Schizophrenia

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Post-mortem brain analysis reveals altered monoaminergic system in the dorsolateral prefrontal cortex and hippocampus in chronic schizophrenia

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Schizophrenia is a multifactorial psychiatric disorder in which monoaminergic neurotransmission, particularly dopamine (DA), serotonin (5-HT), and norepinephrine (NE), is implicated in both pathophysiology and response to treatments. However, the regional composition and functional interaction among these neurotransmitters and their catabolites remain unclear. In this study, using high-performance liquid chromatography (HPLC), we quantified DA, 5-HT, NE and their metabolites, HVA, DOPAC, and 5-HIAA, along with protein levels of key monoamine-metabolizing enzymes (MAO-A, MAO-B, and COMT isoforms) in post-mortem dorsolateral prefrontal cortex (DLPFC) and hippocampus from chronic schizophrenia patients and matched healthy controls. Although absolute monoamine and enzyme levels did not differ between cases and controls, partial correlation analyses revealed remarkable schizophrenia-specific interactions. In particular, patients exhibited distinct 5-HT-DOPAC correlations in both regions, suggesting an abnormal coupling between serotonergic and dopaminergic metabolism. In the DLPFC, the DA-NE correlation was markedly attenuated in schizophrenia, potentially reflecting disrupted catecholaminergic integration relevant to cognitive function and salience processing. Furthermore, an increased 5-HIAA-5-HT correlation emerged in the hippocampus of schizophrenia patients, indicative of abnormal serotonin turnover. In conclusion, our observations indicate that while the cerebral concentrations of monoamines and their catabolites remain relatively stable in patients with schizophrenia, their interactions are significantly dysregulated compared to those reported in healthy controls. These postmortem findings provide new insights into the monoaminergic neurotransmission crosstalk in the schizophrenia brain.

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INTRODUCTION

Schizophrenia (SCZ) is a complex psychiatric disorder affecting approximately 1% of the global population, characterized by a diverse array of positive, negative, and cognitive symptoms[1–3]. It is widely recognized as a neurodevelopmental, polygenic, and multifactorial condition characterized by altered synaptic plasticity, abnormal cortical-subcortical neurotransmission, and dysregulated brain connectivity, which collectively contribute to its complex clinical phenotype[4–7].

Among the neurotransmitter systems implicated in SCZ, monoaminergic dysfunctions, including dopamine (DA), serotonin (5-HT), norepinephrine (NE), along with their receptors, transporters, and metabolizing enzymes, such as monoamine oxidase A and B (MAO-A and MAO-B) and catechol-O-methyltransferase (COMT), have been consistently implicated in the pathogenesis of SCZ and resistance to antipsychotic treatments[8–15]. Specifically, dysregulation of DA, 5-HT, and NE, especially in key brain regions such as the dorsolateral prefrontal cortex (DLPFC) and hippocampus, has been implicated in cognitive and executive dysfunctions observed in individuals with SCZ, including planning, decision-making and working memory[16–22]. In this framework, decreased dopamine D1 receptor (DRD1) signaling in the DLPFC has emerged as a robust neurobiological correlate of impaired executive functions and information maintenance in SCZ[7,23,24]. Furthermore, variations in the genes encoding the enzymes responsible for DA, 5-HT, and NE catabolism have been associated with increased vulnerability to psychotic symptoms and cognitive deficits, further reinforcing the hypothesis of a monoamine neurotransmission abnormality in SCZ pathophysiology[8,9,25–31].

Dopamine-mediated neurotransmission, particularly in the striatum, remains the first-line intervention for SCZ treatment[13,32,33]. Both first- and second-generation antipsychotics primarily act through DA D2 receptor (DRD2) blockade, although most of them also exhibit affinity for other aminergic receptors[34–42]. More recently, the approval of Cobenfy, a muscarinic M1-M4 agonist, has introduced a DA-sparing mechanism that acts independently of DRD2 blockade[43]. Altogether, these findings support a broader view of SCZ pathophysiology, in which monoaminergic imbalance remains pivotal, affecting synaptic transmission and neural circuitry connectivity across different

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Table 1. Demographic characteristics of schizophrenia and non-psychiatric control subjects.

