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Final Project

Methylone and MDMA mice model frontal cortex neuroplastogen activity

– An analysis –

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List of Abbreviations and Symbols

MDMA	3,4-Methylenedioxymethamphetamine
PTSD	Post Traumatic Stress Disorder
SSRI	Selective serotonin reuptake inhibitor
MDMA-AT	MDMA Assisted Therapy
MDD	Medically diagnosed depression
GPCRs	G-protein-coupled-receptors
GEO Database	Gene Expression Omnibus Database
FC	Frontal Cortex
GO	Gene Ontology
KEGG	Kyoto Encyclopedia of Genes and Genomes
$\log_2$FC	$\log_2$ fold change
FDR	False discovery Rate
AMY	Amygdala

Abstract

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Keywords:

1 Introduction

Post-traumatic stress disorder (PTSD) is a mental condition that develops most often as result of exposure to war, sexual assault and other types of trauma. PTSD greatly lowers the quality of life of a patient. It has been reported that patients suffering from the condition have higher likelihood of having alcohol and drug abuse problems, other anxiety disorders and even affective disorders like mania and dysthymia (a type of long-term depression)[1]. Strangely enough there seems to be a strong correlation between the previously married (separated, divorced, or widowed) than the currently married for both men and women, controlling for age. Suicide attempts have been reported, via questionary, to suffer more than a ten fold increase between non PTSD and PTSD patients (0.5 and 6.5% respectively)[2].

There are several treatment types for PTSD using psychological therapies, pharmacotherapy or both[3]. Common pharmacotherapies for PTSD include SSRIs (antidepressants) that are second-generation antidepressants like introduced in the 1980s, these were a major advancement over first-generation drugs due to their higher selectivity and fewer side effects[4]. Some notable examples of second generation SSRIs include S-Citalopram (Escitalopram), Citalopram and Fluoxetine (Prozac).

MDMA-AT has recently been subject of discussion for the treatment of PTSD, showing promising results in patient outcomes[5].

However available pharmacotherapies are limited, take weeks to show modest benefit (in the case of SSRIs it can be from 2-6 weeks)[4] and remain ineffective for up to 40% of patients.

Methylone, an MDMA analogue, is being studied as a potential PTSD treatment due to its rapid and long-lasting antidepressant and anti-anxiety effects seen in

1 Kessler, R. C., Sonnega, A., Bromet, E., Hughes, M., & Nelson, C. B. (1995). Posttraumatic stress disorder in the National Comorbidity Survey. Archives of general psychiatry, 52(12), 1048–1060. https://doi.org/10.1001/archpsyc.1995.03950240066012

2 Sareen, J., Cox, B. J., Stein, M. B., Afifi, T. O., Fleet, C., & Asmundson, G. J. (2007). Physical and mental comorbidity, disability, and suicidal behavior associated with posttraumatic stress disorder in a large community sample. Psychosomatic medicine, 69(3), 242–248. https://doi.org/10.1097/PSY.0b013e31803146d8

3 Martin, A., Naunton, M., Kosari, S., Peterson, G., Thomas, J., & Christenson, J. K. (2021). Treatment Guidelines for PTSD: A Systematic Review. Journal of clinical medicine, 10(18), 4175. https://doi.org/10.3390/jcm10184175

4 Patrick, G. L. (2017). An introduction to medicinal chemistry. Oxford University Press.

5 Mitchell, J.M., Ot'alora G., M., van der Kolk, B. et al. MDMA-assisted therapy for moderate to severe PTSD: a randomized, placebo-controlled phase 3 trial. Nat Med 29, 2473–2480 (2023). https://doi.org/10.1038/s41591-023-02565-4

preclinical research. This work examined how methylone affects gene activity and brain pathways in the frontal cortex of mice brains tied to PTSD and MDD. We also compared its effects to MDMA, which has shown promise in PTSD treatment, to identify similarities and differences between the two compounds[6]. **Figure 1** shows the chemical structures of the two compounds.

