The Converging Effects of Different Categories of Antidepressants on the Brain: A Systematic Meta-Analysis of Public Transcriptional Profiling Data from the Hippocampus and Cortex

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Competing Interests

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CRediT statement

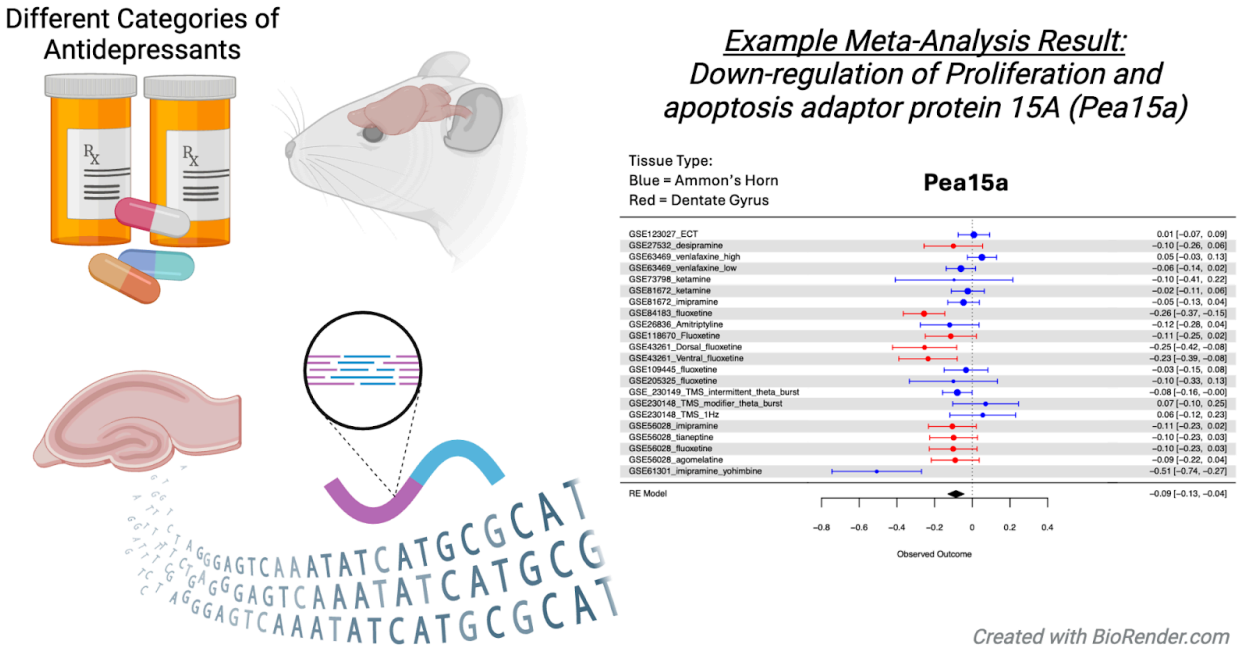
EMG - Conceptualization, Methodology, Software, Formal analysis, Investigation, Data Curation, Writing - Original Draft, Writing - Review & Editing, Visualization
MHH: Conceptualization, Methodology, Software, Writing - Review & Editing, Visualization, Supervision, Project administration
EH: Conceptualization, Methodology, Software, Formal analysis, Investigation, Data Curation, Writing - Original Draft, Writing - Review & Editing, Visualization
SE: Conceptualization, Methodology, Software, Writing - Review & Editing
EIF: Conceptualization, Methodology, Writing - Review & Editing
PTN: Writing - Review & Editing, Data Curation
AS: Writing - Review & Editing, Data Curation
MRB: Conceptualization, Methodology, Writing - Review & Editing
SM: Conceptualization, Methodology, Writing - Review & Editing
SJW: Writing - Review & Editing, Funding acquisition
HA: Writing - Review & Editing, Supervision, Funding acquisition
RH: Conceptualization, Methodology, Writing - Review & Editing, Supervision, Funding acquisition

Abstract

Background: Depression can be treated with traditional antidepressant pharmaceuticals targeting monoaminergic function, as well as with a variety of non-traditional drug classes and neuromodulatory interventions, such as electroconvulsive therapy.
Objective: To identify mechanisms of action shared across antidepressant treatment categories, we performed a systematic meta-analysis of public transcriptional profiling data from adult laboratory rodents (rats, mice). Our scope included clinically-used and clinically-effective antidepressant treatments, both pharmacological and neuromodulatory. The outcome variable was gene expression, as measured by microarray or RNA-Seq, from bulk dissected tissue from two brain regions linked to depression, the hippocampus and cortex.
Methods: Relevant datasets were identified in the Gemma database of curated, reprocessed transcriptional profiling data using pre-defined search terms and inclusion/exclusion criteria (*hippocampus:* 6-24-2024, *cortex:* 7-10-2024). Differential expression results were extracted for all available genes, minimizing bias. For each gene, a random effects meta-analysis model was fit to the antidepressant vs. control effect sizes (Log2 Fold Changes) from each study for each brain region, with exploratory analyses examining traditional and non-traditional antidepressant categories separately.
Results: For the hippocampus, 15 relevant studies were identified, containing 22 antidepressant vs. control group comparisons. These treatment comparisons represented a collective n=313 samples, approximately half of which received traditional versus non-traditional antidepressants. Of the 16,439 genes with stable meta-analysis estimates, 58 were consistently differentially expressed (False Discovery Rate (FDR)<0.05) following treatment. Antidepressant effects were enriched in gene sets related to stress regulation, brain growth and plasticity, vascular and glial function, and immune function. Comparisons with findings from single nucleus RNA-Seq confirmed antidepressant effects on specific hippocampal cell types, including promoting an immature phenotype in dentate granule neurons. For the cortex, 14 studies were identified, containing 17 antidepressant vs. control group comparisons (collective n=260). Of the 14,344 genes with stable meta-analysis estimates, only one was consistently differentially expressed (FDR<0.05: *Atp6v1b2*), but the overall pattern of expression correlated with that observed in the hippocampus.
Conclusion: Genes and pathways that are consistently differentially expressed across treatment categories may serve as linchpins for antidepressant efficacy, providing promising targets for novel therapies. Future work should explore the relevance of these findings to human clinical populations, and explore potential heterogeneity introduced by sex, region, and drug category.

Keywords: Antidepressant, Hippocampus, Meta-analysis, RNA-Seq

Graphical Abstract



Key Points

- Depression can be treated with traditional antidepressants targeting monoaminergic function, as well as multiple other drug classes and non-pharmaceutical interventions.
- Understanding the congruent effects of different types of antidepressant treatments on sensitive brain regions, such as the hippocampus and cortex, can highlight essential mechanisms of action.
- A meta-analysis of public transcriptional profiling datasets identified genes and functional gene sets that are differentially expressed across antidepressant categories.

Plain Language Summary

Major depressive disorder is characterized by persistent depressed mood and loss of interest and pleasure in life. Worldwide, an estimated 5% of adults suffer from depression, making it a leading cause of disability. The current standard of care for depressed individuals includes psychotherapy and antidepressant medications that enhance signaling by monoamine neurotransmitters, such as serotonin and norepinephrine. Other treatments include non-traditional antidepressants that function via alternative, often unknown, mechanisms. To identify mechanisms of action shared across different categories of antidepressants, we performed a meta-analysis using public datasets to characterize changes in gene expression (mRNA) following treatment with both traditional and non-traditional antidepressants. We focused on the hippocampus and cortex, which are two brain regions that are sensitive to both depression and antidepressant usage. We found 59 genes that had consistently higher or lower levels of expression (mRNA) across antidepressant categories. The functions associated with these genes were diverse, including regulation of stress response, the immune system, brain growth and adaptability. These genes are worth investigating further as potential linchpins for antidepressant efficacy or as targets for novel therapies.

Introduction

Major Depressive Disorder (MDD) is a serious psychiatric disorder characterized by consistent depressed mood or markedly diminished pleasure in daily activities [1]. Worldwide, an estimated 5% of adults suffer from depression, making it a leading cause of disability [2,3] and increased mortality [4]. The current standard of care includes evidence-based psychotherapies and specific classes of drugs, which may be guided by blood biomarkers predictive of treatment outcomes [5,6]. Although antidepressant treatments are effective for some patients, around 30-40% of patients fail to respond to first-line treatment [7].

To improve individualized treatment options, the mechanisms of action for different antidepressant treatments needs to be investigated further to understand both their congruent and differing neurobiological effects. Many first-line antidepressants enhance monoamine neurotransmitter function, such as the serotonergic, noradrenergic, and dopaminergic systems. These include selective serotonin reuptake inhibitors (SSRIs), serotonin-norepinephrine reuptake inhibitors (SNRIs), tricyclic antidepressants (TCAs) and monoamine oxidase inhibitors (MAOIs) [8–10]. However, monoamine potentiation may not be the direct mechanism of action of these drugs, as monoamine potentiation occurs rapidly and clinical improvement usually requires several weeks [9].

Other classes of pharmacological treatments have shown promise for treating depression. Ketamine, a N-Methyl-D-aspartate receptor (NMDAR) antagonist, has rapid-acting antidepressant effects at subanesthetic doses [11–13]. Agomelatine has synergistic effects as an agonist of melatonin and some serotonin receptors [7,14,15]. Tianeptine is an atypical tricyclic believed to have antidepressant effects via μ-opioid receptor agonism [16,17]. Other promising new classes include psychedelics (MDMA, psilocybin)[18] and second generation antipsychotics (e.g., quetiapine)[19]. Finally, alpha-2 adrenergic antagonists may accelerate TCA treatment response times by reversing treatment-related alpha-2 adrenoceptor desensitization [20,21]. Depression is also treated using neuromodulatory brain stimulation therapies, such as electroconvulsive therapy (ECT), deep brain stimulation (DBS) and transcranial magnetic stimulation (TMS) [22–27]. Altogether, the diversity of pathways targeted by these new treatments suggests that monaminergic effects may not be central to antidepressant response, or may lie upstream of other critical pathways.

In addition to diverse molecular targets, there are multiple brain regions that may mediate antidepressant response. Antidepressant treatments are often theorized to alleviate depressive symptoms via effects on the hippocampus. Hippocampal volume reduction is common in patients with depression. This atrophy may be due to prolonged exposure to stress hormones (glucocorticoids) and subsequent reduction in neurotrophic factors and adult hippocampal neurogenesis (AHN) in the dentate gyrus (DG) [28], both of which precede mood disturbance [29–31]. Antidepressants reverse or prevent hippocampal atrophy in depression. Growing evidence suggests that both traditional, monoaminergic-targeting antidepressants (*e.g.,* SSRIs, TCAs) [32,33] and other antidepressant treatments (*e.g.,* ECT) [34,35] reverse hippocampal atrophy by stopping stress-induced dendritic retraction, increasing growth factor signalling (*e.g.,* brain-derived neurotrophic factor (BDNF) [11,14,15,36], and increasing hippocampal neuroplasticity and AHN [32,37,38]. However, it is still unclear whether increases in hippocampal neuroplasticity and AHN are a central mechanism for all antidepressant classes.