	Control	Schizophrenia	Statistic	*p* value
Subjects (total number)	20	20	-	-
Sex (M/F)	16/4	12/8	$\chi^2 = 1.071$ (df=1)	0.301[a]
Age (years, median [IQR])	73.50 [66.00–80.25]	52.50 [39.50 − 61.25]	$t = 4.819$ (df = 38)	<0.001[b]
PMI (hours, median [IQR])	12.90 [11.80−16.32]	15.25 [12.52 − 24.58]	$t = -2.426$ (df = 38)	0.020[c]
pH (median, [IQR])	6.54 [6.49–6.63]	6.50 [6.42 − 6.56]	$t = 0.708$ (df = 26)	0.485[c]

Demographic characteristics (age, PMI, pH) are shown as medians with interquartile range (first-third quartile). Data were tested for normality using the Shapiro–Wilk test. Variables that deviated significantly from normality ($p < 0.05$) were log-transformed for statistical analyses. Comparisons of demographic and clinical characteristics (age, PMI, pH, sex) between schizophrenia and control groups were performed using two-sample t-tests for continuous variables and Chi-Square (χ^2) statistic with Yates's correction for categorical variables. *M/F* number of males/females, *PMI* post-mortem interval, *IQR* Interquartile Range, *df* degrees of freedom;
[a]Chi-Square test (with Yates's correction).
[b]two sample *t* test.
[c]two sample *t* test on log-transformed values.

brain regions, ultimately influencing complex clinical phenotypes of SCZ and related treatment responses in patients.

Despite the significance of this topic, postmortem studies investigating monoamine composition in the brain of individuals with SCZ have yielded inconsistent findings. These discrepancies are likely due to several confounding factors, including antipsychotic treatments, postmortem interval and tissue storage conditions, differing experimental methodologies, age ranges, sex distribution, illness duration, and the uneven distribution of cases and controls across brain regions or diagnostic groups, as well as differences in sample size across studies[44–47]. Accordingly, while some studies reported increased levels of monoamines in striatal regions of SCZ brains compared to healthy individuals[45,48,49], others found comparable levels of DA, 5-HT, NE, or their metabolizing enzymes[44,45,50,51]. In the present study, we sought to overcome these limitations by examining two non-striatal regions critically involved in SCZ pathophysiology, the DLPFC and hippocampus, and by integrating the quantitative assessment of monoamines and their catabolites with the evaluation of enzymatic pathways. Furthermore, we conducted correlation analyses as a higher-level investigation to explore potential region-specific patterns of monoaminergic crosstalk and functional interplay dysregulation. To this end, we conducted a comprehensive HPLC analysis of DA, NE, 5-HT and their catabolites, HVA, DOPAC, and 5-HIAA, in the postmortem DLPFC and hippocampus from SCZ patients (n = 20) and non-psychiatric control subjects (n = 20). Using the same autopsy samples, we also assessed the protein levels of MAO-A, MAO-B, and COMT isoforms, including s-COMT (soluble form, 24 kDa) and mb-COMT (membrane-bound form, 30 kDa) by Western blotting. Despite comparable monoamine and enzyme levels between diagnostic groups across both brain regions, correlation analyses revealed distinct, region-specific patterns among NE, DA, 5-HT, and their catabolites in SCZ patients compared with controls.

METHODS

Tissue collection

DLPFC and hippocampus samples from the same post-mortem brain cohort of non-psychiatric controls and SCZ patients (n = 20/brain region/clinical condition) were obtained from The Human Brain and Spinal Fluid Resource Center (Los Angeles Healthcare Center, Los Angeles, CA, USA) (Table. 1). All tissue collection and processing were carried out in accordance with the regulations and licenses of the Human Tissue Authority and the Human Tissue Act of 2004. Clinical diagnosis of SCZ was made according to DSMIII-R criteria. Frozen tissues were pulverized in liquid nitrogen and stored at −80 °C for subsequent processing.

HPLC analysis

Frozen DLPFC and hippocampus tissues were added with 80 µl of 0.2 N perchloric acid. Tissues were homogenized with ultrasounds and centrifuged at 11,000 × *g* for 10 min at 4°C. The supernatant was transferred into Spin-X microcentrifuge filters (0.22 µm nylon filter), centrifuged at 11,000 × *g* for 5 min at 4°C and then stored at -80°C until HLPC analysis.

Twenty µl samples (5 µl of hydrophilic phase from brain tissue samples +15 µl of 0.2 N PCA) were injected without purification into an HPLC equipped with a reverse phase column (LC-18 DB, 15 cm, 5 µm particle size, Supelco, Waters, Milford, MA, USA) and a coulometric detector (ESA, Coulochem II, Bedford, MA, USA) to quantify molecules such as, DA, NE, HVA, DOPAC, 5-HT, 5-HIAA.

