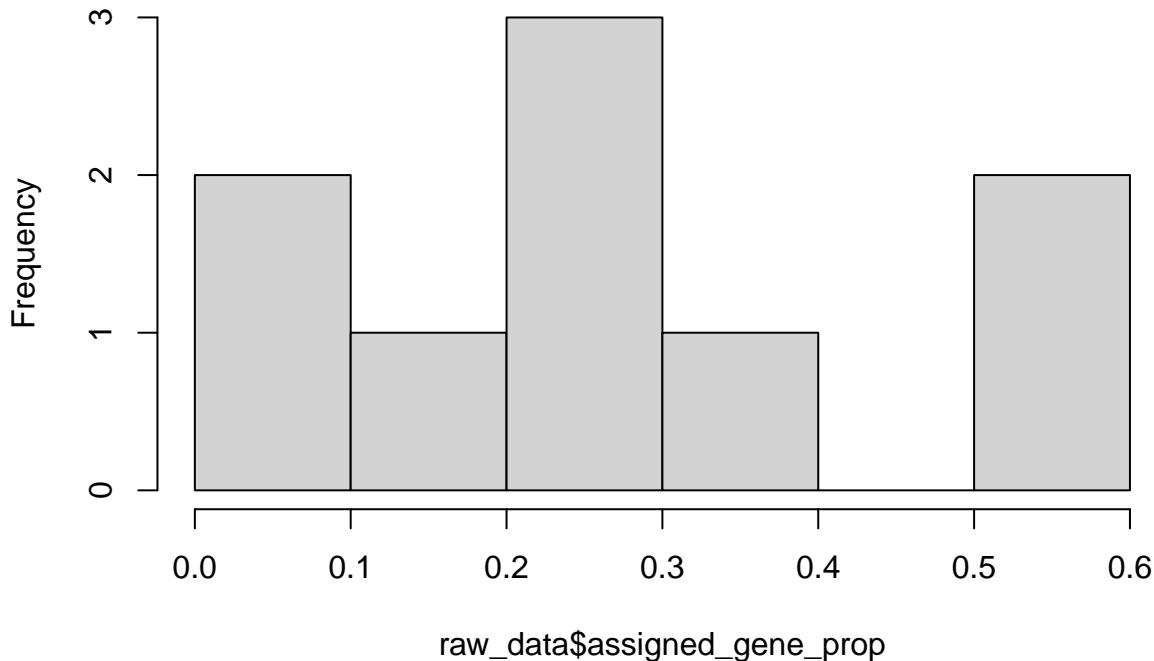
raw_data$assigned_gene_prop <- raw_data$recount_qc.gene_fc_count_all.assigned / raw_data$recount_qc.gen
summary(raw_data$assigned_gene_prop)

##      Min. 1st Qu. Median      Mean 3rd Qu.      Max.
## 0.08963 0.17329 0.23192 0.28844 0.32822 0.59110

```

Data cleaning

Histogram of raw_data\$assigned_gene_prop



```
gene_means <- rowMeans(assay(raw_data, "counts"))
summary(gene_means)
```

```
##      Min.    1st Qu.     Median      Mean    3rd Qu.      Max.
##      0.0      0.0      0.1     169.7     15.8 494766.1
```

```
## Eliminamos genes
data <- raw_data[gene_means > 0.1, ]
```

```
## Dimensiones finales
dim(data)
```

```
## [1] 34797      9
```

```
## Porcentaje de genes que retuvimos
round(nrow(data) / nrow(raw_data) * 100, 2)
```