Some antidepressants may also target the cortex. Depressed patients have documented cortical abnormalities, including thinner cortical gray matter [39] and reduced prefrontal cortex (PFC) volume [40]. Rodent models of depression show similar structural and physiological changes, including dendritic shrinkage and reductions in myelination in the PFC [41]. Antidepressant treatments may reverse these changes by directly modulating cortical function. For example, TMS is thought to facilitate synaptic plasticity within the target site, which is most commonly the dorsolateral prefrontal cortex (DLPFC) [26] , by depolarizing neurons and increasing cortical activity [42], and antidepressant DBS targets cortical white matter tracts [22]. Antidepressant pharmaceuticals can also modulate cortical activity. As an NMDA receptor antagonist, ketamine decreases cortical GABAergic interneuron activity, causing downstream

disinhibition [12] and increasing synaptic plasticity [11–13]. Similarly, monoaminergic antidepressants (SSRIs, SNRIs) have been shown to increase activity or metabolism in frontal cortical regions [43]. However, whether cortical plasticity is a central mechanism for all antidepressant classes remains unknown.

In order to better understand the effects of antidepressant treatments on the hippocampus and cortex, we conducted a meta-analysis of publicly available rodent transcriptional profiling data. Transcriptional profiling technologies, such as ribonucleic acid-sequencing (RNA-Seq) and microarray, quantify gene expression in tissues. By conducting a meta-analysis across studies using different classes of antidepressants, we hoped to identify patterns of gene expression common to a wide variety of antidepressant treatments while also increasing statistical power to provide stronger insights into gene expression patterns underlying functional changes.

Methods

The meta-analysis project was conducted using a standardized pipeline (protocol: [44], validation: [45], *not pre-registered*). The analysis code (R v.4.4.1 and RStudio v.2024.9.9.365 [46,47]) is available at: *https://github.com/evageoghegan/AntidepressantMetaAnalysis*.

Information source

The Gemma Database was used to access curated and reprocessed public transcriptional profiling data [48,49]. Gemma contains differential expression results for nearly 20,000 publicly available transcriptional profiling datasets, representing approximately 600,000 samples [49,50]. We focused on this database to provide consistency in our meta-analysis input: Gemma adheres to a strict, standardized pipeline for dataset curation, preprocessing, and re-analysis [48,49]. During preprocessing, Gemma realigns the datasets to an updated genome. Quality control measures include identification and removal of outlier samples, removal of genes (rows) with minimal variance in expression values (either zero variance or <70% distinct values), and manual curation for common issues such as batch effects [48]. Differential expression is calculated using the *limma* or *limma-voom* pipeline, and statistical output is available for the full model (omnibus) and individual contrasts.

Dataset identification and search

For our meta-analysis, we focused on treatments that specifically target depressive mood symptoms [51]. A list of common traditional antidepressants, corresponding brand names and drug classes was compiled (**Fig. 1**). Non-traditional antidepressant treatments were added if there was approved clinical use or high-quality peer-reviewed clinical evidence available for potential antidepressant effects. These included ECT, TMS, DBS, ketamine, 3,4-methylenedioxymethamphetamine (MDMA), psilocybin, tianeptine, quetiapine, and agomelatine [14,18,19,23,27,52–55]. The scope for the search included transcriptional profiling datasets from laboratory rats (*Rattus norvegicus*) or laboratory mice (*Mus musculus*) using bulk tissue dissections that targeted the full or large representative portion of the transcriptome. Search terms were initially generated by the first, second, and third author (EMG, EH, and SE) and decisions reviewed and approved by co-authors (MHH, RH, SE, MRB, SM). The search terms were then structured with Boolean operators (PRISMA diagram: **Fig. 1**) and the search conducted using Gemma's application programming interface (API, packages *gemma.R* (v.3.1.6), *plyr* (v.1.8.9) and *dplyr* (v.1.8.9) [49,56,57]).

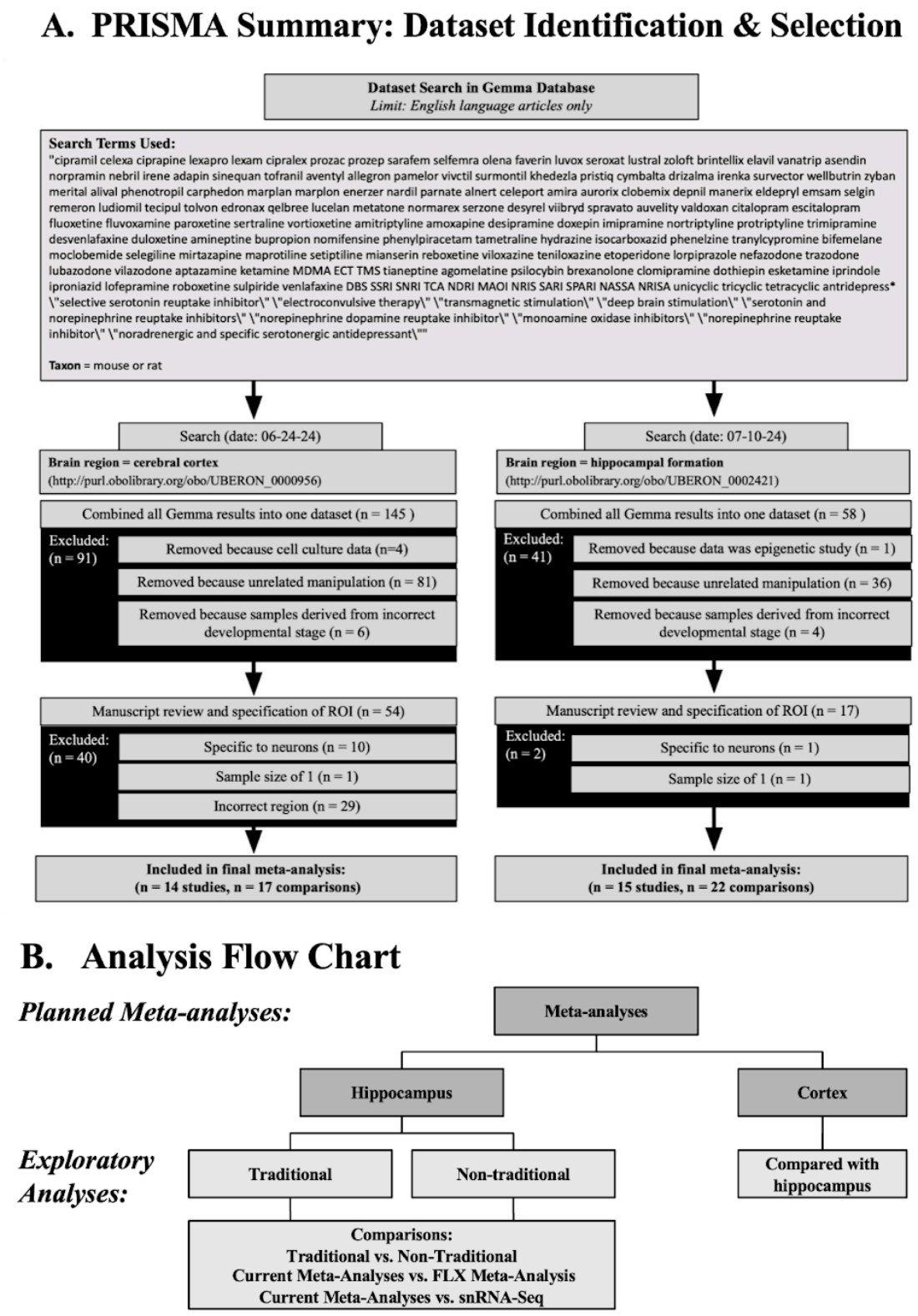


Figure 1. A PRISMA diagram overviewing hippocampal and cortical dataset search and selection. A. *We identified transcriptional profiling datasets of laboratory rodents treated with antidepressants within*

the Gemma database using pre-specified keywords. The search encompassed tissue from the hippocampal formation. The titles, abstracts, and metadata for the datasets were initially scanned and filtered using pre-specified inclusion/exclusion criteria, including indications that the dataset was an epigenetic study or cell culture, a manipulation unrelated to antidepressants or samples originated from the incorrect developmental stage. The secondary screening step included a detailed review of the metadata on Gemma and published methodology, followed by a final specification of the region of interest (ROI: Hippocampus). Datasets were excluded in the secondary dataset filtering if they did not include a sufficient sample size, were cell-specific or sampled a different region. ***B.*** *A flow chart overviewing the analyses, including planned meta-analyses examining the converging effects of antidepressants on the hippocampus and cortex, and exploratory analyses examining effects within particular antidepressant categories (traditional monoaminergic-targeting vs. non-traditional mechanisms) and comparisons between meta-analysis results and previous work, including a previous meta-analysis examining the effects of FLX on the hippocampus* [58] *and snRNA-Seq studies examining the effect of FLX and ECT on hippocampal cell types* [59,60]*.*

Initial filtering

The metadata for the identified datasets was filtered by "organism part" using the formal hierarchical ontology term for the hippocampus (UBERON_0002421) or cerebral cortex (UBERON_0000956). Metadata records were then filtered down to publicly available datasets that were not labeled as problematic within the Gemma database. The identified datasets were scrutinized for inclusion/exclusion based on systematic criteria [44]. Initial filtering for the hippocampal meta-analysis was performed by the first author (EMG), and then decisions were reviewed and approved by co-authors (MHH, RH). Forty-one studies were removed because either 1) treatment was administered during development, 2) brain collection was during development, 3) the focus was epigenetic, or 4) the manipulation was unrelated to antidepressant treatment. Initial filtering for the cortical meta-analysis was performed by two researchers (SE, EMG), and then decisions were reviewed and approved by co-authors (MHH, RH, MRB). Ninety-one studies were removed because either 1) the manipulation was unrelated to antidepressant treatment, 2) brain collection was during development, or 3) the studies were in cell culture.

Secondary filtering

For the remaining studies, metadata for the differential expression results were downloaded and reviewed to ensure that there was an experimental manipulation relevant to antidepressant treatment and related statistical contrasts. The publications associated with the datasets were also referenced, focusing exclusively on methodological information to determine inclusion/exclusion. Following this review, two datasets were removed from the hippocampal meta-analysis: one due to being neuron-specific and another due to having a sample size of n=1 per subgroup. For the cortical meta-analysis, forty datasets were removed: ten due to being neuron-specific, twenty-nine due to focus on non-cortical regions, and one due to having a sample size of n=1 per subgroup. At this stage, all inclusion/exclusion decisions were reviewed and finalized by the entire 2024 cohort of the *Brain Data Alchemy Project* (MHH, SE, EIF, SM, RB, LTC, AL, DMN, TQD) and laboratory principal investigator (RH). Overall, the risk of systematic bias within the datasets from the individual transcriptional profiling studies was deemed low for any particular gene-level result, as the original publications conducted full genome analyses and the data was re-analyzed using a standardized pipeline. That said, it should be noted that the bias in the literature against negative results may inflate effect sizes represented in publicly-released datasets, but in a manner that should be impartial to direction-of-effect.

