Supplemental Online Content

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This supplementary material has been provided by the authors to give readers additional information about their work.

eMethods 1. Study Procedures

The prescreening procedure, conducted via telephone after receiving verbal consent from the participant, included a safety screening for magnetic resonance imaging (MRI), as well as the screening part of the structural clinical interview for DSM disorders (SCID-IV). Subsequently, participants completed an online battery of psychological and physiological questionnaires. Following this telephone screening, an approximately five-hour long face-to-face session was conducted at the Children's Hospital Zürich, at the start of which participants gave written consent and which included interviews of psychological rating scales conducted by a trained psychologist, such as the full SCID-IV interview.[1] This session also included assessments using the Hamilton Depression Rating Scale (HAM-D),[2] the Montgomery–Åsberg Depression Rating Scale (MADRS),[3] and Beck's Depression Inventory (BDI).[4] Finally, blood samples were collected, and participants underwent MRI scanning, including magnetic resonance spectroscopy (MRS).

eMethods 2. MRI Data Acquisition and Analyses

Measurements included a localizer and a 3D T1-weighted spoiled gradient recalled sequence for the assessment of global and local brain volumes (162 slices of 256x256 voxels, 1x1x1 mm resolution; TR=11 ms, TE=5 ms, TI = 600 ms, flip angle 8°), used for the localization of the MR spectra. The centre of the spectrum was localised by a standardised set of measurements,[5,6] on a slice 1 mm above the superior margin of the lateral ventricles. On this slice, the length of the midline was measured, and the width of the left hemisphere was calculated at a point 1/3 of the distance from the anterior margin of the brain, along the midline. The midpoint of a perpendicular line crossing from the midline to the lateral margin of the left hemisphere was used to define the voxel center (see Figure 1A and B for a picture of the voxel and the spectra respectively). Editing pulses were applied at 1.9 and 7.5 ppm. Since the GABA findings were not separated from the co-edited macromolecular peak at 3 ppm, the results refer to GABA+ rather than pure GABA. The MRS spectra were processed with the LCModel 6.3-1 H, using a simulated basis set. Spectra were inspected visually for artefacts, and CRLB cutoffs for GABA, Glu, and Gln were set at 20 %, 20 %, and 30 % respectively, resulting in n=372, 372, 248, and 373 datasets for GABA, Glu, Gln, and Glx, passing the quality criteria. Linewidth data from LCModel were used as an additional index of spectral quality. Metabolite levels were referenced to the unsuppressed water peak and derived in institutional units after correction for atrophy and differing water concentrations within the brain and CSF compartments.[5] We also adjusted for grey matter fraction in all correlation analyses to minimise any confounds from differences in tissue composition, and there were no significant between-group differences of grey matter fraction for any of the MDD diagnosis groups (χ^2=2.52, p=.283). There was, however, a trend-level

difference between the sexes (χ^2=3.54, p=.06, $\bar{x}_{Female(n)}$=.325(250), $\bar{x}_{Male(n)}$=.316(136)).

eResults 1. Comorbidity Analyses

Results of metabolite differences between the depression groups remained unchanged with the introduction of the diagnosis of anxiety disorders, OCD, PTSD, and eating disorders as covariates in a ANCOVA for Glu ($F(2,368)=3.3$, $p=.028$), with significantly lower Glu concentrations in the past MDD group (Cohen's $d=.365$, $p=.008$, mean difference Healthy-Past$_{(SEM)}$=.349(.130)), and for GABA ($F(2,369)=6.233$, $p=.002$), with significantly lower concentrations in the past (Cohen's $d=.458$, $p<.001$, mean difference Healthy-Past$_{(SEM)}$=.225(.067)), as well as current MDD groups (Cohen's $d=.353$, $p=.042$, mean difference Healthy-Current$_{(SEM)}$=.173(.085)). There were no significant between group differences for Glx. The effect for Gln diminished to trend-level with the introduction of anxiety disorders as a covariate ($F(2,244)=2.772$, $p=.065$), but remained significant when anxiety disorders were not included, even if the other disorders where included as covariates ($F(2,244)=4.359$, $p=.014$), in which case the results showed significantly higher Gln concentrations in the past MDD group (Cohen's $d=-.406$, $p=.009$, mean difference Healthy-Past$_{(SEM)}$=-.209(.079)), as well as the current MDD group (Cohen's $d=-.416$, $p=.050$, mean difference Healthy-Current$_{(SEM)}$=-.214 (.109)) when compared to the healthy controls.