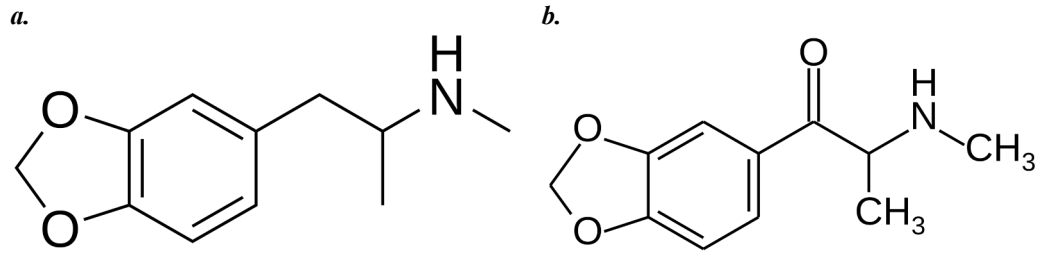


Fig. 1: a. MDMA b. Methylone
Source: Wikipedia

1.1 Base Study

All our data was extracted from (Warner-Schmidt et al., 2024)[6], in this study monoamine binding, uptake and release along with a high-throughput screen to assess agonist and antagonist activities across 168 GPCRs in vitro were done. Our focus however will be on the RNA-seq data produced in the experiments. RNA-seq was used to examine changes in the amygdala and frontal cortex, two brain regions linked to emotional learning and commonly impacted by PTSD and MDD. Rats were treated with either a single dose of methylone or MDMA (both at 10 mg/kg, administered via intraperitoneal injection), and their responses were compared to control groups (vehicle), the brains were harvested and frozen 8h post injection. All the groups were composed of 6 individual rats (Vehicle, n=6; MDMA, n=6; Methylone, n=6). The purpose of the RNA-seq data was to identify which genes, pathways, and/or functions were commonly regulated by methylone and MDMA, with the hypothesis that they might underlie therapeutic activity. In contrast, genes and pathways regulated by either drug alone might reflect off-target effects.

[6] Warner-Schmidt, J., Stogniew, M., Mandell, B., Rowland, R. S., Schmidt, E. F., & Kelmendi, B. (2024). Methylone is a rapid-acting neuroplastogen with less off-target activity than MDMA. Frontiers in Neuroscience, 18, 1353131. doi:10.3389/fnins.2024.1353131

2 Methods

The data produced in the (Warner-Schmidt et al., 2024) study was obtained from the GEO Database with the following ascension number: **GSE253280**. We extracted the 18 available samples from the database related to the FC (Frontal Cortex) experiments. Samples were named FC_"condition"_x. Where "condition" could be either Methylone, MDMA or Vehicle and x is the number of the sample (1 through 6). These are .txt files that contain the GeneID, location, strand length, gene count and more. Since we didn't use the raw data from the study these files had been previously analyzed in the matters of quality of raw data by the authors. However, for our analysis we created corresponding files for each of the sample files containing only the GeneID and respective count number (first and last column of each row in the .txt files). In the course of our work we also used the dplyr[7] R package to handle our datasets. All our files and R code can be found on GitHub under the repository: https://github.com/joao-matias0/omics.

2.1 Data Analysis

For our analysis we used the R-Studio to treat and analyze the data. The first part of the analysis consisted on the merging of all the data from the individual .txt files into one table with the following columns: Common Name (gene); condition (x16); GeneID. After having our data correctly assembled we proceeded to do a differential expression analysis, using the DESeq2[8] R package followed by Gene Set Enrichment Analysis (GSEA). Then with the *clusterProfiler*[9] R package and the org.Rn.eg.db[10] library from Bioconductor we proceeded with pathway analysis of the

[7] Wickham H, François R, Henry L, Müller K, Vaughan D (2023). dplyr: A Grammar of Data Manipulation_. R package version 1.1.4, <https://CRAN.R-project.org/package=dplyr>.

[8] Love MI, Huber W, Anders S (2014). "Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2." Genome Biology, 15, 550. doi:10.1186/s13059-014-0550-8.