Reprocessing

Four studies required reprocessing: GSE118670, GSE26836, GSE84183 and GSE43261. For GSE118670, GSE26836, and GSE43261, the differential expression model used by Gemma included multiple tissue types rather than just hippocampal tissue. For GSE43261, the differential expression model also used treatment sensitivity phenotype as the variable of interest rather than the treatment itself. For GSE84183, the expression data appeared log(2) fold transformed twice during Gemma's preprocessing.

To re-analyze these datasets, we followed Gemma's analysis pipeline as much as possible to maintain consistency. We used the *Gemma.R* (v.0.99.41) and *tidyverse* (v.2.0.0) [48,50] packages to import preprocessed log(2) expression data and sample metadata from the Gemma database (import date: 10/22/2024). Each dataset was subsetted to only our brain regions of interest, and the Gemma Diagnostics tab was consulted to determine whether outlier samples or batch effects were previously identified (*none found*). For GSE118670, adult and developing comparison mice were also excluded, as they did not receive antidepressant or control treatment conditions.

Genes (rows) were excluded that lacked log(2) expression data or had insufficient variability within the antidepressant or control groups. The control group for the antidepressant treatment was defined as the treatment factor reference level (intercept). Any additional experimental variables present in the metadata were included as co-variates (GSE84183: stress treatment, with a reference level of no stress, GSE118670, GSE43261, and GSE26836: no co-variates). The differential expression model was fit using the *limma* pipeline (package: *limma* v.3.17) with correction for heteroskedasticity (mean-variance trend) and an empirical Bayes correction [61].

Result extraction & meta-analysis

Differential expression results for the antidepressant treatment vs. control statistical contrasts from each included study were extracted. The Log(2) Fold Change (Log2FC) was extracted for each gene from each study, and standard error (SE) and sampling variance calculated. If more than one result represented a gene, the Log2FC and SE were averaged. Results were then aligned across species using EntrezID and the Jackson Labs Mouse Ortholog Database [62] trimmed down to the best supported orthologs. For a gene to be included in the hippocampal meta-analysis, a minimum number of 11 results (contrasts) needed to be available. For a gene to be included in the cortical meta-analysis, a minimum number of 5 results (contrasts) needed to be available, as there were fewer contrasts available. A simple (intercept-only) random effects meta-analysis was then fit to the Log2FC estimates from each contrast (package: *metafor* [63] (v. 4.8.0)). A false discovery rate (FDR) correction using the Benjamin-Hockberg method was applied to the nominal meta-analysis p-values (*multtest* v.1.32.0) [64]. Differentially expressed genes (DEGs) were defined using a threshold of FDR<0.05, and visualized using forest plots (package: *metafor* [63] (v. 4.8.0)).

Exploratory follow-up analysis

In order to explore the effects of different treatment categories on the hippocampus, two subgroup meta-analyses were run using only the contrasts characterizing the effects of: 1) traditional, monoaminergic-targeting antidepressants, 2) non-traditional antidepressants (**Table 1**). Based on dataset availability, for a gene to be included in the traditional subgroup meta-analysis, differential expression results needed to be available from a minimum of 12 antidepressant vs. control comparisons, whereas for the non-traditional subgroup meta-analysis the minimum was 7 antidepressant vs. control comparisons.

To compare the pattern of antidepressant differential expression identified in the different meta-analyses (planned hippocampal and cortical meta-analyses, exploratory traditional and non-traditional antidepressant meta-analyses), Pearson's product moment correlation was calculated and visualised using scatterplots, and ranked gene lists compared using Spearman's rank correlation. To explore other potential sources of heterogeneity, hierarchically clustered heatmaps were created using the Log2FCs from each of the included studies for the following ranked gene lists: planned hippocampal meta-analysis (all 58

significant DEGs), planned cortical meta-analysis and exploratory subgroup meta-analyses (top 50 genes, as ranked by p-value).

Dataset ID	Total *n*	HPC *n*	CTX *n*	Species	Sex	Authors	Year	Platform	Tissue	Model of Stress/ Depression	Treatment
GSE109445	12	12 (9)	NA	Rat	M	Wang *et al.,*	2018	Illumina (RNA-Seq)	Ammon's horn	CUMS, Ctrl	**FLX (3), Ctrl (6)**
GSE123027	24	24	NA	Mouse	M	Rimmerman *et al.*,	2021	Illumina (RNA-Seq)	Ammon's horn	CUMS	*ECT (10), Ctrl (14)*
GSE205325	21	21	NA	Rat	M	Demin *et al.*,	2022	Illumina (RNA-Seq)	Ammon's horn	CUMS, CUMS-LPS, Ctrl	**FLX (9), Ctrl (12)**
GSE230148	40	40	NA	Rat	M	Weiler *et al.*,	2023	Agilent (microarray)	Ammon's horn	Aged w/ or w/o cog. impairment*, Adult Ctrl	*TMS-mod (14), TMS-1 Hz (12), Ctrl (14)*
GSE230149	56	28	28	Rat	M	Weiler *et al.*,	2023	Agilent (microarray)	Ammon's horn, Parietal cortex	Aged w/ or w/o without cog. impairment*, Adult Ctrl	HPC: *TMS (13), Ctrl (15)* CTX: TMS (14), Ctrl (14)
GSE27532	16	16	NA	Mouse	M	Lisowski *et al.*,	2011	Illumina (microarray)	DG	Bred for High/ Low Stress Analgesia	**Desipramine (8), Ctrl (8)**
GSE56028	21	21	NA	Rat	M	Patrício *et al.*,	2015	Agilent (microarray)	DG	CUMS, Ctrl	**Imipramine (3), FLX (3),** *Agomelatine (3), Tianeptine (3),* ***Ctrl (9)***
GSE61301	8	8	NA	Rat	M	Husain *et al.*,	2015	Agilent (microarray)	Ammon's horn	Ctrl	Imipramine+yohimbine (4), Ctrl (4)
GSE63469	6	6	NA	Mouse	M	Proft *et al.*,	2019	Affymetrix (microarray)	Ammon's horn	Stress sensitive (DBA/2) mice	**Venlafaxine- 100kg/d/kg (2), Venlafaxine- 30kg/d/kg (2), Ctrl (2)**
GSE73798	60	60 (24)	NA	Mouse	M	Ficek *et al.*,	2019	Illumina (microarray)	Ammon's horn	Ctrl	*Ketamine (12),Ctrl (12)*
GSE81672	99	24	25	Mouse	M	Bagot *et al.*,	2018	Agilent (microarray)	Ammon's horn, PFC	CSDS, Ctrl	HPC: *Ketamine (6),* **Imipramine (7),** ***Ctrl (11)*** CTX: Ketamine (6), Imipramine (7), Ctrl (12)
GSE43261	38	38	NA	Mouse	M	Samuels *et al.*,	2014	Affymetrix (microarray)	DG	CORT*	HPC-ventral: **FLX (11), Ctrl (8)** HPC-dorsal: **FLX (11), Ctrl (8)**
GSE84183	64	32	32	Mouse	M	Hervé *et al.*,	2017	Agilent (microarray)	DG, Anterior cingulate	CUMS*, Ctrl	HPC: **FLX (16), Ctrl (16)** CTX: FLX (16), Ctrl (16)
GSE118670	44	16	28 (16)	Mouse	M	Hagihara *et al.*,	2019	Affymetrix (microarray)	DG, PFC	Ctrl	HPC: **FLX (8), Ctrl (8)** CTX: FLX (8), Ctrl (8)
GSE26836	12	6	6	Mouse	M	Chadwick *et al.*,	2015	Illumina (microarray)	Ammon's horn, Frontal cortex	Aged Alzheimer's genetic model*	HPC: **Amitriptyline (3), Ctrl (3)** CTX: Amitriptyline (3), Ctrl (3)
GSE28644	60	NA	60	Mouse	M	Benton *et al.*,	2012	Affymetrix (microarray)	Cerebral cortex	30 inbred strains	FLX (30), Ctrl (3)
GSE93041	9	NA	9 (6)	Mouse	M	Orozco-Solis *et al.*,	2018	Affymetrix (microarray)	Anterior cingulate	Ctrl	Ketamine (3), Ctrl (3)
GSE150264	36	NA	36	Mouse	F	Rajkumar *et al.*,	2020	Agilent (microarray)	Anterior cingulate	Genetic depression model, Ctrl	Imipramine (19), Ctrl (17)
GSE168172	15	NA	15	Mouse	M	Funayama *et al.*,	2022	Illumina (RNA-Seq)	PFC	Stress sensitive (BALB) mice	Duloxetine (6), Sertraline (6), Ctrl (3)
GSE138802	6	NA	6	Mouse	M	Kokkinou *et al.*,	2021	Illumina (RNA-Seq)	PFC	Ctrl	Ketamine (3), Ctrl (3)
GSE129359	24	NA	24 (12)	Mouse	M	Lukić *et al.*,	2019	Illumina (RNA-Seq)	PFC	Ctrl	Duloxetine (6), Ctrl (6)
GSE45229	20	NA	10 (6)	Mouse	M	Kondo *et al.*,	2013	Affymetrix (microarray)	Frontal cortex	Ctrl	Quetiapine-10 mg/kg (2), Quetiapine-100 mg/kg (2), Ctrl (2)
GSE253280	36	NA	18 (12)	Rat	M	Warner-Schmidt, *et al.*,	2024	Illumina (RNA-Seq)	Frontal cortex	Ctrl	MDMA (6), Ctrl (6)

Table 1. Overview of datasets included in the meta-analyses. *Color refers to datasets included in specific meta-analyses: hippocampal (yellow), hippocampal and cortical (green), cortical (blue). Experiment ID refers to the Gene Expression Omnibus (GEO) accession number for each dataset. The Author column refers to the first author of the publication where available* [65–85]*. The Year column refers to the dataset release date listed on Gemma. All experimental details describe the samples used in the transcriptional profiling experiment (e.g., species, biological sex). The Total n indicates the number of tissue samples in the full dataset, whereas the HPC n and CTX n indicate the number of hippocampal and cortical samples included in the full (omnibus) statistical model for each brain region, respectively, with the sample size in parentheses indicating the number of samples represented in the antidepressant treatment-related statistical contrasts (if different from the full model). The Platform column indicates the transcriptional profiling technology used. The Tissue column indicates the specific region/subregion sampled. Model of Stress/Depression lists relevant subject characteristics or chronic stress conditions included in the experiment (acute stress due to behavioral assays or routine procedures not noted). Treatment column lists each treatment group (with # of samples) included in the treatment vs. control statistical contrasts used in the meta-analysis. Treatment categories included SSRIs (FLX, sertraline), TCAs (imipramine, desipramine, amitriptyline), SNRIs (venlafaxine, duloxetine), NMDA receptor antagonists (ketamine), Alpha-2 adrenergic antagonists combined with TCAs (yohimbine and imipramine), atypical antidepressants (tianeptine), MASSAs (agomelatine), atypical antipsychotics (quetiapine), serotonin–norepinephrine–dopamine releasing agent (SNDRA) (MDMA), and neuromodulatory brain stimulation (ECT, TMS). Within all hippocampal datasets (yellow and green), bolded treatments indicate inclusion in the traditional-only subanalysis and italicized treatments indicate inclusion in the non-traditional only subanalysis. Abbreviations: DG: Dentate Gyrus, PFC: Prefrontal Cortex, FLX: fluoxetine, Ctrl: Control, CUMS: chronic unpredictable mild stress, CSDS: chronic social defeat stress, OBX: olfactory bulbectomy, LPS: lipopolysaccharide (LPS)-induced depression-like model, SIA: stress-induced analgesia, CSDS: chronic social defeat stress*

Functional patterns

Gene set enrichment analysis was used to determine whether the hippocampal and cortical meta-analysis results, ranked by effect size (Log2FC), were enriched with down-regulation or upregulation in sets of genes associated with known biological pathways and previous experimental results. Both directional (Log2FC) and non-directional (abs(Log2FC)) versions of the analysis were carried out using the *fgsea* (v.1.32.0) package [86] (parameters: nperm=10000, min size=10, max size=1000) and a gene set database including both traditional gene ontology gene sets and custom brain-related gene sets (*Brain.GMT*: [87]). P-values were corrected for FDR using the Benjamini-Hochberg method.