To detect catecholamines and their catabolites, the first electrode of the detector was set to +125 mV (oxidation) and the second to −175 mV (reduction). The composition of the mobile phase was (in mM): 50 NaH_2PO_4, 0.1 Na_2-EDTA, 0.5 n-octyl sodium sulfate, 15% (v/v) methanol, pH 3.7[52,53]. For 5-HT and 5-HIAA the first electrode of the detector was set at +280 mV (oxidation) and the second at −120 mV (reduction). The composition of the mobile phase was (in mM): 120 CH_3-COONa, 100 citric acid, 0.3 EDTA, 5% (v/v) methanol, pH 4.9[54]. For quantitative determination of each neurotransmitter, calibration curves were run using standards (SIGMA–ALDRICH, MO, USA). The software AZUR (Euroservice, Ge, Italy) was used for data acquisition. Catecholamine and their catabolite values were expressed as pmol/g of tissue, while the ratios were calculated by dividing the amount of each catabolite by that of its corresponding amine.

Western blotting

Frozen samples from post-mortem DLPFC and hippocampus tissues were powdered, sonicated in 1% SDS and boiled for 10 min. Two µl of homogenate were used to determine protein concentration using a Bio-Rad Protein Assay kit. Thirty µg of total proteins from each sample were loaded on pre-cast 4-20% acrylamide gradient gel (BioRad Laboratories). We used Precision Plus Protein™ All Blue Prestained Protein Standards (Cat. No. 1610373) as the molecular weight marker. Proteins were separated by SDS–PAGE and transferred to PVDF membranes (GE Healthcare) using Trans Blot Turbo System. Membranes were immuno-blotted overnight with primary antibodies for MAO-A (1:1000; Abcam Cat. No. ab126751), MAO-B (1:1000; Abcam Cat. No. ab137778) or COMT (1:1000; Abcam Cat. No. ab126618), detecting both soluble (s-COMT) and membrane-bound (MB-COMT) isoforms. Given the gel format (18 wells per gel, 16 for samples and 2 for molecular weight markers), the total of 40 samples were distributed evenly across three gels for each

protein of interest. Each gel included an equal number of control and SCZ cases. Separate western blots were run for MAO-A, MAO-B and COMT. MAO-A and MAO-B were run on independent membranes, as the two enzymes have nearly identical molecular weights (~60 kDa). After incubation with anti-rabbit horseradish peroxidase-conjugated secondary antibodies, immunoreactivity was detected using enhanced chemiluminescence (ECL, GE-Healthcare) and quantified by densitometric analysis with Quantity One software (Bio-Rad). Each blot was subsequently re-probed with GAPDH (1:1000; Santa Cruz Cat. No. sc-32233) as a loading and transfer control. For each sample, protein signals were normalized to the corresponding GAPDH value. To standardize data across different immunoblots, the normalized intensity of each sample was divided by the mean normalized intensity of the control samples run on the same gel, and expressed as a percentage. The resulting relative values (% of the control mean) were then used for statistical comparisons. All blots were processed in parallel using identical experimental conditions.

Univariate statistical analysis

Demographic characteristics, presented as medians with interquartile ranges, were tested for normality using the Shapiro-Wilk test. Variables that deviated significantly from normality ($p < 0.05$) were log-transformed for statistical analyses. Comparisons of demographic and clinical characteristics (age, PMI, pH) and sex distribution between schizophrenia and control groups were performed using two-sample *t*-tests for continuous variables and Chi-Square statistic with Yates's correction for categorical variables. Significant differences were observed for the age and *post-mortem* interval (PMI) when comparing SCZ patients and controls (Table 1); these variables were treated as confounders in the following analyses. Statistical comparisons of catecholamines and enzymes levels between groups were performed by ANCOVA models, including age and PMI as covariates. To account for multiple comparisons, p-values were corrected using the Bonferroni method.

Partial correlation analysis of monoaminergic interactions

To investigate the functional interactions within the monoaminergic system and identify potential alterations in SCZ, we conducted partial correlation analyses in both the DLPFC and hippocampus. First, cases with missing values were excluded via listwise deletion to ensure complete data for all analyses (one patient and two controls in the DLPFC; two controls in the hippocampus). Partial correlation coefficients were then computed for each pair of monoamines and their metabolites while controlling for potential confounding variables. Specifically, we employed, for each pair of molecules, a linear regression model in which each variable was regressed onto all other measured monoamines (excluding the variables of interest). Additionally, age and PMI were included as covariates in the regression models, as these factors significantly differed between the two groups. The residuals from these models were extracted, and Pearson's correlation coefficient (r) was computed between these residuals, yielding partial correlation estimates that reflect the direct association between each pair of variables. Since monoamines and their metabolites belong to the same pathway, simple bivariate correlations might be confounded by indirect effects. By excluding the influence of all other variables, we aimed to capture the unique association between each pair of molecules. A detailed account of each partial correlation for the two brain regions is available on the Open Science Framework platform (https://osf.io/vbe28/?view_only=3e9cee1786314bb0adbf248d4a90653c).