Exclusion of n=10 subjects who took psychoactive medication during testing did not change the results of the between-group analyses. GABA concentrations were significantly lower in the past ($r=.184$, $p=.003$, $\bar{x}_{Healthy(SEM,\ n)}=2.70(.03,\ 232)$, $\bar{x}_{Past\ MDD(SEM,\ n)}=2.48(.05,\ 88)$, adjusted for FDR) as well as the current MDD group ($r=.172$, $p=.008$, $\bar{x}_{Healthy(SEM,\ n)}=2.70(.03,\ 232)$, $\bar{x}_{Current\ MDD(SEM,\ n)}=2.47(.07,\ 34)$, adjusted for FDR) when compared to healthy controls, while Glu concentrations were significantly lowered in the past MDD group ($r=.163$, $p=.010$, $\bar{x}_{Healthy(SEM,\ n)}=7.52(.06,\ 230)$, $\bar{x}_{Past}$

$_{MDD(SEM, n)}$=7.23(.11, 89), adjusted for FDR). Gln concentration was significantly higher in the past MDD group (r=.165, p=.043, $\bar{x}_{Healthy(SEM, n)}$=1.64(.04, 149), $\bar{x}_{Past\ MDD(SEM, n)}$=1.85(.08, 64), adjusted for FDR).

eResults 2. ANCOVA Correcting for Gray Matter Ratio, Age, and Sex

In additional ANCOVA analyses with post-hoc pairwise comparisons covarying for grey matter ratio, age, and sex, and for multiple testing using Bonferroni correction we found that the results for the comparison of GABA concentrations between the healthy control group and the past MDD group stayed significant (Cohen's d=.436, $p_{bonferroni}$=.002, mean $difference_{Healthy\text{-}Past\ MDD}$=.216, $n_{Healthy;Past\ MDD}$=234,93), while the comparison between healthy controls and current MDD group was reduced to nonsignificance (Cohen's d=.299, $p_{bonferroni}$=.221, mean $difference_{Healthy\text{-}Current\ MDD}$=.148, $n_{Healthy;Past\ MDD}$=236,44). See tables 1a and 1b for an overview.

For the other metabolites, the analyses mirrored those described in the manuscript, with no effects found for Glx, significantly lower Glu concentrations in the past MDD group when compared to the control group (Cohen's d=.309, $p_{bonferroni}$=.045, mean $difference_{Healthy\text{-}past\ MDD}$=.292, $n_{Healthy;Past\ MDD}$=236,92), and significantly higher Gln concentrations in the past MDD group when compared to the healthy controls (Cohen's d=-.406, $p_{bonferroni}$=.027, mean $difference_{Healthy\text{-}past\ MDD}$=-.205, $n_{Healthy;Past\ MDD}$=153,66). See tables 2a and 2b, 3a and 3b, and 4a and 4b respectively.

Table 1. GABA Concentrations

Table 1a: ANCOVA - GABA Concentrations

	Sum of Squares	df	Mean Square	F	p	η^2
Overall model	3.22296	5	0.64459	2.70796	0.020	
MDD Diagnosis	3.19435	2	1.59717	6.54229	0.002	0.035
Grey Matter Ratio	0.00202	1	0.00202	0.00829	0.927	0.000
Age	0.00701	1	0.00701	0.02871	0.866	0.000
Sex	0.01958	1	0.01958	0.08019	0.777	0.000
Residuals	87.15467	357	0.24413			

Table 1b: Post Hoc Comparisons – GABA Concentrations by MDD Diagnosis

Comparison								
MDD Diagnosis		MDD Diagnosis	Mean Difference	SE	df	t	$p_{bonferroni}$	Cohen's d
Healthy	-	Past MDD	0.2156	0.0626	357	3.445	0.002	0.436
	-	Current MDD	0.1476	0.0823	357	1.794	0.221	0.299
Past MDD	-	Current MDD	-0.0680	0.0925	357	-0.735	1.000	-0.138

Note. Comparisons are based on estimated marginal means, corrected for multiple comparisons using the Bonferroni method.