```
## [1] 54.49
```

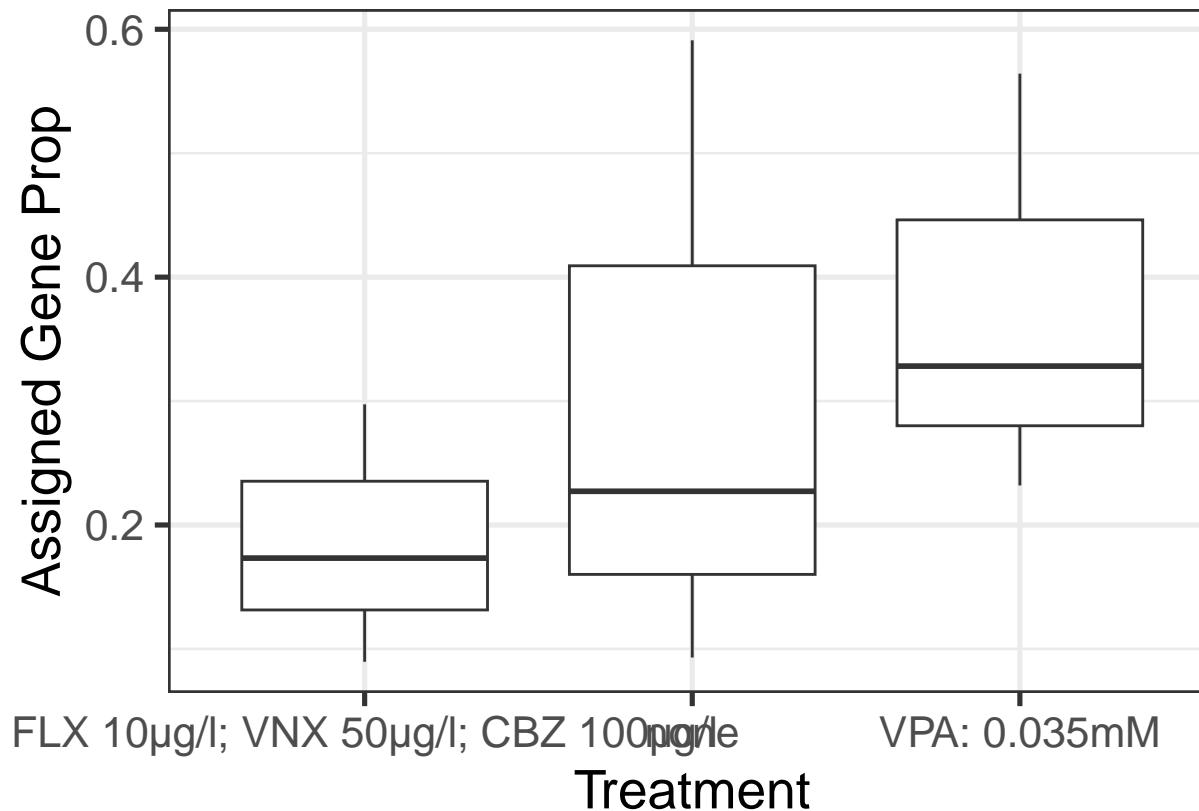
Data normalization

```

dge <- DGEList(
  counts = assay(data, "counts"),
  genes = rowData(data)
)
dge <- calcNormFactors(dge)

```

Expression analysis



```

mod <- model.matrix(~ data$sra_attribute.treatment + assigned_gene_prop,
  data = colData(data)
)
colnames(mod)