To identify differential correlations between SCZ patients and controls, we performed Fisher's z-test and controlled p-values using a modified Bonferroni method according to the Gao method[55].

To compare statistically partial correlation coefficients between the schizophrenia and control groups, we employed Fisher's z-test using the "diffcor.two" function from the *diffcor* package[56] in RStudio (RStudio: Integrated Development for R. RStudio, PBC, Boston, MA http://www.rstudio.com/). Fisher's z-test evaluates whether two correlation coefficients are significantly different by transforming them into a normally distributed z-score. Effect sizes for correlation differences were quantified using Cohen's q, which measures the magnitude of change between two correlation coefficients.

Given the large number of statistical comparisons, we applied a correction for multiple testing. However, standard Bonferroni correction assumes independent tests, which may not be valid in the context of interrelated variables[55,57]. Instead, we estimated the effective number of independent tests using principal component analysis (PCA) on the partial correlation matrix, through the "meff" function in the *poolr* R package[58]. The maximum number of independent components across groups was used to adjust p-values, balancing control over Type I (false positives) and Type II (false negatives) errors[55].

To visualize the results, partial correlation matrices were created for each group and represented using the *ggplot2* R package (Wickham H (2016). ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York. ISBN 978-3-319-24277-4, https://ggplot2.tidyverse.org). Additionally, correlation difference matrices were used to visualize the statistical significance (p-values) of pairwise differences in correlation coefficients between patients and controls. These matrices were plotted using the *corrplot* R package (Wei T, Simko V, Levy M, Xie Y, Jin Y, Zemla JJRpv. corrplot: Visualization of a correlation matrix. 2013; 230(11)).

RESULTS

Demographic and clinical characteristics of the study cohort

Demographic and clinical data for SCZ patients and control subjects are summarized in Table 1. Groups did not differ significantly in sex distribution. However, significant differences were observed in age and post-mortem interval (PMI) between SCZ and control individuals. These variables were therefore included as covariates in the subsequent ANCOVA analyses to control for their potential confounding effects on monoamine and metabolite levels and protein expression. No significant group differences were detected in brain pH.

HPLC quantification of monoamines and their metabolites in the DLPFC and hippocampus of schizophrenia and control subjects

In the DLPFC, statistical analyses reported no significant changes in the levels of 5-HT, its primary metabolite 5-HIAA, and 5-HIAA/5-HT turnover ratio between SCZ and control groups (5-HT: $F_{(1,35)} = 0.0165$, $p = 0.8985$; 5-HIAA: $F_{(1,35)} = 1.0989$, $p = 0.3017$; 5-HIAA/5-HT: $F_{(1,35)} = 0.7189$, $p = 0.4023$; ANCOVA; Fig. 1a–d; Suppl. Table 1). Similarly, comparable levels between groups were observed in DA ($F_{(1,35)} = 0.6264$, $p = 0.4340$; ANCOVA; Fig. 1e, f; Suppl. Table 1), along with its derivatives DOPAC, HVA, and the related neurotransmitter NE (DOPAC: $F_{(1,34)} = 0.9538$, $p = 0.3357$; HVA: $F_{(1,35)} = 0.3243$, $p = 0.5727$; NE: $F_{(1,34)} = 0.5738$, $p = 0.4539$; ANCOVA; Fig. 1e, g, h, k; Suppl. Table 1). Moreover, DA turnover, as assessed by DOPAC/DA and HVA/DA ratios, did not differ significantly between SCZ patients and controls (DOPAC/DA: $F_{(1,33)} = 0.7179$, $p = 0.4029$; HVA/DA: $F_{(1,34)} = 0.4904$, $p = 0.4885$; Fig. 1i, j; Suppl. Table 1).