Table 2. Glx Concentrations

Table 2a: ANCOVA - Glx Concentration

	Sum of Squares	df	Mean Square	F	p	η^2
Overall model	15.9837	5	3.1967	2.7613	0.018	
MDD Diagnosis	1.6879	2	0.8439	0.7245	0.485	0.004
Grey Matter Ratio	12.5938	1	12.5938	10.8118	0.001	0.029
Age	0.0561	1	0.0561	0.0481	0.826	0.000
Sex	1.6459	1	1.6459	1.4130	0.235	0.004
Residuals	417.0066	358	1.1648			

Table 2b: Post Hoc Comparisons – Glx Concentrations by MDD Diagnosis

Comparison								
MDD Diagnosis		MDD Diagnosis	Mean Difference	SE	df	t	$p_{bonferroni}$	Cohen's d
Healthy	-	Past MDD	0.16157	0.136	358	1.1859	0.709	0.14971
	-	Current MDD	0.00813	0.180	358	0.0452	1.000	0.00753
Past MDD	-	Current MDD	-0.15344	0.202	358	-0.7610	1.000	-0.14217

Note. Comparisons are based on estimated marginal means, corrected for multiple comparisons using the Bonferroni method.

Table 3. Glu Concentrations

Table 3a: ANCOVA - Glu Concentration

	Sum of Squares	df	Mean Square	F	p	η^2
Overall model	13.4874	5	2.6975	3.2373	0.007	
MDD Diagnosis	5.3219	2	2.6609	2.9819	0.052	0.016
Grey Matter Ratio	5.6171	1	5.6171	6.2946	0.013	0.017
Age	2.4902	1	2.4902	2.7906	0.096	0.008
Sex	0.0583	1	0.0583	0.0653	0.798	0.000
Residuals	317.6829	356	0.8924			

Table 3b: Post Hoc Comparisons – Glu Concentrations by MDD Diagnosis

Comparison								
MDD Diagnosis		MDD Diagnosis	Mean Difference	SE	df	t	$p_{bonferroni}$	Cohen's d
Healthy	-	Past MDD	0.2915	0.119	356	2.442	0.045	0.3086
	-	Current MDD	0.0762	0.157	356	0.484	1.000	0.0806
Past MDD	-	Current MDD	-0.2154	0.176	356	-1.220	0.670	-0.2280

Note. Comparisons are based on estimated marginal means, corrected for multiple comparisons using the Bonferroni method.

Table 4. Gln Concentrations

Table 4a: ANCOVA - Gln Concentration

	Sum of Squares	df	Mean Square	F	p	η^2
Overall model	5.284	5	1.057	3.998	0.002	
MDD Diagnosis	2.186	2	1.093	4.300	0.015	0.034
Grey Matter Ratio	0.217	1	0.217	0.855	0.356	0.003
Age	2.761	1	2.761	10.865	0.001	0.043
Sex	0.120	1	0.120	0.471	0.493	0.002
Residuals	59.477	234	0.254			

Table 4b: Post Hoc Comparisons – Gln Concentrations by MDD Diagnosis

Comparison								
MDD Diagnosis		MDD Diagnosis	Mean Difference	SE	df	t	$p_{bonferroni}$	Cohen's d
Healthy	-	Past MDD	-0.2046	0.0777	234	-2.632	0.027	-0.4057
	-	Current MDD	-0.1926	0.1057	234	-1.822	0.209	-0.3820
Past MDD	-	Current MDD	0.0119	0.1172	234	0.102	1.000	0.0237

Note. Comparisons are based on estimated marginal means, corrected for multiple comparisons using the Bonferroni method.

eResults 3. Analyses in Female and Male Groups

Within the female subgroup, we observed significantly lower GABA concentration in subjects with past MDD when compared to healthy controls (r=.173, p=.033, $\bar{x}_{Healthy(SEM,n)}$=2.68(.04, 153), $\bar{x}_{Past\ MDD(SEM,n)}$=2.49(.05, 75), adjusted for FDR). Likewise, Glu concentrations were significantly lower in the past MDD group when compared to the control group (r=.187, p=.019, $\bar{x}_{Healthy(SEM,n)}$=7.52(.08, 153), $\bar{x}_{Past\ MDD(SEM,n)}$=7.16(.12, 75), adjusted for FDR). Analyses left DLPFC Gln concentrations revealed significantly higher levels in subjects with past MDD when compared to healthy controls (r=.258, p=.009, $\bar{x}_{Healthy(SEM,n)}$=1.63(.05, 153) $\bar{x}_{Past\ MDD(SEM,n))}$=1.92(.09, 75), adjusted for FDR).