To explore cell type specificity, gene set enrichment analysis was also used to compare the results of the hippocampal meta-analyses (full, traditional and non-traditional antidepressant meta-analyses) to previously-documented effects of FLX on hippocampal cell types [59] or electroconvulsive shock (ECS) on hippocampal neurons [60] as measured by single nucleus RNA-Seq (snRNA-Seq). Gene sets were constructed representing all genes that were significantly upregulated or downregulated in response to FLX (5 days of treatment or 3 weeks of treatment) or 10 sessions of ECS (20 days of treatment) in each of the hippocampal cell type clusters (46 gene sets total). Two gene sets were also constructed representing the "developmental-like state" that was shown to increase in response to FLX and ECS, as measured using the top 100 genes upregulated and down-regulated in p5 vs. p132 DG granule cells [88]. Enrichment

within this custom gene set database was calculated using fGSEA using the procedure described above, but allowing for larger gene sets (max size=2500), followed by FDR correction.

Comparison with previous FLX meta-analysis (Ibrahim et al. 2022)

The effects of the FLX on the hippocampus [58] were recently characterized using meta-analyses of transcriptional profiling results from either stressed (7 studies, n=75) or stress-naive rodents (7 studies, n=56). Due to differing inclusion/exclusion criteria, only 66% of the samples included in their meta-analyses were in our own meta-analysis (n=87 of 131), contributing 28% to our final sample size (n=87 of 313). Therefore, their results can provide insight that is partially independent from our own. Their publication provided results for the top DEGs identified using two methods of integrating findings across studies (integration method and portrait method) and consensus scores indicating whether those DEGs were predominantly upregulated or downregulated for the stressed (*Table 2 & 3 in* [58]) and stress-naive animals (*Table 6 & Table 7 in* [58]). For comparison with our results, consensus scores from these tables were converted to a categorical variable indicating "Up" or "Down" direction of effect, which was then tested as a predictor for effects within our meta-analyses (Log2FCs) using standard bivariate linear regression.

Results

Hippocampus

Characteristics of the datasets included in the hippocampal meta-analysis:

Fifty-eight datasets were identified using prespecified antidepressant search terms (**Fig. 1**). Of these, fifteen datasets survived inclusion/exclusion criteria, leaving a final collective sample size of *n*=313 hippocampal samples from antidepressant-treated and control subjects. Most datasets were collected using microarray technology; the remaining three were by RNA-seq. Two-thirds were derived from whole Ammon's horn tissue, with the remaining specified as DG. The datasets included 22 antidepressant vs. control comparisons, representing eleven types of treatments, including pharmacological treatments (SSRIs, TCAs, SNRIs, NMDA receptor antagonists, Alpha-2 adrenergic antagonists with TCAs, atypical antidepressants, and MASSAs) and non-pharmacological treatments (ECT, TMS) (**Table 1**).

The antidepressant treatments were tested using a variety of rodent depression models, including chronic unpredictable mild stress (CUMS), lipopolysaccharide-induced depression-like behavior, chronic corticosterone treatment, stress-susceptible strains, and chronic social defeat stress (CSDS) [66,69,70,73–75,89]. Three datasets included aged subjects, some with transgenically-induced cognitive impairment [68,77]. Three datasets utilized no depression model [71,72,76]. For species, most datasets used *Mus musculus*, with six using *Rattus norvegicus*. All samples were male (**Table 1**).

Hippocampal meta-analysis reveals consistent differential expression across antidepressant categories:

To be included in the meta-analysis, a gene needed to be present in a minimum of eleven antidepressant vs. control comparisons. 16,494 genes fulfilled this requirement, and 16,439 genes produced stable meta-analysis estimates (*full results:* **Table S1**). Fifty-eight genes were significantly differentially expressed (FDR<0.05, "DEGs", **Table S2, Table S3**), of which 23 were consistently upregulated and 35 consistently downregulated following antidepressant treatment (*example forest plots:* **Fig. 2**) often in 19 or more comparisons. A hierarchically-clustered heatmap of the top 58 DEGs did not show any obvious pattern in treatment type, platform or tissue type (**Fig. 3**).

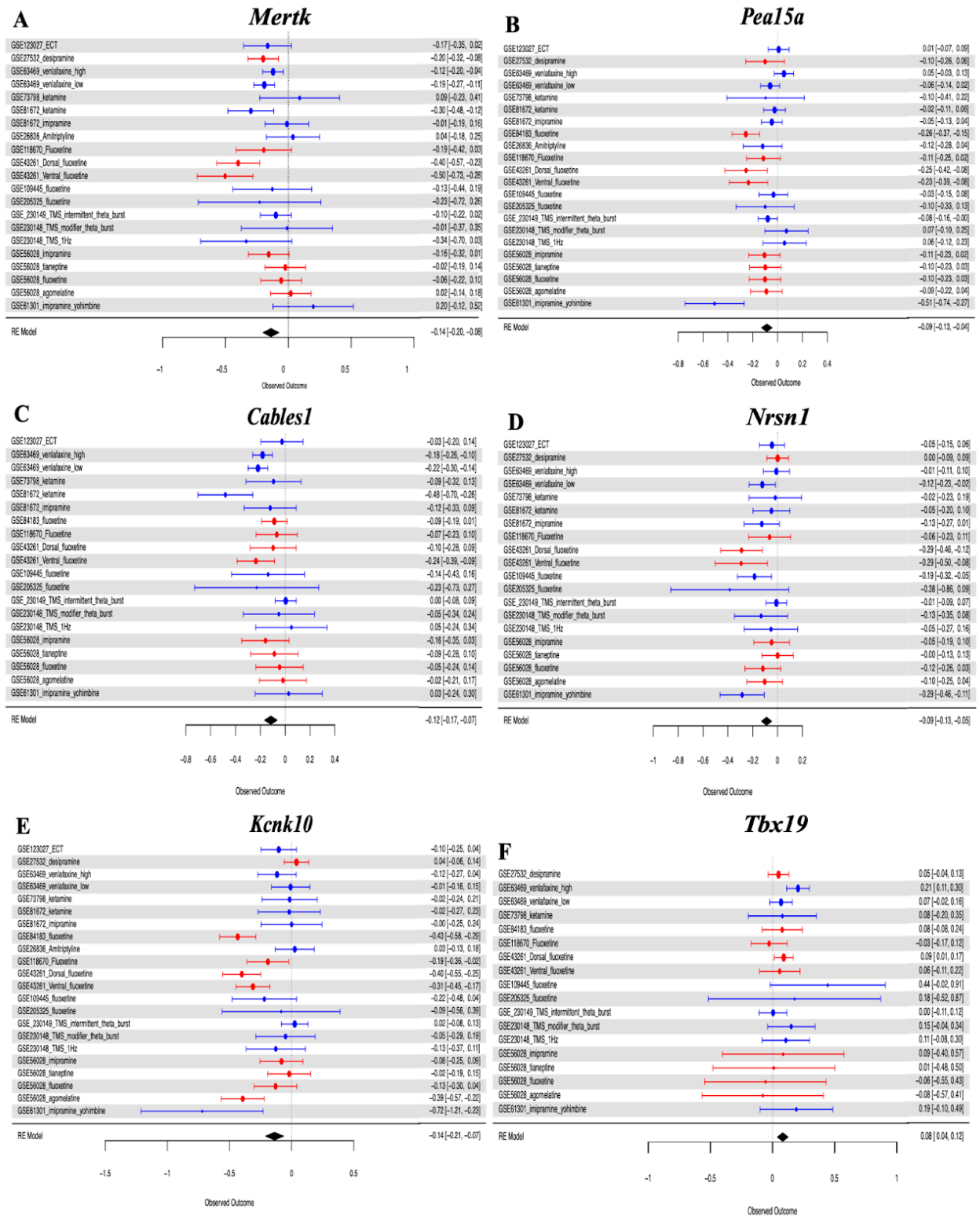


Figure 2. Hippocampal meta-analysis reveals consistent differential expression across antidepressant categories: Example forest plots for six of the top DEGs (FDR<0.05). *Rows illustrate Log2FC (circles)*

with 95% confidence intervals (whiskers) for each of the datasets and the meta-analysis random effects model ("RE Model"). Color indicates tissue type, with DG in red and Ammon's horn in blue. Forest plots allow for visual inspection of the consistency and magnitude of effects across all comparisons. ***A.*** *MER proto-oncogene tyrosine kinase (Mertk). Significantly differentially expressed across 21 datasets, downregulated.* ***B.*** *Proliferation and apoptosis adaptor protein 15A (Pea15a). Significantly differentially expressed across all 22 comparisons, downregulated.* ***C.*** *Cdk5 And Abl Enzyme Substrate (Cables1). Significantly differentially expressed across 20 datasets, downregulated.* ***D.*** *Neurensin 1 (Nrsn1). Significantly differentially expressed across 20 datasets, downregulated.* ***E.*** *Potassium Two Pore Domain Channel Subfamily K Member 10 (Kcnk10). Significantly differentially expressed across all 22 comparisons, downregulated.* ***F.*** *T-box Transcription Factor 19 (Tbx19). Significantly differentially expressed across 18 datasets, upregulated.* ***Definitions:*** *Log2FC = Log2 Fold Change (Antidepressant treatment vs. Control), SE = Standard Error, CI_lb=confidence interval lower bound, CI_ub=confidence interval upper bound, p-value = nominal p-value, FDR = False Discovery Rate (q-value). The #Comparisons represent the number of treatments that included measurements for the gene following quality control.*