[9] Yu, G., Wang, L. G., Han, Y., & He, Q. Y. (2012). clusterProfiler: an R package for comparing biological themes among gene clusters. Omics : a journal of integrative biology, 16(5), 284–287. https://doi.org/10.1089/omi.2011.0118

[10] Carlson, M. (2017). org.Rn.eg.db. doi:10.18129/B9.BIOC.ORG.RN.EG.DB

differentially expressed genes using the Gene Ontology[11,12] database and the KEGG database[13,14,15].

2.2 Differential Expression Analysis

For our differential expression analysis we used a table containing the GeneID and respective count numbers of the genes in the 18 samples. In out table the first 6 columns of the count number are “Vehicle”, 7-12 are “Methylone” and 13-18 are “MDMA”. Using DESeq2 we estimated variance-mean dependence in count data comparing the control condition to both drugs. We filtered significant genes with an adjusted p value cutoff of 0.05 (adjusted p-value < 0.05) and extracted subsets of the differentially expressed genes by $\log_2$ fold change, with upregulated genes considered with a $\log_2FC > 0$ and downregulated genes considered with a $\log_2FC < 0$.

2.3 Pathway Analysis

Using our lists of differentially expressed genes we proceeded with a pathway analysis in order to find specific pathways and functions that are being affected by the presence of both drugs in the tissue. To do this we used the *clusterProfiler* R package to search for enriched pathways using our list of differentially expressed genes in the GO database and KEGG database. To adjust for the FDR we used the Benjamini-Hochberg method in order to limit the amount of FDRs by adjusting p-values. Furthermore a p-value cutoff of 0.05 and a q-value cutoff of 0.2 were used (meaning only results with an FDR below 20% are retained). The list of enriched pathways were saved as separate .csv files.

2.4 Pathway and gene enrichment pathway comparison

To further understand the effects of methylone and mdma on the target tissue we proceeded with a comparison analysis between their enriched pathways by crossing the results from the individual pathway analysis of both drugs. In addition to this, and to better understand the genes that are involved in the therapeutic effect of both drugs we

[11] Ashburner et al. Gene ontology: tool for the unification of biology. Nat Genet. 2000 May;25(1):25-9. DOI: 10.1038/75556

[12] The Gene Ontology Consortium. The Gene Ontology knowledgebase in 2023. Genetics. 2023 May 4;224(1):iyad031. DOI: 10.1093/genetics/iyad031

[13] Kanehisa, M., & Goto, S. (2000). KEGG: kyoto encyclopedia of genes and genomes. Nucleic Acids Research, 28(1), 27–30. doi:10.1093/nar/28.1.27

[14] Kanehisa, Minoru. (2019). Toward understanding the origin and evolution of cellular organisms. Protein Science: A Publication of the Protein Society, 28(11), 1947–1951. doi:10.1002/pro.3715

[15] Kanehisa, Minoru, Furumichi, M., Sato, Y., Kawashima, M., & Ishiguro-Watanabe, M. (2023). KEGG for taxonomy-based analysis of pathways and genomes. Nucleic Acids Research, 51(D1), D587–D592. doi:10.1093/nar/gkac963

decided to cross the lists of differentially expressed genes of both drugs (up and down regulated separately) to obtain list of common differentially expressed genes in both drugs. We also sorted and analyzed the top ten enriched GO terms from both drugs from our pathway analysis results.

3 Results

3.1 Differential Expression Analysis

The summary of our differential expression analysis can be found on **Table 1**, where we list the number or upregulated genes and downregulated genes for both conditions (MDMA and Methylone) in the frontal cortex and the genes regulated by both drugs, the results from the amygdala analysis by (Warner-Schmidt et al., 2024) are also represented.

	Downregulated			Upregulated		
Location	MDMA	Methylone	Both	MDMA	Methylone	Both
FC	939	78	21	495	55	29
AMY	871	111	97	548	10	9

Tab. 1: Comparison of Up and Downregulated genes in FC and AMY
Source: Own creation

The lists of the differentially expressed genes as well as the genes regulated by both drugs can be found in out GitHub repository.