In the hippocampus, we observed no significant differences between SCZ patients and healthy controls in 5-HT levels ($F_{(1,34)} = 2.9126$, $p = 0.09701$; ANCOVA; Fig. 2a, b; Suppl. Table 2). 5-HIAA levels were decreased in SCZ patients, compared to control subjects (median [IQR; 25%–75%]; 5-HIAA: Ctrl = 927.41

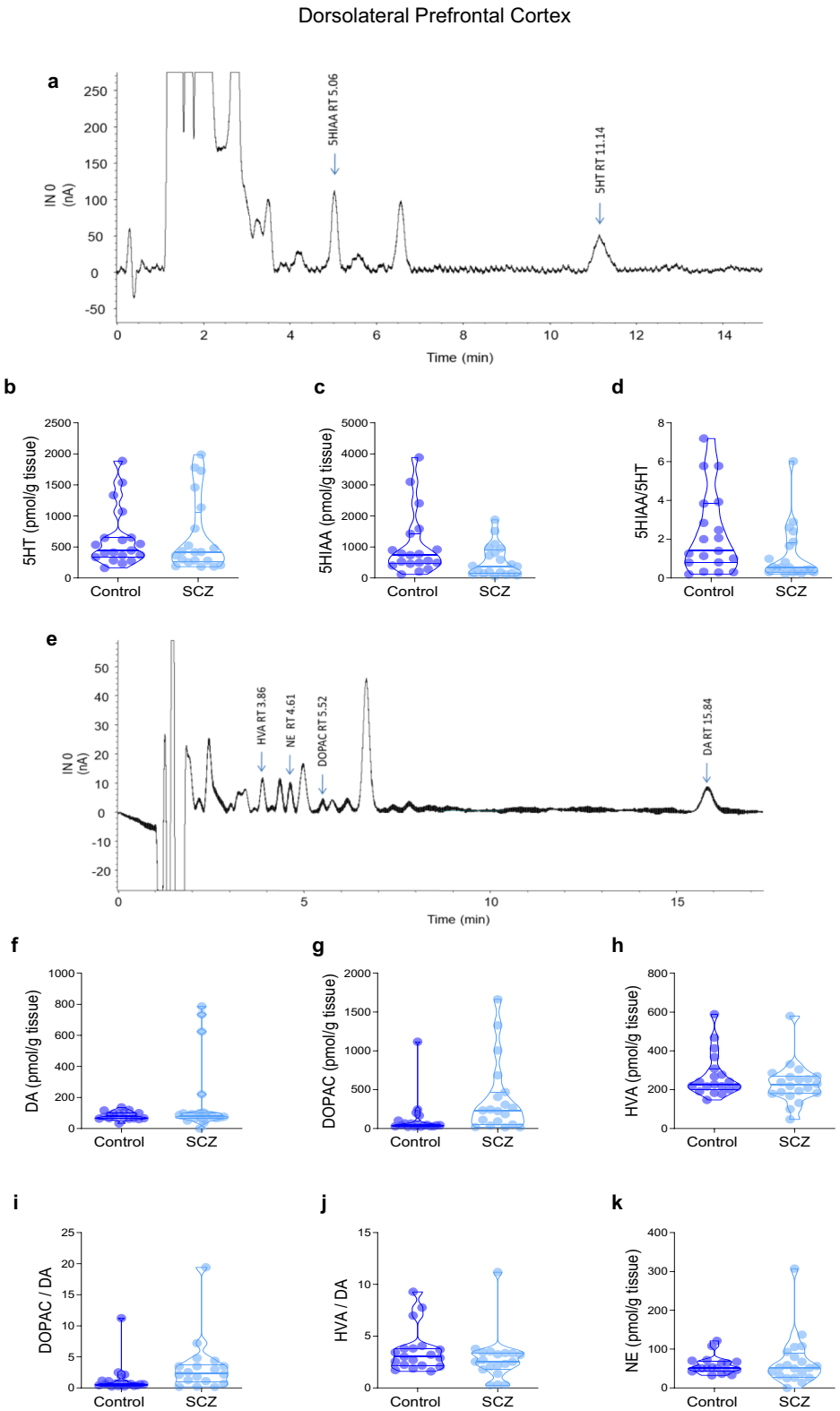

Fig. 1 HPLC analysis of monoamine and their precursors/derivatives levels in the *post-mortem* dorsolateral prefrontal cortex of schizophrenia patients. a, **e** Representative HPLC chromatograms showing monoamine peaks in a dorsolateral prefrontal cortex sample. Quantification of **b** 5-HT, **c** 5-HIAA, **d** 5-HIAA/5-HT ratio, **f** DA, **g** DOPAC, **h** HVA, **i** DOPAC/DA ratio, **j** HVA/DA ratio, **k** NE. In each sample, monoamines and their precursors/derivatives were detected in a single run by HPLC coupled with a coulometric detector and expressed as pmol/g of tissue. The number of examined samples is reported in Supplementary Table 1, for each considered molecule.

