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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
$\log_2\text{FC}$	\log_2 fold change
FDR	False discovery Rate
AMY	Amygdala

Abstract

a

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 questionnaire, 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.

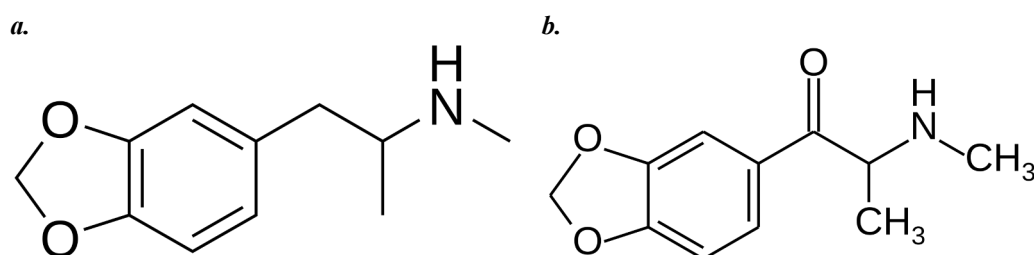


Fig. 1: a. MDMA b. Methylone
Source: Wikipedia

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 Methy-lone, 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 our 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 log₂ fold change, with upregulated genes considered with a log₂FC > 0 and downregulated genes considered with a log₂FC < 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 of 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 our 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**.

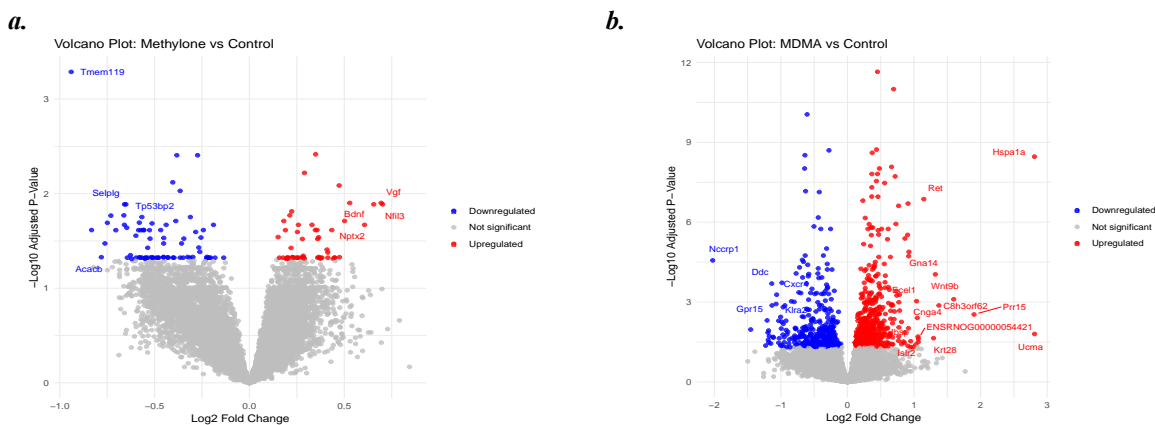


Fig. 2: Volcano plots show significantly regulated genes in the frontal cortex after treatment with (a) methylone or (b) MDMA compared to vehicle-injected controls ($N = 6$ per group). Light gray dots represent genes that were not significantly changed by either treatment.

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**.

Database	Downregulated		Upregulated	
	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**.

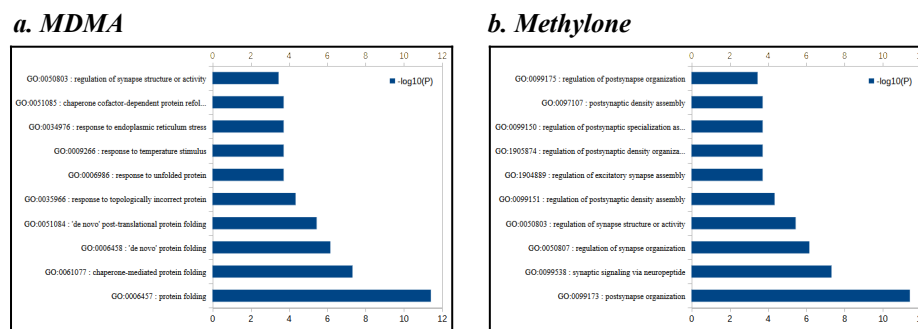
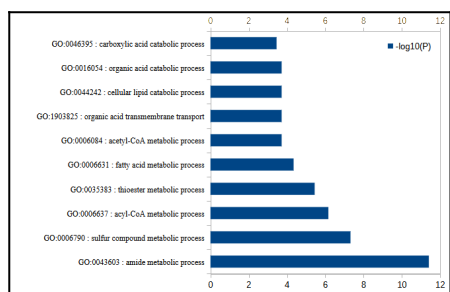