To better understand the regulatory effect of both drugs we produced Volcano Plots using several R packages including ggplot2[16], to capture the differences between expression magnitude and gene significance **Figure 2**.

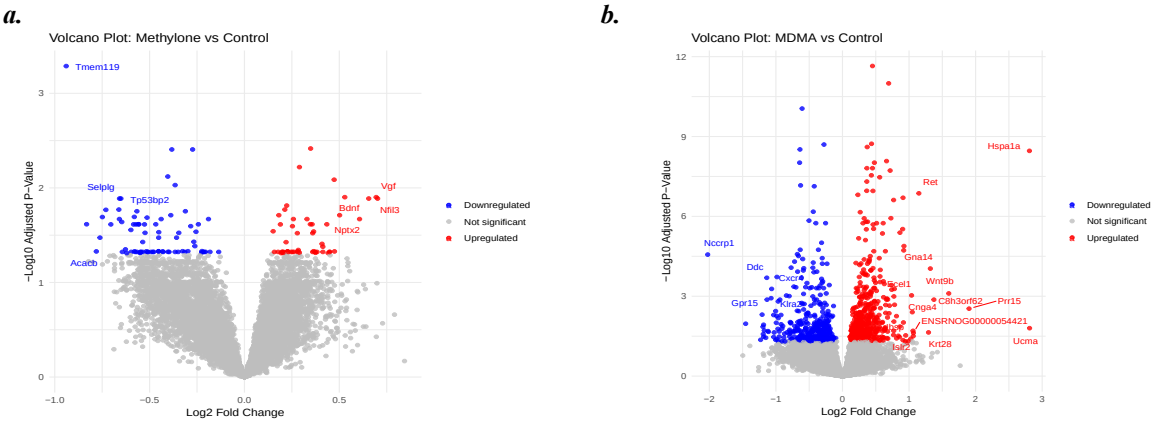


Fig. 2: *Volcano plots show significantly regulated genes in the frontal cortex after treatment with (a) methylone or (b) MDMA compared to vehicle-injected controls (N = 6 per group). Light gray dots represent genes that were not significantly changed by either treatment.*
Source: Own Creation

[16] Ggplot: H. Wickham. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York, 2016.

3.2 Pathway Analysis

Our pathway analysis yielded very different results when using the GO database or KEGG database. The quantification of enriched pathways found in the gene list for each pathway is outlined in **Table 2**.

	Downregulated		Upregulated	
Database	MDMA	Methylone	MDMA	Methylone
KEGG	8	1	4	0
GO	66	38	53	19

Tab. 2: Comparison of Up and Downregulated genes in FC and AMY
Source: Own creation

In general, the KEGG results weren't very elucidative. The GO database results made it clear that MDMA is involved in many more enrichment pathways than methylone. Furthermore, it seems that both drugs upregulate a number of pathways involved in synaptic processes, however MDMA has a much more broad set of effects outside of those expected. Both drugs downregulate pathways involved in metabolic processes of associated with the catabolism of several biomolecules (especially fats) again with MDMA having much more broad effects. The top ten upregulated pathways for both drugs are shown in **Figure 3** and the same is shown for downregulated pathways in **Figure 4**.

a. MDMA ***b. Methylone***

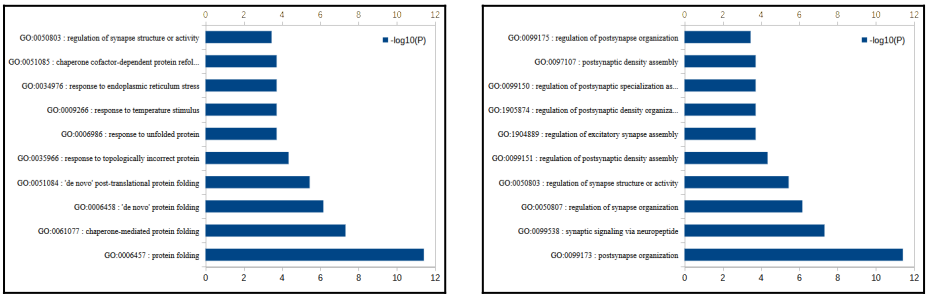
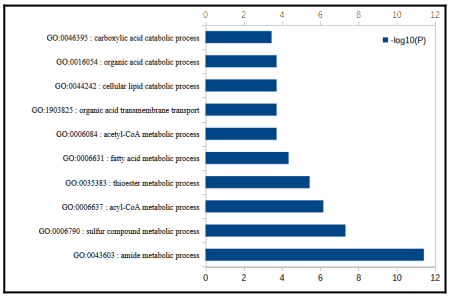