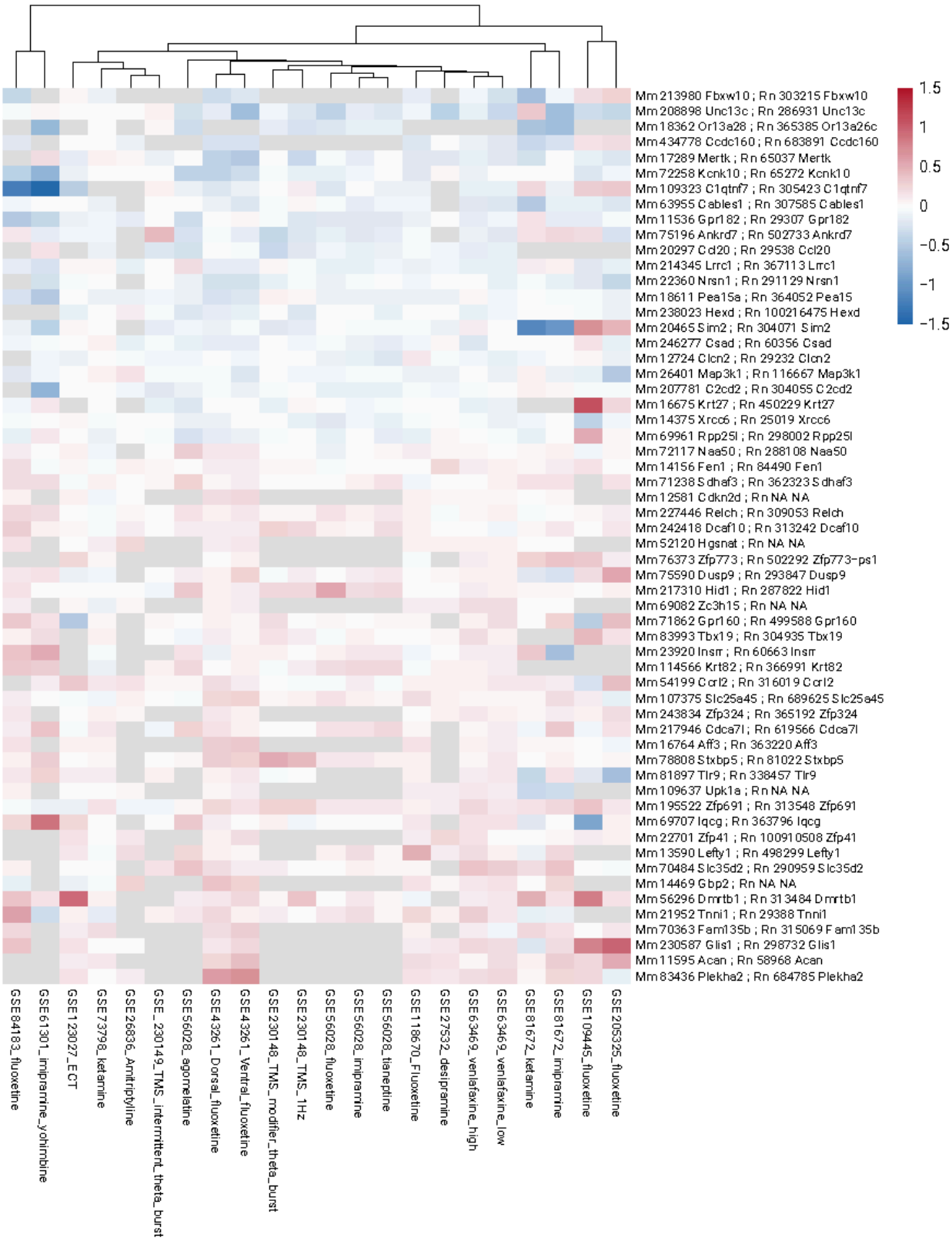


Figure 3. A hierarchically-clustered heatmap of antidepressant effects across hippocampal datasets does not show any obvious pattern related to treatment type, tissue type, or platform. *The heatmap*

allows for visual comparison of the expression patterns of the 58 hippocampal meta-analysis DEGs (FDR<0.05) across individual datasets. The color scale indicates standardized effect size (log_2 fold change), with red denoting upregulation and blue denoting downregulation relative to control samples. Each column represents results from an individual antidepressant treatment (vs. control) comparison from the included datasets, as identified using the Gene Expression Omnibus accession # (GSE…). Each row represents one gene, identified by mouse (Mm) Entrez ID and official mouse gene symbol and rat (Rn) Entrez ID and official rat gene symbol, with NA values indicating a lack of clear ortholog in the species. The dendrogram at the top groups datasets by similarity in their transcriptional profiles, with shorter connecting lines indicating greater similarity in gene expression changes across datasets.

Antidepressant effects on the hippocampus are enriched in many cell types and pathways previously linked to depression:

There were 114 gene sets that were significantly enriched with antidepressant differential expression in the non-directional fGSEA analysis (FDR<0.05, *full results:* **Table S4**), many of which are associated with factors modulating depression risk. Of these enriched gene sets, 62 contained at least one significant DEG amongst the "leading edge" genes driving the enrichment, and thus are of particular interest. The enriched gene sets containing DEGs spanned several categories (summarized in **Table 2**), including cell signalling (*e.g.,* cytokine activity, calcium binding, enzyme-linked kinase receptors), growth and development (*e.g.,* cell proliferation and differentiation, morphogenesis, the DG), central nervous system cell types (*e.g.,* pyramidal, GABA-ergic, and dopaminergic neurons, astrocytes, myelinating cells, ependyma), and vasculature-related cell types (e.g., endothelial cells, pericytes, fibroblasts). There was also enrichment in gene sets derived from previous differential expression studies, including several included in our meta-analysis (2 gene sets, *not shown*), and studies of psychiatric disorders, depression, stress-related animal models, and environmental enrichment. The directional version of the analysis identified 36 gene sets showing a non-significant trend towards enrichment with antidepressant effects (FDR<0.1, *full results:* **Table S5**), twenty-three of which overlapped the non-directional results (**Table 2**).

Gene Sets	DEGs in Leading Edge	Direction of Effect: HPC	Direction of Effect: CTX
Cell Signalling			
Cytokine production (GOBP)	*Mertk, Tlr9, Xrcc6*		
Cytokine receptor activity (GOMF)	*Ccrl2*		
Signaling via enzyme-linked transmembrane serine/threonine kinase receptors	*Mertk, Insrr, Lefty1*		
Calcium ion binding (GOMF)	*Unc13c*		
Receptor regulator activity (GOMF)	*Lefty1, Ccl20*		
Receptor complex (GOCC)	*Mertk, Insrr, Stxbp5, Gpr160*		
Molecular transducer activity (GOMF)	*Mertk, Insrr, Tlr9, Ccrl2, Gpr182, Gpr160*		
Growth and Development			
Negative regulation of cell population proliferation (GOBP)	*Cdkn2d*		
Regulation of cell differentiation	*Xrcc6*		
Epidermis development and morphogenesis	*Krt82, Krt27*		
Epithelial cell differentiation (GOBP)	*Krt82, Krt27, Upk1a*		
Muscle cell proliferation	*Xrcc6*		
Broad Biological Processes			
Aminoglycan metabolic and biosynthetic processes	*Hgsnat, Slc35d2*		
Regulation of cellular component movement (GOBP)	*Ccl20*		
Tissues/Brain Regions:			
DG vs. Other Hippocampal Regions (Hipposeq)	*Plekha2*		Down
Nervous System Cell Types:			
Neurons (generic)	*Nrsn1, Cdca7l, Unc13c*		
Neuron Pyramidal (CA1, Cortical)	*Stxbp5, Aff3*	Down	Up
Gaba-ergic interneurons	*Stxbp5, Aff3, Nrsn1, Cdkn2d, Csad*		
Dopaminergic neurons	*Stxbp5, Cdkn2d*		
Astrocytes	*Cables1, Mertk, Pea15a, Gbp2*	Down	Down
Myelinating glial lineage (OPCs, Schwann cells)	*Map3k1, Pea15a, Cdca7l, Ccdc160*		
Ependyma	*Iqcg, Plekha2*		
Radial glia-like cell types	*Map3k1, Pea15a, Iqcg*	Down	Down
Vasculature-related:			
Circulatory System Processes	*Tnni1*		
Endothelial and pericyte markers	*Map3k1, Gpr182, Aff3*		
Fibroblasts and stromal cells	*Gpr182, Krt27, C1qtnf7*		Down
Diagnosis-related:			
Down-regulated genes in the cortex of Bipolar patients (Gandal_2018)	*Cdkn2d*	Down *(*Consistent)*	Up *(*Reversal)*
Down-regulated genes in the cortex of Schizophrenia patients (Gandal_2018)	*Cdkn2d*	Down *(*Consistent)*	Up *(*Reversal)*
DEGs in the hippocampus replicated across bred rodent models of internalizing behavior (Birt, Hagenauer 2021)	*Naa50, Csad*		
Stress-related - recovery from stress:			
Down-regulated genes at timepoint 28d post-stress: 10 days chronic social defeat stress with 1h aggressor re-exposure (Gemma_GSE72343)	*Upk1a, Cables1, Mertk, Nrsn1, Slc35d2, Zfp773, Gpr160*	Up *(*Reversal)*	Up *(*Reversal)*
Upregulated genes at timepoint 28d post-stress: 10 days chronic social defeat stress with 1h aggressor re-exposure (Gemma_GSE72343)	*Glis1, Tlr9*		Down *(*Reversal)*
Down-regulated genes at timepoint 28d post-stress: 10 days chronic social defeat stress (Gemma_GSE72343)	*Mertk, Nrsn1, Slc35d2, Plekha2, Gpr160*	Up *(*Reversal)*	Up *(*Reversal)*
Upregulated genes at timepoint 28d post-stress: 10 days chronic social defeat stress (Gemma_GSE72343)	*Glis1*	Down *(*Reversal)*	Down *(*Reversal)*
Down-regulated genes from dentate gyrus neurons following environmental enrichment (Gemma_GSE95740)	*Kcnk10*	Down *(*Consistent)*	

Table 2. Antidepressant effects on the hippocampus are enriched in many cell types and pathways, some of which have been previously linked to depression. *Gene sets were identified as significantly enriched with antidepressant-related differential expression in the hippocampal meta-analysis via non-directional functional gene set enrichment analysis (fGSEA, FDR<0.05), and narrowed down to gene sets containing significant hippocampal antidepressant DEGs (FDR<0.05) amongst the leading genes driving the enrichment. Bold text indicates gene sets that are also significant (FDR<0.05) in the non-directional fGSEA analysis for the cortex. The direction of effect for the gene set is provided if the directional hippocampal or cortical fGSEA analysis trended towards significance (FDR<0.10). Gene sets have been organized into groupings based on function, and filtered to reflect brain-relevant functions. Gene set names have been reformatted and simplified for easy viewing, removing highly redundant terms with similar leading genes. Color indicates direction of effect; red: upregulated, blue: downregulated.*

Exploratory analysis: Traditional and non-traditional antidepressants have overlapping effects on the hippocampus

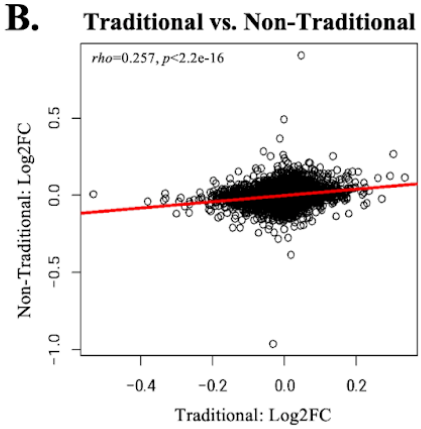
As an exploratory follow-up analysis, we compared the patterns of gene expression associated with traditional, mono-aminergic targeting antidepressants and non-traditional antidepressants, both pharmacological and neuromodulatory (**Table 1**, **Fig. 4A**). In the traditional antidepressant meta-analysis, ten datasets were included, with a collective sample size of *n*=177 samples used in thirteen antidepressant vs. control comparisons. In the non-traditional antidepressant meta-analysis, six datasets were included, with a collective sample size of *n*=148 samples used in eight antidepressant vs. control comparisons. For a gene to be included in the traditional subgroup meta-analysis, differential expression results needed to be available from a minimum of 12 antidepressant vs. control comparisons. Of the 9,628 genes fulfilling this requirement, 9,612 produced stable meta-analysis estimates. Sixty-five of these genes were significantly differentially expressed (FDR<0.05: 42 upregulated, 23 downregulated, *full results*: **Table S6**). For the non-traditional subgroup meta-analysis, a gene needed to be present in a minimum of 7 antidepressant vs. control comparisons. Of the 12,203 genes fulfilling this requirement, 12,154 produced stable meta-analysis estimates. None of these genes were significantly differentially expressed (*full results:* **Table S7**), although the pattern of non-traditional antidepressant-related expression moderately resembled that observed in the traditional subgroup meta-analysis (*R*=0.228, *df*=12,614, *rho*=0.257, *p*<2.2e-16) (**Fig. 4B**).