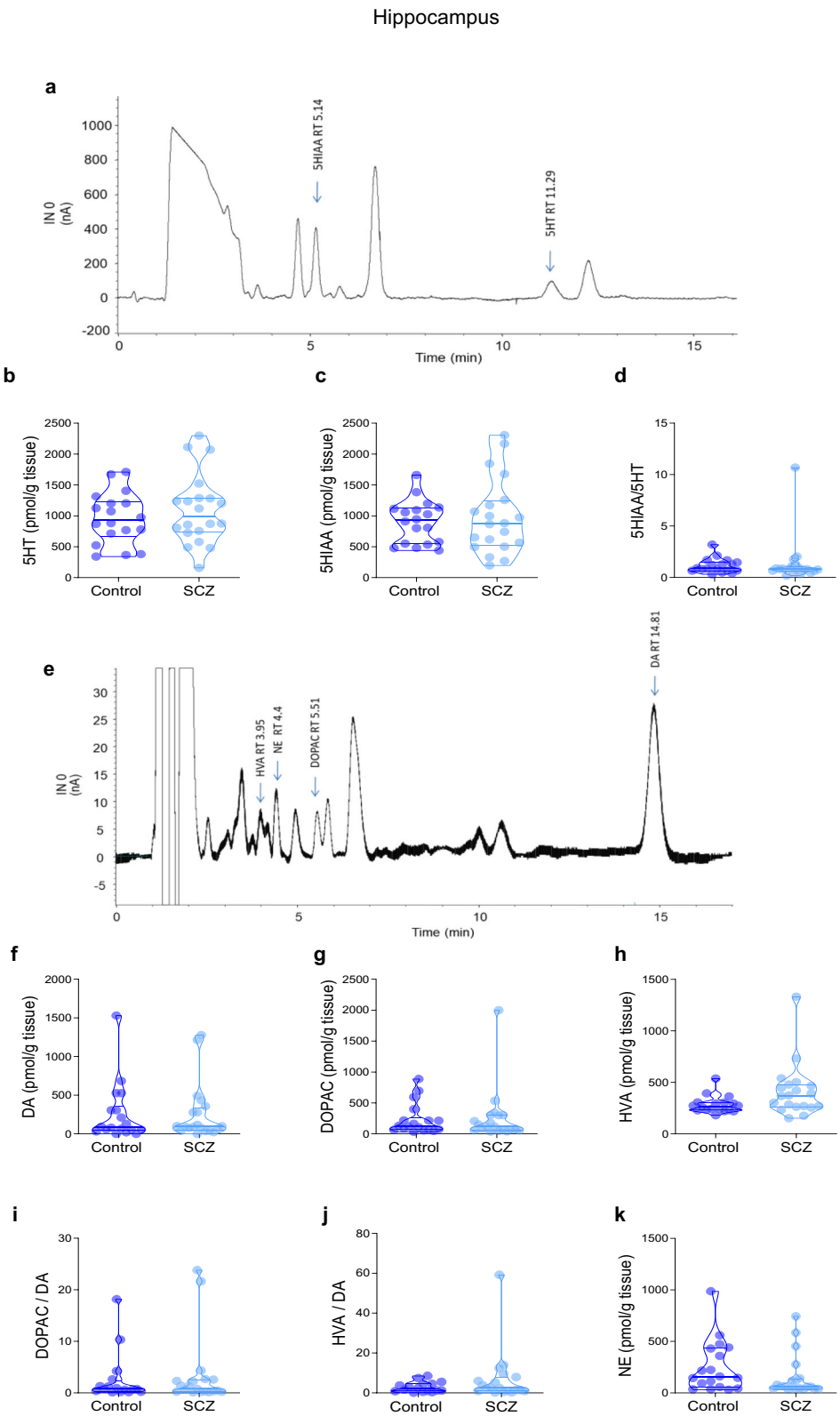

Fig. 2 HPLC analysis of monoamine and their precursors/derivatives levels in the *post-mortem* hippocampus of schizophrenia patients. **a**, **e** Representative HPLC chromatograms showing monoamine peaks in a hippocampus sample. Quantification of **b** 5-HT, **c** 5-HIAA, **d** 5-HIAA/5-HT ratio, **f** DA, **g** DOPAC, **h** HVA, **i** DOPAC/DA ratio, **j** HVA/DA ratio, **k** NE. In each sample, all the monoamines and their precursors/derivatives were detected in a single run by HPLC coupled with a coulometric detector and expressed as pmol/g of tissue. The number of examined samples is reported in Supplementary Table 2, for each considered molecule.