Between-group analyses in the male subgroup revealed significantly lower GABA concentrations in subjects with past MDD when compared to healthy controls (r=.189, p=.046, $\bar{x}_{Healthy(SEM,n)}$=2.73(.06, 98), $\bar{x}_{Past\ MDD(SEM,n)}$= 2.48(.13, 23)). The result in the male subgroup diminished to non-significant levels when adjusted for FDR, although the effect size was similar to that seen in the (larger) female subgroup.

In the male subgroup, we found significant associations between GABA levels and MADRS (rho=-.252, p=.005, n=124), BDI (rho=-.261, p=.004, n=122).

eResults 4. Exploratory Factor Analysis of BDI Items

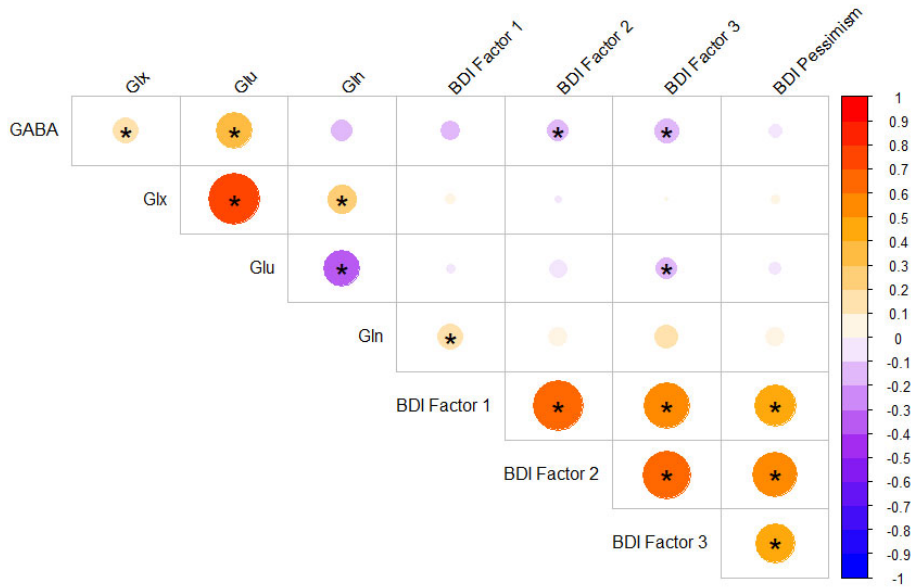
Exploratory factor analysis revealed 4 factors for the twenty-two items of the BDI. Factor 1 was comprised of 5 items of the BDI, mostly associated with a negative self-image that explained 4.7% of the variance with factor loadings from .473 to .748. A second factor, mostly associated with 9 items relating to sadness, anhedonia and negative affect explained 40.3% of the variance with factor loadings between .341 and .695. A third factor, comprised of 5 items associated with somatic symptoms and feelings of being punished, explained 5.1% of the variance and showed loadings from .346 to .608. A final factor comprised of the single BDI item measuring pessimism explained another 3.8% of the variance, with a factor loading of .559 (see Figure 1 for an overview).

In a partial Spearman analysis, controlling for sex and grey matter ratio, GABA concentration was significantly and negatively associated with factors 2 (rho=-.110, p=.037, n=361) and 3 (rho=-.155, p=.003, n=361) identified in the exploratory factor analysis. Glx was not associated with any of the factors. Glu was significantly and negatively associated with factor 3 (rho=-.111, p=.036, n=360). Finally, Gln showed a significant positive association with factor 1 (rho=.150, p=.021, n=239), see Figure 2 for an overview.