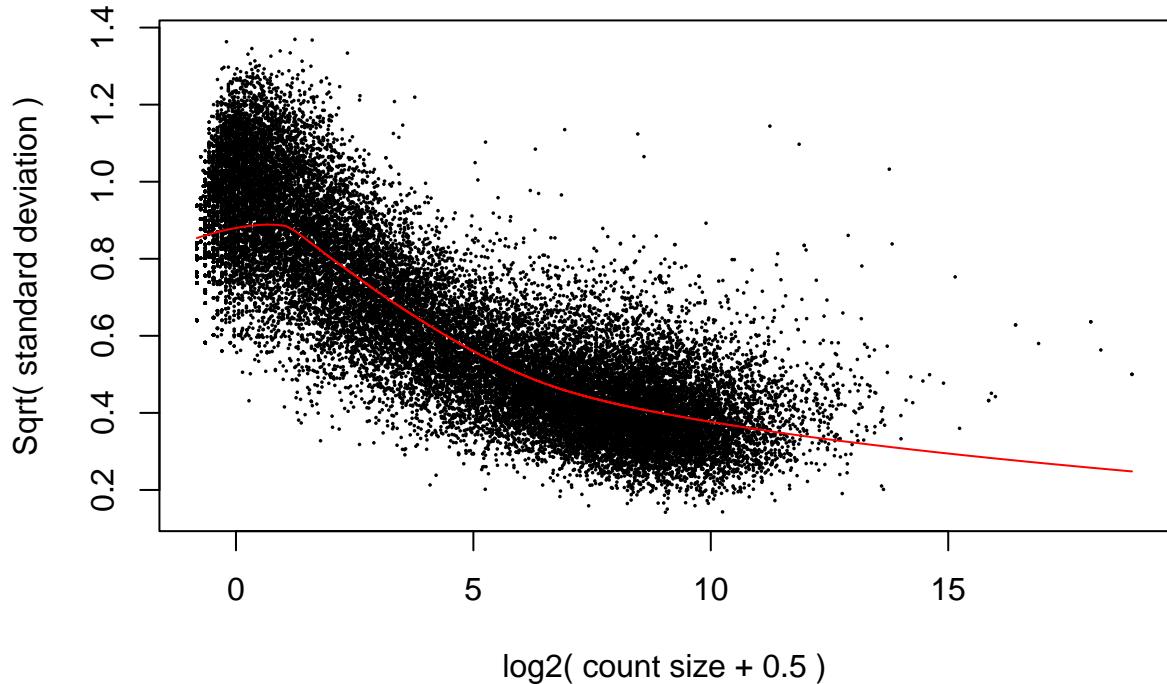
```

```

## [1] "(Intercept)"
## [2] "data$sra_attribute.treatmentnone"
## [3] "data$sra_attribute.treatmentVPA: 0.035mM"
## [4] "assigned_gene_prop"

```

voom: Mean–variance trend

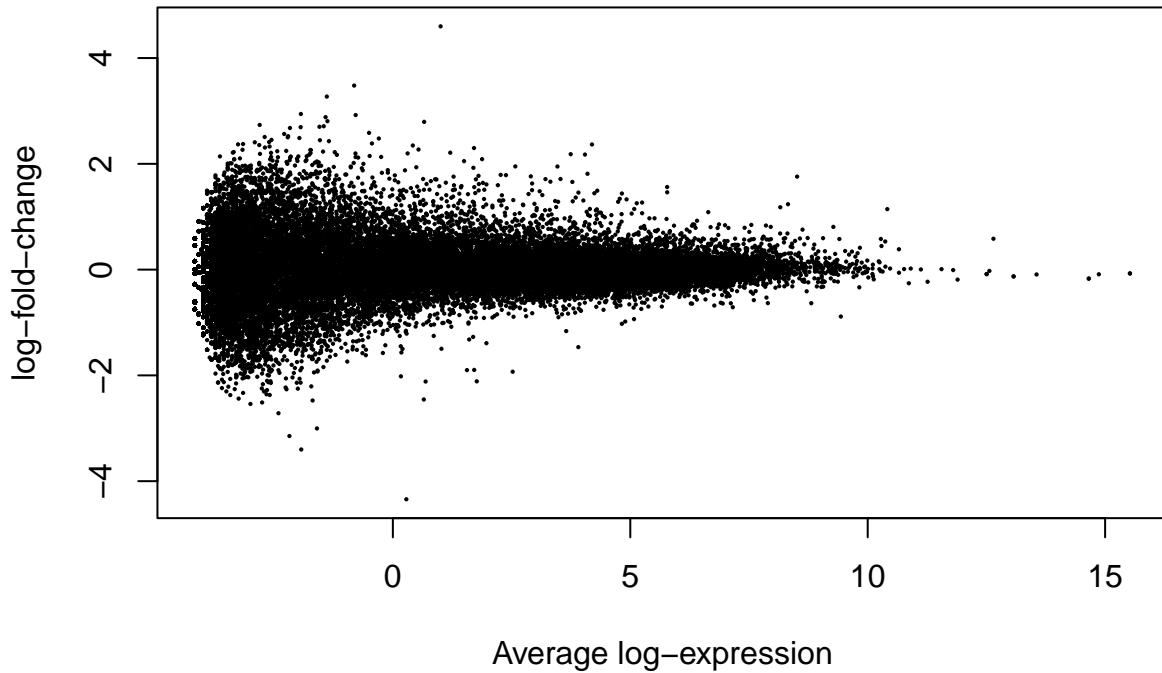


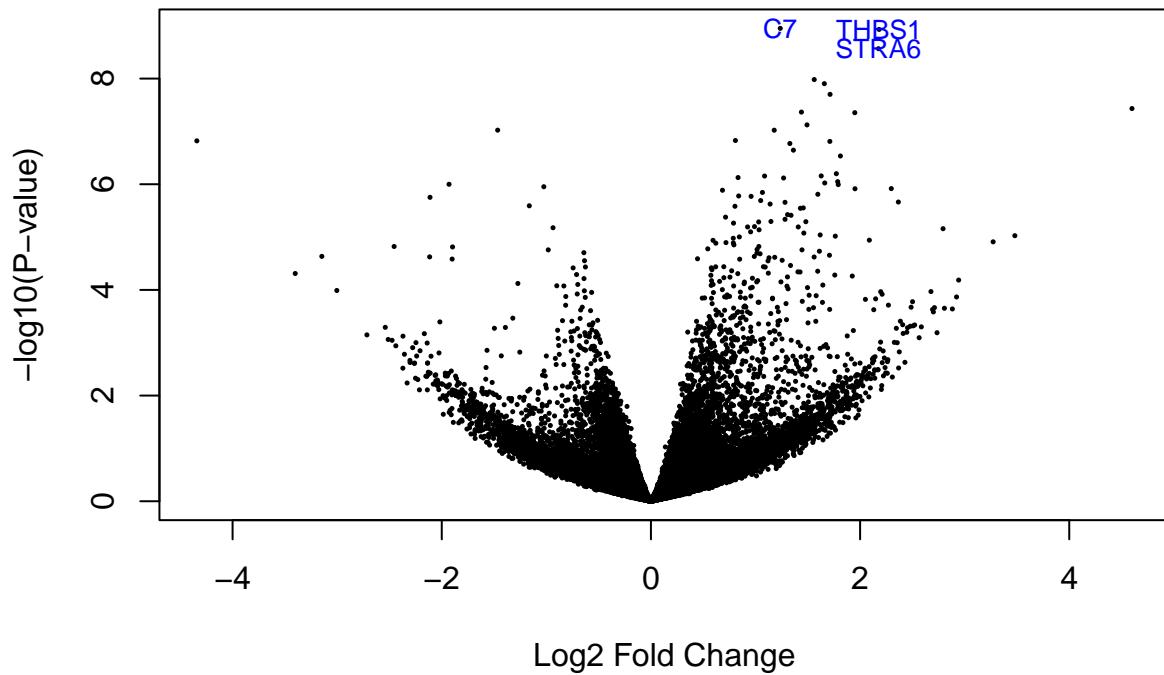
```
eb_results <- eBayes(lmFit(vGene))