Exploratory analysis: The pattern of effects detected in previous FLX meta-analyses appear partially generalizable to other antidepressants:

The effects of FLX on hippocampal gene expression in stressed and stress-naive animals were recently characterized using meta-analyses of public transcriptional profiling studies [58]. When comparing our meta-analysis findings to their results, we found reasonable convergence, despite only partially overlapping samples (28%). Overall, we tended to observe the same direction of effect (**Table S8**), with more positive Log2FCs observed in our meta-analyses for genes previously found to be upregulated with FLX vs. down-regulated with FLX (**Fig. 4D**, *full meta-analysis*: R^2=0.438, β+/-SE=0.149+/-0.0146, T(135)=10.25, *p*<2.2e-16). This similarity was stronger with the results of our meta-analysis using only traditional antidepressants (**Fig. 4E**, R^2=0.447, β+/-SE=0.237+/-0.0254, T(105)=9.311, *p*<2.15e-15), which had an overlapping sample, but still present - although weaker - in our meta-analysis using non-traditional antidepressants (**Fig. 4F**, R^2=0.0969, β+/-SE=0.0227+/-0.00627, T(122)=3.618, *p*=0.000433). There was no overlap with our top DEGs, but 22 of the FLX genes showed the same direction of effect and nominal significance (p<0.05) in our full hippocampal meta-analysis, and only one showed the opposite direction of effect and nominal significance, providing suggestive support.

A.

	Traditional Monoaminergic Targets	Non-Traditional or Unknown Targets
Treatment Categories Represented in Exploratory Hippocampal Meta-Analyses	• **SSRIs**: *Fluoxetine* • **SNRIs**: *Venlafaxine*, **TCAs**: *Desipramine, Imipramine, Amitriptyline*	• **ECT** • **TMS** • **NMDAR antagonism**: *Ketamine* • **μ-opioid receptor agonism**: *Tianeptine* • **MASSA**: *Agomelatine*
Sample Size (*n*)	177	148
# Contrasts	13	8
# of DEGs	65	0



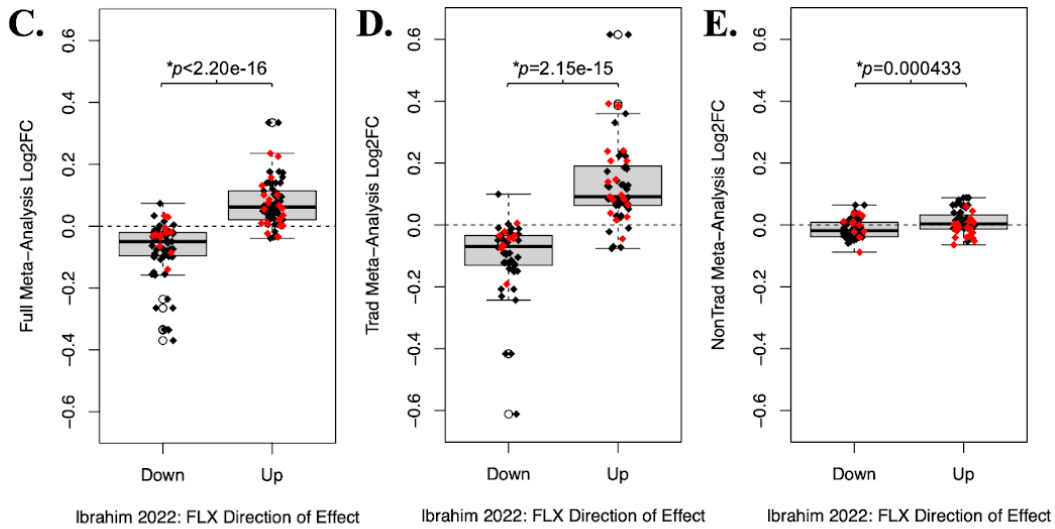


Figure 4. Exploratory analysis: Traditional and non-traditional antidepressants have overlapping effects on the hippocampus. A. *Table summarizing the types of antidepressant treatments, sample sizes, and number of antidepressant vs. control contrasts included for the exploratory meta-analyses examining the effects of traditional (n=177, 13 contrasts) and non-traditional (n=148, 8 contrasts) antidepressants on the hippocampus.* ***B.*** *Scatterplot comparing log_2 fold changes from the traditional and non-traditional antidepressant treated hippocampus across all genes.* ***C-E.*** *Boxplots with overlaid jittered data points illustrating that our meta-analyses replicated the direction of effect observed within previous meta-analyses of the effects of fluoxetine (FLX) on the hippocampus [58]. The previous meta-analyses used a sample that only partially overlapped with our own (28%) and thus provide semi-independent insight. The y-axis shows the Log2FC estimated within our antidepressant meta-analyses (**D:** full meta-analysis, **E:** traditional antidepressant meta-analysis, **F:** non-traditional antidepressant meta-analysis) for each of the top genes identified within the previous FLX meta-analyses of transcriptional profiling results from stressed (red) and stress-naive (black) samples using both reported methods ("portrait" or "integration"). The x-axis indicates the direction of effect identified by the consensus score in the FLX meta-analyses. Boxes = first quartile, median, and third quartile; whiskers = range or 1.5× the interquartile range; open dot = outlier datapoint falling beyond the whiskers of the boxplot.*

Exploratory analysis: Antidepressant effects in the current hippocampal meta-analyses reproduce effects detected in individual cell types using snRNA-seq

To follow up on the enrichment of antidepressant effects within gene sets associated with particular hippocampal cell types, we compared our meta-analysis results to the effects of antidepressant treatments observed in two snRNA-Seq studies: the effects of FLX (5 days or 3 weeks of treatment) on all hippocampal cell types [59] and the effects of ECS (10 sessions over 20 days of treatment) on hippocampal neuronal cell types [60]. We found that differential expression within the antidepressant meta-analyses was enriched within sets of genes that responded to three weeks of FLX and ECS treatment in multiple hippocampal cell type clusters (**Table S9**), including DG granule neurons, astrocytes, and other hippocampal neuron types (CA1, CA3, Mossy, GABA-ergic), and within sets of genes responding to 5 days of FLX treatment in microglia and oligodendrocytes. Furthermore, the enrichment of upregulation or downregulation within the meta-analyses results always paralleled the direction of effect observed in the snRNA-Seq experiments for treatments with demonstrable antidepressant efficacy (3 weeks of FLX and ECS). There was also an enrichment of antidepressant effects within two gene sets previously identified as signifying the re-activation of a "developmental-like state" in DG granule neurons, which was theorized to mediate the antidepressant effects of FLX [59] and ECS [60] (**Fig. 5**).

There was evidence of treatment heterogeneity: in general, the traditional antidepressant meta-analysis results tended to more closely resemble the FLX snRNA-Seq results than the non-traditional meta-analysis results (**Table S9, Fig. 5**). This might be expected due to FLX targeting traditional monoaminergic systems (SSRI). However, a similar bias was not observed for the ECS-derived gene sets, and the full meta-analysis results were strongly enriched with differential expression in gene sets derived from both FLX and ECS snRNA-Seq experiments. Notably, genes that were down-regulated in DG granule neurons following 3 weeks of FLX or ECS showed enrichment within all three of our hippocampal meta-analyses (full, traditional, and non-traditional).

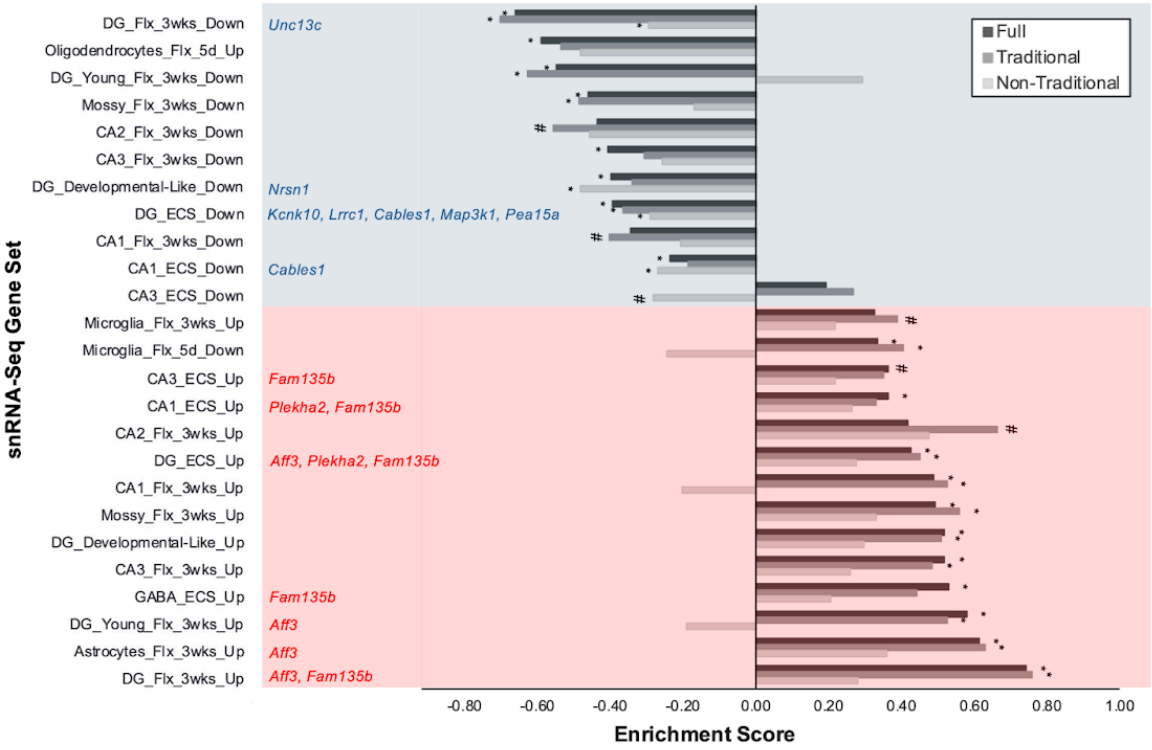


Figure 5. Meta-analyses of antidepressant effects identified in bulk hippocampal tissue uncover similar patterns of differential expression as those previously identified in specific hippocampal cell types using snRNA-Seq. *Sets of genes that were found to be differentially expressed in hippocampal cell types in response to fluoxetine (FLX: 5 days (5d) or 3 weeks (3wks) of treatment) or electroconvulsive shock (ECS, 10 days) as measured by snRNA-Seq were enriched with similar differential expression in our hippocampal antidepressant meta-analyses (full meta-analysis, exploratory meta-analysis focused on traditional antidepressants, exploratory meta-analysis focused on non-traditional antidepressants). For ease of visualization, only gene sets with at least nominal enrichment (#p<0.05) of differential expression in at least one of the meta-analyses were included in the figure, with asterisks indicating enrichment surviving false discovery rate correction (*FDR< 0.05). In general, the direction of effect for the enrichment in the antidepressant meta-analyses tends to parallel the direction of effect observed in the original snRNA-Seq results. Abbreviations: DG: Dentate Gyrus (granule cells), Mossy: Mossy Cells, CA: Cornus Ammonis (neurons), GABA: GABAergic interneurons.*