Figure 1. Factor Loadings

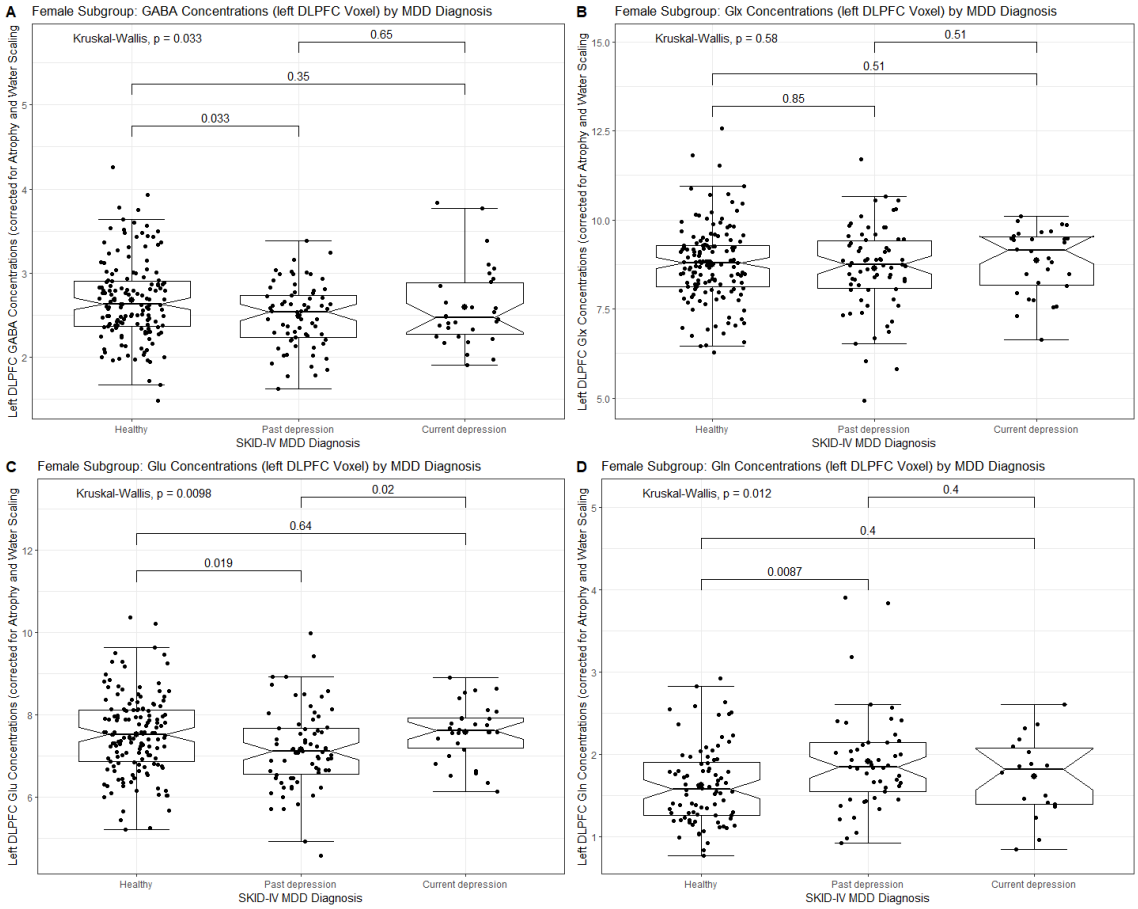
Factor Loadings					
	Factor				
	1	**2**	**3**	**4**	**Uniqueness**
BDI8_SelfCriticism	**0.748**				0.387
BDI7_SelfDislike	**0.719**				0.375
BDI3_PastFailure	**0.682**				0.331
BDI5_Guilt	**0.549**				0.496
BDI14_Worthlessness	**0.473**				0.606
BDI12_LossOfInterest		**0.695**			0.455
BDI4_LossOfPleasure		**0.650**			0.410
BDI17_Tiredness		**0.610**			0.438
BDI22_InterestInSex		**0.580**			0.618
BDI13_Indecisiveness		**0.501**			0.486
BDI15_EnergyLoss	0.330	**0.400**			0.523
BDI1_Sadness		**0.396**		0.326	0.466
BDI10_Crying		**0.353**			0.653
BDI11_Irritability		**0.341**			0.715
BDI18_Appetite			**0.608**		0.543
BDI21_HealthWorry			**0.507**		0.666
BDI19_WeightLoss			**0.499**		0.739
BDI6_PunishmentFeelings	0.317		**0.479**		0.601
BDI9_SuicidalThoughts			**0.346**	0.310	0.479
BDI16_SleepChanges					0.657
BDI2_Pessimism				**0.559**	0.311
Note. 'Minimum residual' extraction method was used in combination with a 'oblimin' rotation					