de_results <- topTable(
  eb_results,
  coef = 2,
  number = nrow(data),
  sort.by = "none"
)
dim(de_results)
```

```
## [1] 34797    16
```

data\$sra_attribute.treatmentnone

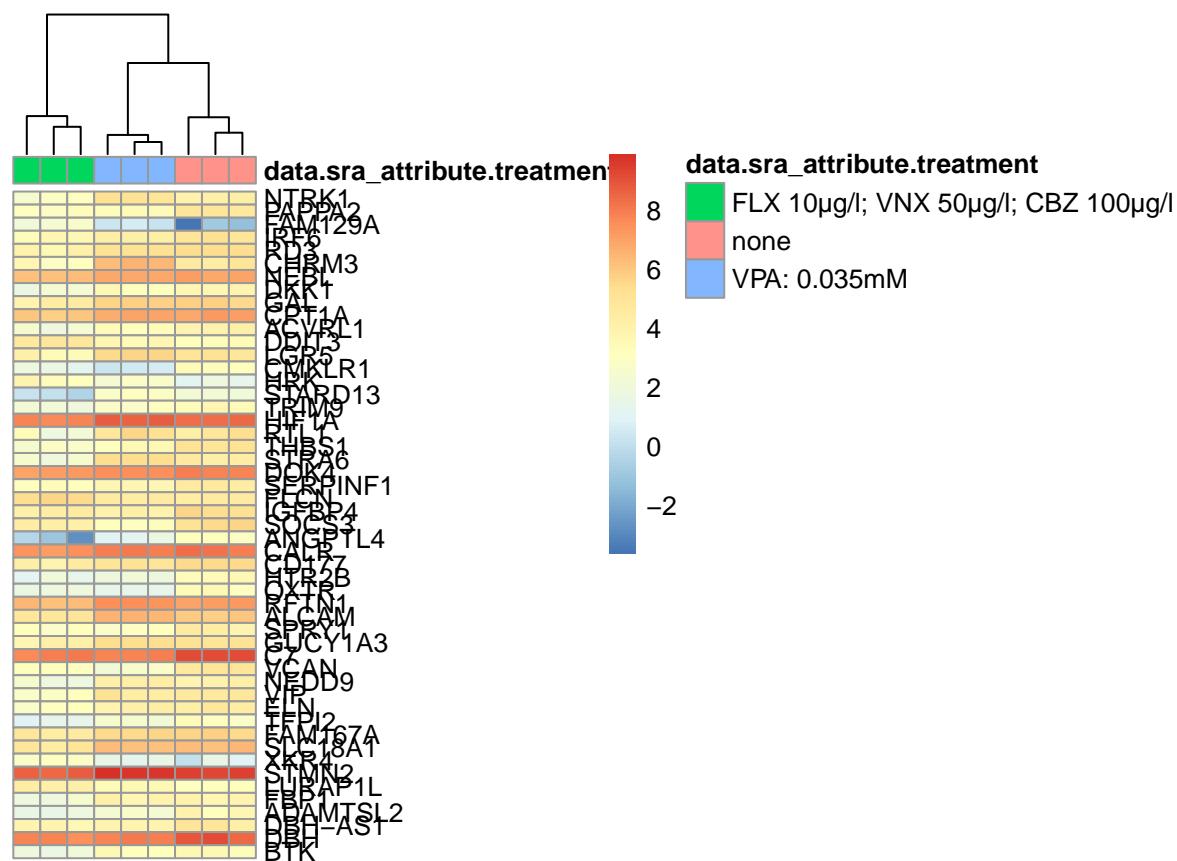