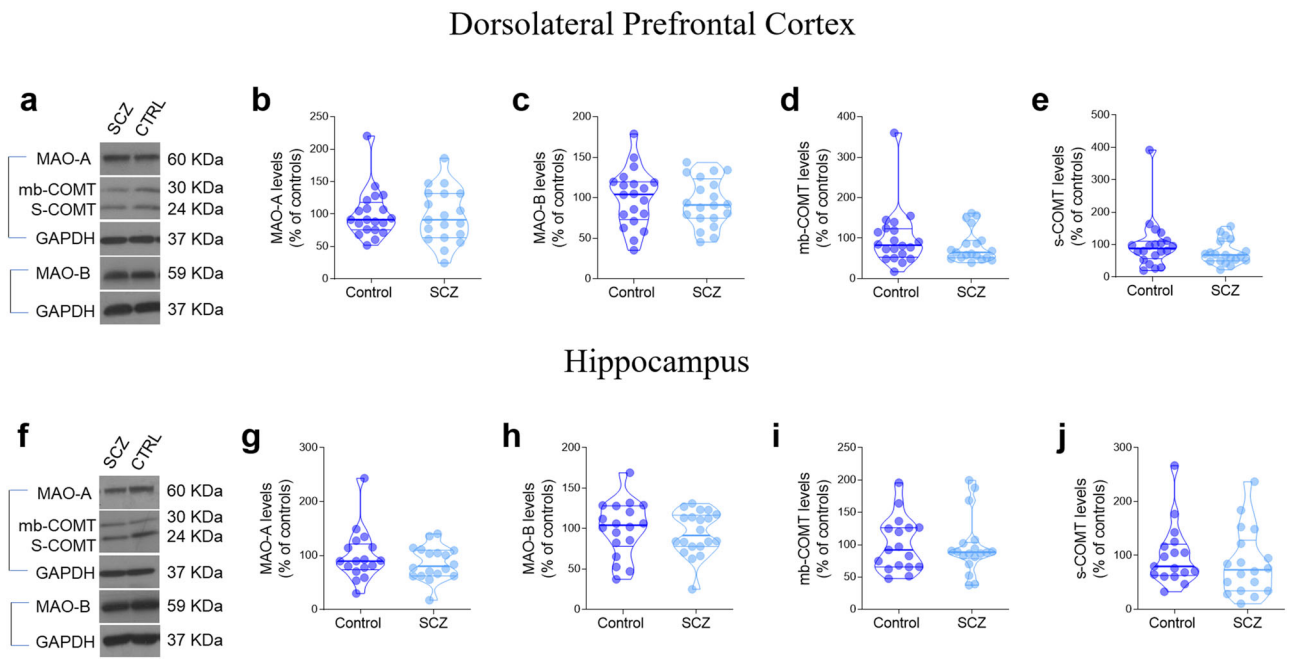

Fig. 3 Expression levels of the enzymes involved in monoamine catabolism in the post mortem dorsolateral prefrontal cortex and hippocampus of schizophrenia patients. a, **f** Representative immunoblots of the monoamine catabolism-related enzymes performed in the dorsolateral prefrontal cortex and hippocampus of non-psychiatric controls (CTRL) and patients with schizophrenia (SCZ). MAO-A and MAO-B were run on separate membranes due to their similar molecular weights. Quantification of **b**, **g** MAO-A, **c**, **h** MAO-B, **d**, **i** mb-COMT and **e**, **j** s-COMT protein levels in the **b**–**e** dorsolateral prefrontal cortex and **g**–**j** hippocampus of schizophrenia patients and control subjects. Protein variations are expressed as a percentage relative to the control group, which was set to 100%. Protein expression was determined by dividing each normalized sample intensity by the mean intensity of the control group, then multiplying by 100. GAPDH was used to normalize variations in loading and transfer. The number of examined samples is reported in Supplementary Table 3, for each considered protein.