Figure 2. Partial Spearman Correlation Analyses



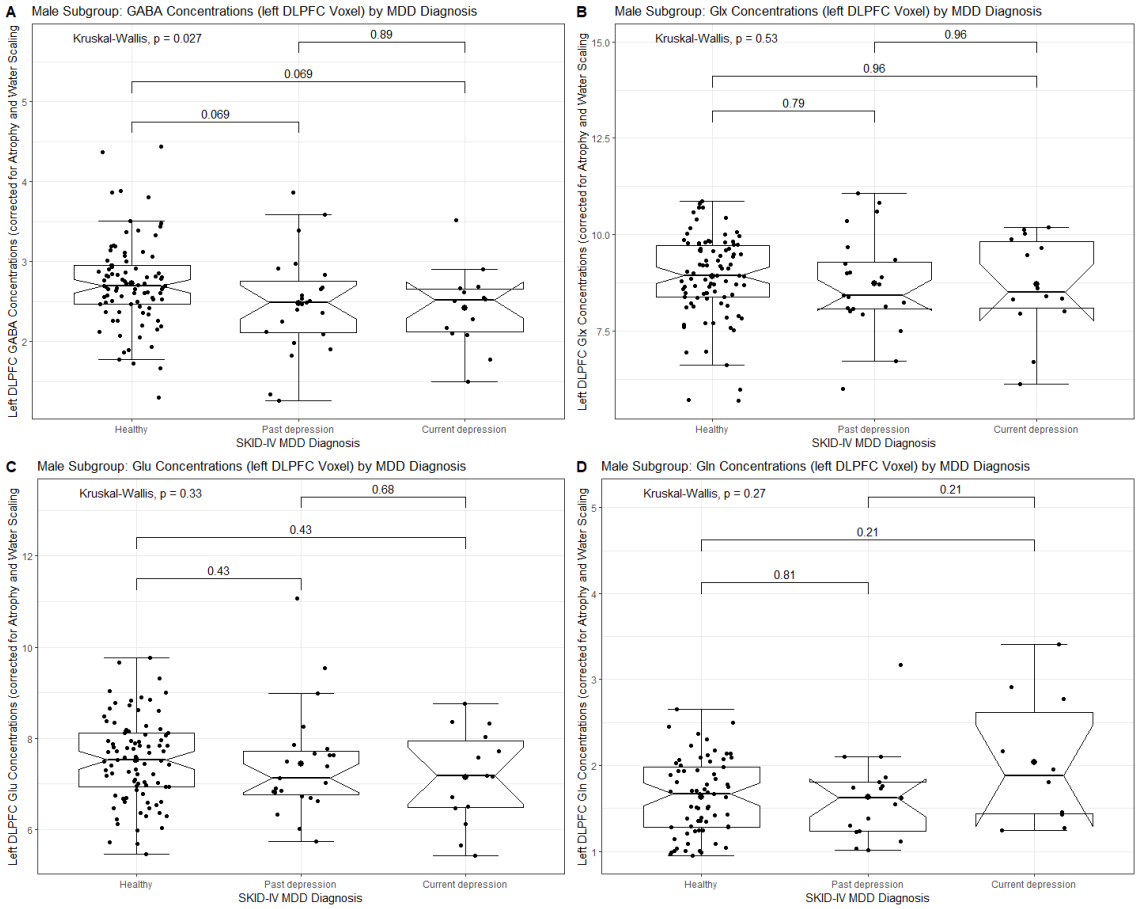
Partial Spearman Correlation Analyses of Left DLPFC Neurotransmitter Concentrations (GABA, Glx, Glu, Gln) and the Factors Identified in the Exploratory Factor Analysis of BDI Items, controlled for sex and grey matter ratio. Note: * p < .05, ** p < .01, *** p < .001, the Legend Depicts Color-coded Effect Sizes (Spearman's Rho).

eFigure 1. Female Subgroup Boxplots



Female Subgroup. Boxplots of left DLPFC neurotransmitter concentrations by MDD diagnosis (corrected for false discovery rate). **A)** Kruskal-Wallis chi-squared = 6.7967, df = 2, p-value = 0.03343; **B)** Kruskal-Wallis chi-squared = 1.0877, df = 2, p-value = 0.5805; **C)** Kruskal-Wallis chi-squared = 9.2515, df = 2, p-value = 0.009797; **D)** Kruskal-Wallis chi-squared = 8.8965, df = 2, p-value = 0.0117.

eFigure 2. Male Subgroup Boxplots



Male Subgroup. Boxplots of left DLPFC neurotransmitter concentrations by MDD diagnosis (corrected for false discovery rate). **A)** Kruskal-Wallis chi-squared =7.2183, df = 2, p-value = 0.02708; **B)** Kruskal-Wallis chi-squared = 1.2595, df = 2, p-value = 0.5327; **C)** Kruskal-Wallis chi-squared = 2.1971, df = 2, p-value = 0.3334; **D)** Kruskal-Wallis chi-squared = 2.6282, df = 2, p-value = 0.2687.

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