```
de_results[de_results$gene_name %in% c("C7", "THBS1", "STRA6"), ]
```

```
##           source type bp_length phase          gene_id
## ENSG00000137801.10 HAVANA gene      9158     NA ENSG00000137801.10
## ENSG00000137868.18 HAVANA gene      7690     NA ENSG00000137868.18
## ENSG00000112936.18 HAVANA gene      5223     NA ENSG00000112936.18
##          gene_type gene_name level          havana_gene
## ENSG00000137801.10 protein_coding    THBS1      2 OTTHUMG00000133665.3
## ENSG00000137868.18 protein_coding    STRA6      2 OTTHUMG00000138998.4
## ENSG00000112936.18 protein_coding    C7        1 OTTHUMG00000150340.3
##          tag      logFC AveExpr       t      P.Value
## ENSG00000137801.10 overlapping_locus 2.181716 3.745250 11.60503 1.173941e-09
## ENSG00000137868.18 <NA>            2.175588 4.045306 10.99333 2.720446e-09
## ENSG00000112936.18 <NA>            1.236592 8.321555 11.63969 1.120511e-09
##          adj.P.Val      B
## ENSG00000137801.10 2.042481e-05 11.99853
## ENSG00000137868.18 3.155445e-05 11.11361
## ENSG00000112936.18 2.042481e-05 12.51118
```

Visualization



Results:

C7 (Complement C7)

Function: The C7 gene encodes a protein that is part of the complement system, a critical part of the immune response. The complement system helps defend against pathogens and clear dead cells, but its overactivation can lead to inflammation.

Relevance to Brain Degeneration: Dysregulation of the complement system has been implicated in several neurodegenerative diseases, including Alzheimer's disease and multiple sclerosis. Complement proteins, including C7, can contribute to neuroinflammation, which is a key factor in the progression of cerebral degeneration. ## THBS1 (Thrombospondin 1) **Function:** THBS1 encodes a glycoprotein involved in the extracellular matrix (ECM) and plays a role in cell adhesion, migration, and tissue remodeling. It has a significant impact on synaptic function and neurogenesis.

Relevance to Brain Degeneration: Thrombospondin 1 has been linked to neurodegenerative diseases such as Alzheimer's disease, where it influences the formation of amyloid plaques and regulates synaptic activity. Overexpression of THBS1 is thought to contribute to the pathological changes seen in neurodegeneration, especially by affecting the blood-brain barrier and promoting the degeneration of neural tissue. ## STRA6 (Stimulated by Retinoic Acid 6) **Function:** STRA6 is involved in the cellular uptake of retinol (vitamin A) and is essential for the retinoic acid signaling pathway. Retinoic acid plays a critical role in brain development, neurogenesis, and synaptic plasticity.

Relevance to Brain Degeneration: Retinoic acid signaling is crucial for brain function, and its disruption has been implicated in various neurodegenerative disorders, including Parkinson's disease and Alzheimer's disease. Altered STRA6 expression can impair retinol uptake, which may contribute to neurodegeneration by affecting neural development and the ability to repair damaged neurons.

Conclusions

The gene expressions of C7, THBS1, and STRA6 were significantly altered by psychoactive pharmaceuticals at lower concentrations. These genes are associated with neurological disorders and play essential roles in brain function and degeneration. The findings suggest that exposure to psychoactive pharmaceuticals may impact the expression of genes linked to neurodegenerative diseases, highlighting the need for further research on the potential neurological effects of environmental contaminants.

References

- Kaushik, Gaurav, Yu Xia, Luobin Yang, and Michael A. Thomas. 2016. "Psychoactive Pharmaceuticals at Environmental Concentrations Induce in Vitro Gene Expression Associated with Neurological Disorders." *BMC Genomics* 17 (3): 435. <https://doi.org/10.1186/s12864-016-2784-1>.