Cortex

Characteristics of the datasets included in the cortical meta-analysis:

Using prespecified antidepressant search terms, 147 datasets were identified (**Fig. 1**). Of these datasets, fourteen survived inclusion/exclusion criteria, leaving a final collective sample size of *n*=260 cortical samples from antidepressant-treated and control subjects. Most datasets were collected using microarray technology, with four using RNA-Seq. Most datasets focused on the frontal cortex (12), but dissection varied considerably: four datasets focused on the PFC, one on the prelimbic subregion, and four on the anterior cingulate. There was also a dataset from the parietal cortex, and "cerebral cortex," broadly defined (**Table 1**).

These fourteen datasets featured a variety of antidepressants including pharmacological treatments (SSRIs, SNRIs, TCAs), atypical antipsychotics, NMDA receptor antagonists, serotonin–norepinephrine–dopamine releasing agent (SNDRA) and non-pharmacological treatments (TMS). Most studies used no depression models [68,76–85], but sometimes represented other characteristics including aged transgenic mice [77] and inbred strains[78]. The remainder used CSDS and CUMS [73,75], a genetic depression model (*Brd1*+/- mice)[80], and stress-sensitive strain[81]. For species, all but two of the datasets used *Mus musculus*; the remainder used *Rattus norvegicus.* With one exception, the datasets used only males (**Table 1**).

Cortical meta-analysis reveals one gene consistently upregulated by antidepressants (Atp6v1b2):

To be included in the meta-analysis, a gene needed to be present in a minimum of five antidepressant vs. control comparisons. 14,449 genes fulfilled this requirement, and 14,344 genes produced stable meta-analysis estimates (**Fig 6B**, *full results:* **Table S10**). One gene, *ATPase H+ transporting V1 subunit B2* (*Atp6v1b2*) was upregulated across sixteen comparisons (FDR<0.05, **Fig 6D**). A further five genes trended towards significant differential expression (FDR<0.1) (**Table S11**). The heatmap of the top 50 genes as ranked by p-value showed no obvious pattern related to treatment type, sequencing platform or tissue type (**Suppl. Fig. 3**).

Antidepressant effects are enriched in microglial-related gene sets containing Atp6v1b2:

There were 171 gene sets that were significantly enriched with antidepressant differential expression in the non-directional fGSEA analysis (FDR<0.05, *full results:* **Table S12**). Four included *Atp6v1b2* amongst the "leading edge" genes driving the enrichment. A directional version of the analysis identified 113 gene sets that trended towards significant enrichment (FDR<0.1, *full results:* **Table S13**), with *Atp6v1b2* included in the leading edge of five, two of which were associated with developing microglia, upregulated, and significant in the non-directional analysis (FDR<0.05).

Antidepressant effects in the cortex show some resemblance to the hippocampus:

There was a positive correlation between the hippocampal and cortical meta-analysis results (R=0.343, df=13,942, rho=0.292, p<2.2e-16, **Fig 6C**), but no shared DEGs. Functional patterns overlapped: 52 gene sets were enriched with antidepressant-related differential expression in both brain regions. These included stress-related gene sets, extracellular matrix and structural support related gene sets, glial, fibroblast and stromal cell types, and gene sets related to psychiatric disorder (bipolar disorder, schizophrenia, and rodent internalizing behavior) (*examples:* **Table 2**).

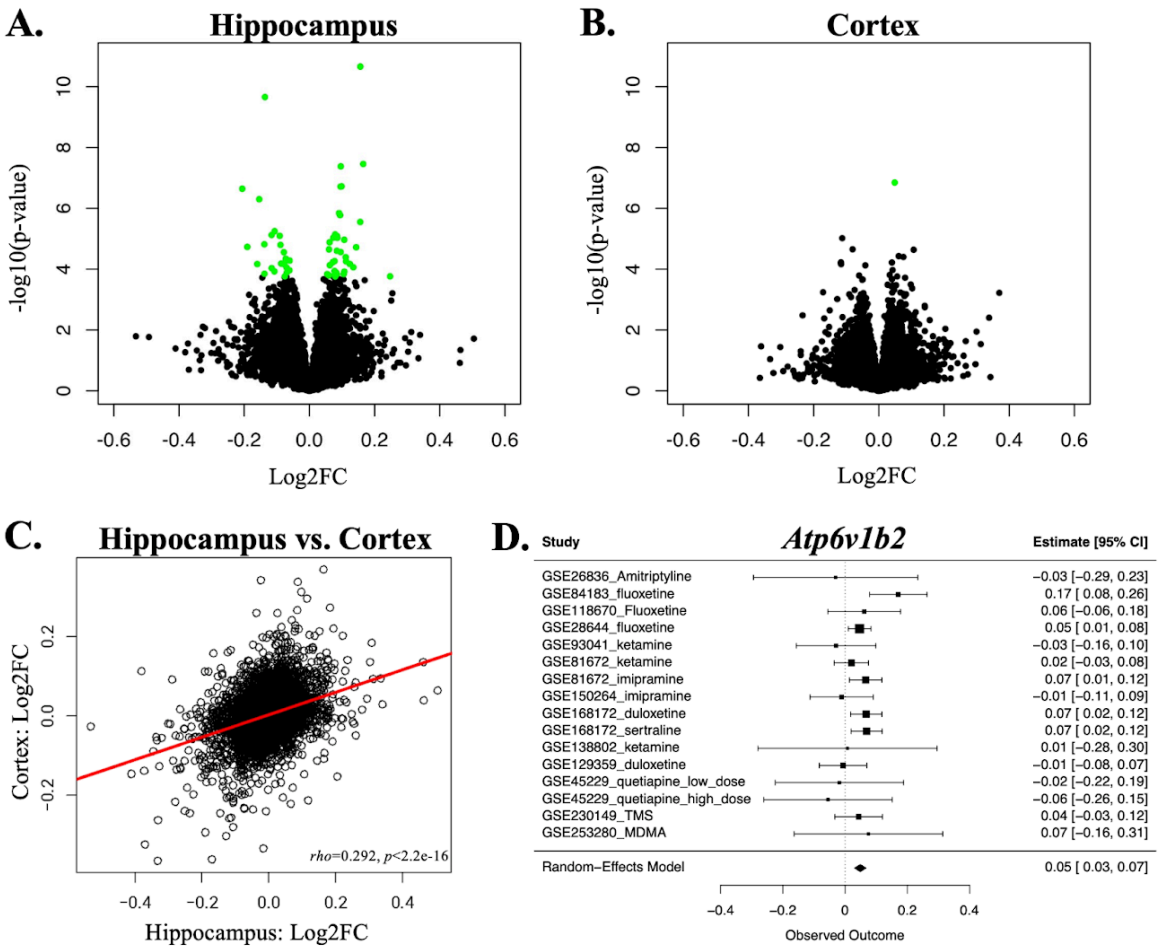


Figure 6. The cortex has fewer antidepressant effects, but shows some parallels with the hippocampus. ***A–B.*** *Volcano plots display differential expression results for hippocampal (A) and cortical (B) datasets, with $\log_2$ fold change plotted against $-\log_{10}$(p-value). Genes reaching significance at FDR < 0.05 are highlighted in green.* ***C.*** *Scatterplot comparing $\log_2$ fold changes in the hippocampus and cortex across all genes.* ***D.*** *Volcano plot of the cortex highlighting Atp6v1b2, the one gene that reached significance at FDR < 0.05. Rows illustrate Log2FC (circles) with 95% confidence intervals (whiskers) for each of the datasets and the meta-analysis random effects model ("RE Model"). Upregulated across 16 comparisons.*

Discussion

Our meta-analyses provide a view into the shared mechanisms of action of diverse antidepressant treatments in the hippocampus and cortex. A wide variety of treatments were represented, including SSRIs, SNRIs, TCAs, NMDA receptor agonists, alpha-2 adrenergic antagonists combined with TCAs, MASSAs, ECT and TMS. This aggregation of data produced enhanced statistical power, with a collective sample size of *n*=313 (hippocampus) and *n*=260 (cortex). The hippocampal meta-analysis identified 58 DEGs, many of which were associated with gene sets mediating factors known to modulate depression risk, such as stress, environmental enrichment, and other psychiatric disorders, as well as pathways previously linked to antidepressant efficacy, including the DG granule neurons, cell proliferation,

morphogenesis, vasculature, glia, synaptic plasticity, and immune signalling. Our hippocampal meta-analysis also confirmed patterns of gene expression identified in previous antidepressant studies, including a meta-analysis of the effects of the FLX on the hippocampus [58] and snRNA-Seq studies characterizing the effects of FLX and ECS on hippocampal cell types [59,60], demonstrating that these effects can be generalized to a broad swath of antidepressants. In the cortex, antidepressant effects resembled the hippocampus in some ways, but there was only one significant DEG, *Atp6v1b2*. We discuss these results in the context of the wider literature below.

Hippocampus: Differential expression related to the neurogenic niche in the DG

Neuroimaging studies have consistently shown hippocampal volume reduction in patients with depression, with stress playing a key role [28,32], and antidepressants producing a reversal [32,37,38]. Several theories of antidepressant mechanism center on the promotion of growth-related processes and AHN [32,33]. Therefore, it is interesting that multiple genes and cell types related to the neurogenic niche in the DG were highlighted in our results. Antidepressant treatment upregulated gene expression specific to the DG, including our most upregulated DEG (*Pleckstrin Homology Domain Containing A2 (Plekha2)*). Antidepressant treatment also altered expression specific to a variety of support cell types that play a critical role in the neurogenic niche, including vasculature-related cells (endothelial cells, pericytes, astrocytes) that secrete factors that promote cell proliferation and differentiation [90–92] and regulate neural stem cell exposure to blood-borne substances [92–94] , and radial glia-like cells, which can serve as quiescent and active neural stem cells [95,96].