Fig. 3: The top ten upregulated pathways for (a) MDMA and (b) methylone
Source: Own Creation

It is clear from these results and from what was previously found by (Warner-Schmidt et al., 2024) that methylone has much more targeted effects on synaptic pathways in mice. Our work however had considerably different outcome on predicted upregulated pathways by methylone in the FC.

a. MDMA



b. Methylone

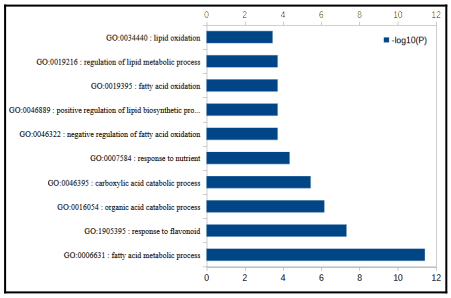


Fig. 4: The top ten downregulated pathways for (a) MDMA and (b) methylone
Source: Own Creation

4 Discussion

Our findings underscore the differential and overlapping effects of methylone and MDMA on gene expression and pathway modulation in the FC of mice, offering insights into their potential therapeutic roles for the treatment of PTSD and MDD.

Both methylone and MDMA exhibited distinct and overlapping gene regulation patterns. Notably, methylone demonstrated a more selective modulation, with fewer genes upregulated or downregulated compared to MDMA. This specificity could indicate a reduced likelihood of off-target effects, making methylone a potentially safer alternative for therapeutic purposes. Conversely, MDMA's broader gene regulation profile suggests a more extensive impact on neural pathways, which may contribute to both its therapeutic efficacy and side effect profile.

Pathway analysis revealed that both compounds upregulate synaptic-related processes, aligning with their proposed neuroplastogenic effects. However, the broader pathway enrichment observed with MDMA suggests additional effects beyond synaptic modulation, including impacts on metabolic and cellular stress response pathways. This divergence highlights methylone’s potential as a targeted treatment with a narrower therapeutic window. The observed downregulation of metabolic pathways, particularly those related to lipid catabolism, may reflect a conserved neuroprotective mechanism aimed at preserving energy homeostasis during acute drug exposure.

The overlap in gene expression and pathway modulation between the two compounds points to shared mechanisms underpinning their neuroplastogenic effects. This commonality supports the hypothesis that shared pathways may underlie their therapeutic benefits in PTSD and MDD. The selectivity of methylone, coupled with its reduced off-target activity as demonstrated by fewer differentially expressed genes, suggests that it may represent a promising candidate for further investigation as a PTSD treatment with potentially fewer adverse effects.

Despite the promising results, this study has limitations. First, the exclusive use of frontal cortex samples may overlook regional brain-specific effects. To better understand how these compounds affect the brain studies like (Warner-Schmidt et al., 2024) are fundamental, due to the inclusion of other regions like the amygdala.

Finally, while pathway analysis provided valuable insights, the limitations of database-driven annotations, particularly for less characterized pathways, should be acknowledged. Further mechanistic studies are required to elucidate the precise roles of the identified genes and pathways in mediating the effects of methylone and MDMA.

The authors hypothesize that the common genes are responsible for the therapeutic activity.

Nota: os nossos volcano plots n tao bem iguais aos do artigo (N QUER DIZER Q ESTAJA MAL!)

5 Conclusion