Fig. 3: The top ten upregulated pathways for (a) MDMA and (b) methylone
Source: Own Creation

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

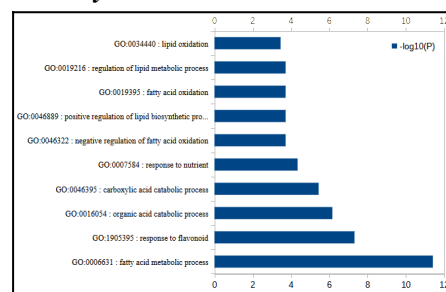


Fig. 4: The top ten downregulated pathways for (a) MDMA and (b) methylone
Source: Own Creation

4 Discussion

In total, the findings of our work show differential and overlapping actions of methylone and MDMA in modulating gene expression and pathways in FC of mice, extending previous evidence for their therapeutic potential to treat PTSD and MDD.

Specific and shared patterns of gene regulation were evidenced with both methylone and MDMA. Notably, methylone showed a more selective pattern of modulation, up- or downregulating fewer genes compared to MDMA. Such specificity of action suggests lower potential for off-target effects and may render it safer in therapeutic use. In contrast, the larger pattern of MDMA gene regulation would suggest an action across many neural pathways, to which therapeutic efficacy and side effect profile might be attributed. Furthermore, genes regulated by both drugs were identified, and as was postulated by (Warner-Schmidt et al., 2024), these genes might be responsible for the therapeutic effect of both drugs, hence further research into pathways in the brain involving these genes might be important for the study of PTSD and MDD.

Pathway analysis indicated that both compounds upregulate synaptic related processes, in agreement with their reputed neuroplastic effects. However, the broader pathway enrichment with MDMA suggested more far reaching effects beyond mere synaptic modulation, including targeting metabolic and cellular stress response pathways. This difference thus highlights the potential of methylone as a targeted treatment with a narrower therapeutic window. Most particularly in the metabolic pathways of lipid catabolism, this downregulation observed could correspond to a conserved neuroprotective mechanism^[17].

The selectivity of methylone, with fewer genes differentially expressed a proxy for off target effects are reasons that this drug deserves further investigation for treating PTSD with fewer side effects.

To better understand how these compounds affect the brain however, studies like (Warner-Schmidt et al., 2024) are fundamental, due to the inclusion of other brain regions like the amygdala that were quantified and thus a more broad sense of the action of these drugs in various areas of brain tissue was possible.

¹⁷ Yang, D., Wang, X., Zhang, L., Fang, Y., Zheng, Q., Liu, X., Yu, W., Chen, S., Ying, J., & Hua, F. (2022). Lipid metabolism and storage in neuroglia: role in brain development and neurodegenerative diseases. *Cell & bioscience*, 12(1), 106. <https://doi.org/10.1186/s13578-022-00828-0>

5 Conclusion

Appendices

Appendix I: List of genes upregulated and downregulated by both drugs

Upregulated	Downregulated
Dnajib5	Acacb
Mier2	Scarb2
Prrt1	Sox9
Nptx2	Abat
C12h12orf43	Tp53bp2
Vgf	Fam210b
Etv5	Acss1
Dgkg	Abcb1a
Camk1g	Acadl
Syt13	Arnt2
Nfil3	S1pr1
Lrfr2	Acsl3
Gne	Rcbtb2
Ddost	Gpam
Arhgap39	Bloc1s5
Shisa7	Cplx3
Ncoa5	Ppfbp2
Egr3	Slc16a1
Zfp428	Atp13a5
Lrfr1	Mlxip1
Psrc1	
Tmem198	
Numbl	
Galnt9	
Bdnf	
Ensa	
Ppp1r12c	
Selenom	

Source: Own creation