As our meta-analysis results reflect bulk dissections, these findings could imply functional changes within those cell types or altered cell type balance. To address this question, we conducted an exploratory follow-up analysis comparing our meta-analysis findings to snRNA-Seq results characterizing the effects of FLX treatment [59] and ECS [60] on hippocampal cell types. Our findings showed a strong enrichment of antidepressant-related expression amongst sets of genes previously shown to be differentially expressed in response to FLX and ECS in DG granule cells. Moreover, our meta-analysis replicated previous results indicating that antidepressants (FLX, ECS) induce a transcriptional remodelling in DG granule neurons resembling a return to a developmental-like state [59,60]. To do this, antidepressants were theorized to reopen critical developmental periods by removing specific forms of the extracellular matrix (ECM), thereby allowing synaptic growth and plasticity [59]. Notably, we also observed differential expression of ECM-related genes (e.g., *Aggrecan* (*Acan*) and *Heparan-alpha-glucosaminide N-acetyltransferase* (*Hgsnat*)) previously shown to modulate anxiety and exploratory activity [97,98], and an enrichment of antidepressant-related effects in gene sets specific to fibroblasts, which secrete ECM components (collagen, proteoglycans) [99]. These findings support the utility of triangulating between highly-powered bulk analyses with diverse samples and smaller, but highly-specific, single-cell or spatial analyses.

Hippocampus: Differential expression related to neurodevelopment and morphogenesis

Many genes and pathways related to morphogenesis and neurodevelopment were also upregulated following antidepressant exposure, both within our hippocampal meta-analyses and previous snRNA-Seq studies [59,60]. For example, *Plekha2* was the most strongly upregulated DEG in our meta-analysis, and previously found to be down-regulated following chronic stress and upregulated in the DG following antidepressant interventions (FLX and exercise), correlating with increased AHN and dendritic spine density [100]. *Plekha2* was also upregulated in DG granule neurons following ECS [60], and encodes a protein (TAPP2) which promotes cell adhesion to the ECM [101,102], suggesting a mechanism by which antidepressants might modulate ECM regulation of “immature-like” and “mature-like” states in DG granule cells. This protein also modulates a signalling axis (PI3K/AKT: [103–105]) proffered as a new antidepressant target [106] because it mediates neurogenic and growth factor (BDNF: [107]) effects in depression models [108–111]. For these reasons, *Plekha2* is a promising candidate for follow-up studies.

Several top DEGs have been directly tied to hippocampal morphology. For example, *Proliferation and apoptosis adaptor protein 15A* (*Pea15a*) was down-regulated by antidepressants in our meta-analysis and in DG granule neurons in response to ECS [60] and encodes an abundant phosphoprotein modulating apoptosis and cell proliferation signaling [112,113] that correlates with ECT-induced hippocampal grey matter volume increases in depressed patients [114]. *ALF transcription elongation factor 3 (Aff3)* was upregulated in our meta-analysis and in multiple hippocampal cell types (DG neurons, astrocytes) following FLX or ECS treatment [59,60]. It encodes a component of the transcriptional super elongation complex (AFF3/LAF4) important for the migration of neural progenitor cells [115]. As loss of *Aff3* produces hippocampal atrophy and enlarged ventricles [116], its upregulation could be important for antidepressant reversal of hippocampal atrophy. *Family With Sequence Similarity 135 Member B (Fam135b)* was also upregulated in our meta-analysis and in hippocampal neurons following FLX or ECS treatment [59,60], and can promote neurite extension and neuron survival [117], suggesting another mechanism for reversing depression-related atrophy.

Hippocampus: Differential expression related to neuronal signaling and neuroplasticity

Multiple genes responsible for neuronal signaling were downregulated by antidepressants in our meta-analysis and in snRNA-Seq datasets. For example, *Neurensin 1 (Nrsn1)* is a brain-specific gene potentially important for neural plasticity and nerve signal transduction [118,119] that is sensitive to chronic stress. *Nrsn1* was part of the enriched gene set signalling the return of DG granule neurons to a "developmental-like" state theorized to be important for antidepressant effects [59,60]. *Potassium Two Pore Domain Channel Subfamily K Member 10* (*Kcnk10*) was down-regulated in our meta-analysis, in DG granule neurons in response to ECS [60], and following environmental enrichment, which has antidepressant-like effects. It encodes potassium channels that can be stimulated by arachidonic acid, which is implicated in the development of depression [120,121]. *Unc-13 homolog C* (*Unc13c*) was down-regulated in our meta-analysis and in DG granule neurons in response to FLX [59] and mediates excitatory neurotransmission [122], whereas another component of hippocampal glutamatergic synapses, *Syntaxin Binding Protein 5* (*Stxbp5*) [123,124] was upregulated. Collectively, these findings suggest molecular targets that may mediate antidepressant effects on neuroplasticity.

Hippocampus: Differential expression related to stress responses

Stress and its effects on the hippocampus are well-documented in the pathophysiology of depression, with the hippocampus acting as both a target and regulator of stress [125]. Several antidepressant DEGs regulate stress responses. For example, *T-box Transcription Factor 19* (*Tbx19*) was upregulated by antidepressants and is an important regulator of the hypothalamus-pituitary (HPA) stress-response axis, correlating with neurotic traits [126]. Moreover, sets of genes previously shown to respond to chronic stress were enriched with antidepressant differential expression, typically in the opposing direction. Individual antidepressant DEGs also often showed stress-opposing effects (*e.g., Plekha2, Uroplakin 1A (Upk1a)*), whereas other DEGs showed antidepressant effects mimicking chronic stress (*e.g., Nrsn1, GLIS family zinc finger 1* (*Glis1*), and corticosteroid-sensitive *Cdk5 And Abl Enzyme Substrate 1* (*Cables1*) [60,127] and *MER proto-oncogene tyrosine kinase* (*Mertk*) [128]). As some of these genes are tied to brain and behavior abnormalities following stress [128], these results may represent an activation of natural compensatory mechanisms. Our findings also suggest possible pathways for antidepressants to reverse stress-effects on growth-related processes, as *Cables1* is responsible for cell cycle progression [127] and *Glis1* can promote the generation of pluripotent stem cells [129].

Hippocampus: Differential expression related to immune function

Immune dysregulation is a prominent theory in depression [130]. Environmental stressors produce an increased immune response in the brain, including activation of hippocampal microglia [131,132]. Several classes of antidepressants, including SSRIs and SNRIs, modulate inflammatory mechanisms and oxidative stress [133]. Therefore, it was interesting that antidepressants upregulated many genes involved in immune cell recruitment, such as *C-C motif chemokine receptor-like 2* (*Ccrl2*), *Plekha2* [105], and

Guanylate Binding Protein 2 (Gbp2) [134]. Many immune-related cell types in the brain can release neurotrophic factors and anti-inflammatory cytokines as well as pro-inflammatory cytokines [135,136]. However, counterintuitively, the observed effects didn't clearly suggest a reduction in inflammation in favor of growth-related processes. For example, *Gbp2* was upregulated by antidepressants, but promotes M1 (pro-inflammatory) microglial polarization [137] and stress-sensitive *Mertk* was down-regulated and mediates anti-inflammatory phagocytosis [128,138,139]. Similarly, the enrichment of antidepressant effects in cytokine-related gene sets could suggest pro-inflammatory or anti-inflammatory effects. *Toll-like receptor 9* (*Tlr9*) was upregulated and can promote both, encouraging immune homeostasis [140,141].

Cortex: Differential expression related to immune cells and neuroplasticity

The potential role for immune cells in mediating the effects of antidepressants on neuroplasticity and growth was also highlighted in the cortex. In the cortex, *Atp6v1b2* was the only significant DEG, upregulated across a variety of antidepressant treatments. However, it is a strong candidate for mediating antidepressant effects, as it contains a risk allele for depression [142,143], and is differentially methylated following antidepressant treatment [144]. *Atp6v1b2* encodes a component of vacuolar ATPase (V-ATPase), a proton pump that acidifies intracellular organelles [145] in a manner important for protein degradation, autophagy, small molecule transport, and receptor-mediated endocytosis [145,146]. *Atp6v1b2* was the leading gene in microglial gene sets upregulated by antidepressants, where it is likely to promote phagocytosis and M2 anti-inflammatory phenotype [147–149]. This could mediate antidepressant function, as microglial activation in the frontal cortex correlates with depression severity [89]. V-ATPase also plays an important role in neurons, generating the electrochemical gradient that facilitates neurotransmitter loading into vesicles [143,150–154], suggesting antidepressants could increase the efficiency of synaptic transmission. Furthermore, V-ATPase can activate the mTOR pathway [155], which may mediate rapid antidepressant effects [156]. Altogether, these findings suggest that *Atp6v1b2* is a promising candidate for follow-up, along with potentially other members of the V-ATPase family [157].

Limitations and future directions

Regional specificity:

There was a positive correlation between the antidepressant effects identified in the hippocampus and cortex, as well as overlapping gene sets enriched with differential expression. Despite that overlap, there was a notable difference in the number of identified DEGs. Considering the high power of the analyses and similar sample sizes, this result likely reflects true biological variance. This may indicate higher sensitivity and responsiveness in the hippocampus to antidepressants, perhaps conferred by the sensitivity of the DG and neurogenic niche, but could also reflect differences in the included subjects and tissue heterogeneity.

Both meta-analyses included significant heterogeneity in targeted tissue. The hippocampal meta-analysis included a mixture of whole hippocampus versus DG datasets, which might have weakened effects tied to DG cell types and function [32–35,37,38], especially within the exploratory meta-analysis characterizing the effects of non-traditional antidepressants which used whole hippocampal tissue in 7 of 9 statistical contrasts. Within our cortical meta-analysis, we included samples from multiple implicated regions, including the PFC and anterior cingulate cortex [41]. These regions are likely to serve different roles in the manifestation of depression symptoms and treatment response [158,159], which may have contributed to the relative lack of identified cortical effects. Future well-powered studies will be needed to assess regional heterogeneity.

Relevance to human clinical populations:

Future work should also explore the relevance of our findings to more diverse human clinical populations. Controlled rodent studies provide insight into causality, as treatment effects in clinical populations are

often confounded by disease severity, but there are important species differences in brain physiology and morphology [160]. Moreover, the scope of our search included datasets not derived from depression models, and antidepressants may affect neural structures and cognition in nondepressed subjects differently than depressed subjects [161,162], including in stress-based animal models [58]. Furthermore, the models represented encompassed highly divergent pathways to depressive-like behavior, including chronic stress, inflammation, and olfactory bulbectomy, which may be optimally reversed by different forms of treatment. Notably, most cortical datasets used no depression model, which could account for weaker cortical antidepressant effects. Finally, unlike clinical populations, our samples were almost all male, despite well-documented sex differences in the neurobiological effects of depression in pre-clinical and clinical studies [163], demanding clear follow-up.

Conclusion

Altogether, our meta-analyses identified 59 genes that were consistently differentially expressed following treatment with a variety of antidepressants, many of which were associated with functions previously implicated in depression. These genes and pathways are worth investigating as potential linchpins for antidepressant efficacy or targets for novel therapies, with triangulation of results across species to infer translatability. For future work, we plan to compare our results with environmental factors that alleviate depression, like environmental enrichment and exercise. Better-powered studies should also follow up on our exploratory analyses examining how categories of antidepressants differ - instead of converge - in their effects, to elucidate treatment resistance and identify predictors of individual response to different treatment categories.

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