[556.31; 1110.23], SCZ = 873.1 [550.18; 1193.36] pmol/g tissue, $F_{(1,34)} = 8.722$, $p = 0.0057$; Fig. 2a, c, Suppl. Table 2). However, correction for multiple testing with Bonferroni did not confirm such a significant change ($p = 0.054$ Suppl. Table 2). Accordingly, the 5-HIAA/5-HT ratio was comparable between diagnosis groups ($F_{(1,34)} = 0.4758$, $p = 0.4950$; ANCOVA; Fig. 2d; Suppl. Table 2). Similar to the DLPFC, in the hippocampus we did not report any significant changes for DA, DOPAC, and NE (DA: $F_{(1,34)} = 0.0031$, $p = 0.9558$; DOPAC: $F_{(1,34)} = 0.2307$, $p = 0.6341$; NE: $F_{(1,34)} = 2.3340$, $p = 0.1358$; Fig. 2e, f, g, k; Suppl. Table 2) between SCZ and control subjects. ANCOVA analysis showed that HVA levels were significantly increased in SCZ patients, compared to control subjects (median [IQR; 25%–75%]; HVA: Ctrl = 266.19 [232.41; 303.38], SCZ = 371.23 [268.42; 470.06] pmol/g tissue, $F_{(1,34)} = 4.907$, $p = 0.0335$; Fig. 2e, h; Suppl. Table 2). However, correction for multiple testing with Bonferroni did not confirm this alteration ($p = 0.3$; Suppl. Table 2). As in the DLPFC, the ratios DOPAC/DA and HVA/DA were comparable between SCZ patients and controls (DOPAC/DA: $F_{(1,31)} = 0.3177$, $p = 0.5770$; HVA/DA: $F_{(1,31)} = 2.5781$, $p = 0.1185$; Fig. 2i, j; Suppl. Table 2).

Overall, our HPLC analyses revealed no significant differences in DA, 5-HT, NE, and their main precursors/derivatives, in both the DLPFC and hippocampus of SCZ patients, compared to control subjects.

Western blotting analysis of MAO-A, MAO-B and COMT in the DLPFC and hippocampus of schizophrenia and control subjects

ANCOVA analysis revealed unaltered protein expression levels of MAO-A, MAO-B, s-COMT and mb-COMT in both the DLPFC (MAO-A: $F_{(1,35)} = 1.0555$, $p = 0.31128$; MAO-B: $F_{(1,36)} = 0.3468$, $p = 0.5595797$; s-COMT: $F_{(1,35)} = 0.8495$, $p = 0.36299$; mb-COMT: $F_{(1,35)} = 1.0880$, $p = 0.3041$; ANCOVA; Fig. 3a–e; Suppl. Table 3) and hippocampus (MAO-A: $F_{(1,31)} = 0.0268$, $p = 0.87101$, MAO-B: $F_{(1,34)} = 0.4310$, $p = 0.5159$; s-COMT: $F_{(1,31)} = 0.5191$, $p = 0.47663$; mb-COMT: $F_{(1,31)} = 0.0604$, $p = 0.8076$; Fig. 3f–j; Suppl. Table 3) between SCZ patients and control subjects.

Our data highlighted that the enzymes regulating monoamine catabolism displayed comparable expression levels between SCZ and control brains in both the DLPFC and hippocampus.

Comparative partial correlation analysis of monoamines and metabolites in the DLPFC and hippocampus of schizophrenia and control subjects

To characterize the interaction patterns within the monoaminergic system, we computed partial correlations separately for SCZ patients and control subjects in both the DLPFC and hippocampus (Figs. 4a and 5a). In the DLPFC, significant differences emerged in the correlation between 5-HT and DOPAC (p-adjusted = 0.042; Cohen's $q = -0.977$), as well as between DA and NE (p-adjusted = 0.018; Cohen's $q = 1.068$), as represented in the differential triangular matrix (Fig. 4b). The following partial correlation analysis revealed that the correlation between 5-HT and DOPAC was stronger in SCZ patients ($r = 0.696$) than in controls ($r = -0.118$) (Fig. 4c). On the other hand, the correlation between DA and NE was weaker in SCZ patients ($r = 0.225$) than in controls ($r = 0.861$) (Fig. 4c). A full summary of effect sizes (Cohen's q) and adjusted/non-adjusted p-values for the DLPFC is available in Supplementary Table 4.

In the hippocampus, we found a significant difference in the correlation between 5-HIAA and 5-HT (p-adjusted = 0.024; Cohen's $q = -1.02$) (Fig. 5b), which was stronger in SCZ patients ($r = 0.681$) than in controls ($r = -0.187$) (Fig. 5c). Similar to the DLPFC, the correlation between 5-HT and DOPAC was significantly different between diagnosis groups (p-adjusted = 0.048; Cohen's $q = 0.939$) (Fig. 5b). Also in this case, we found a stronger correlation in SCZ patients ($r = -0.689$) than in controls ($r = 0.091$) (Fig. 5c). A comprehensive summary of effect sizes (Cohen's q), as

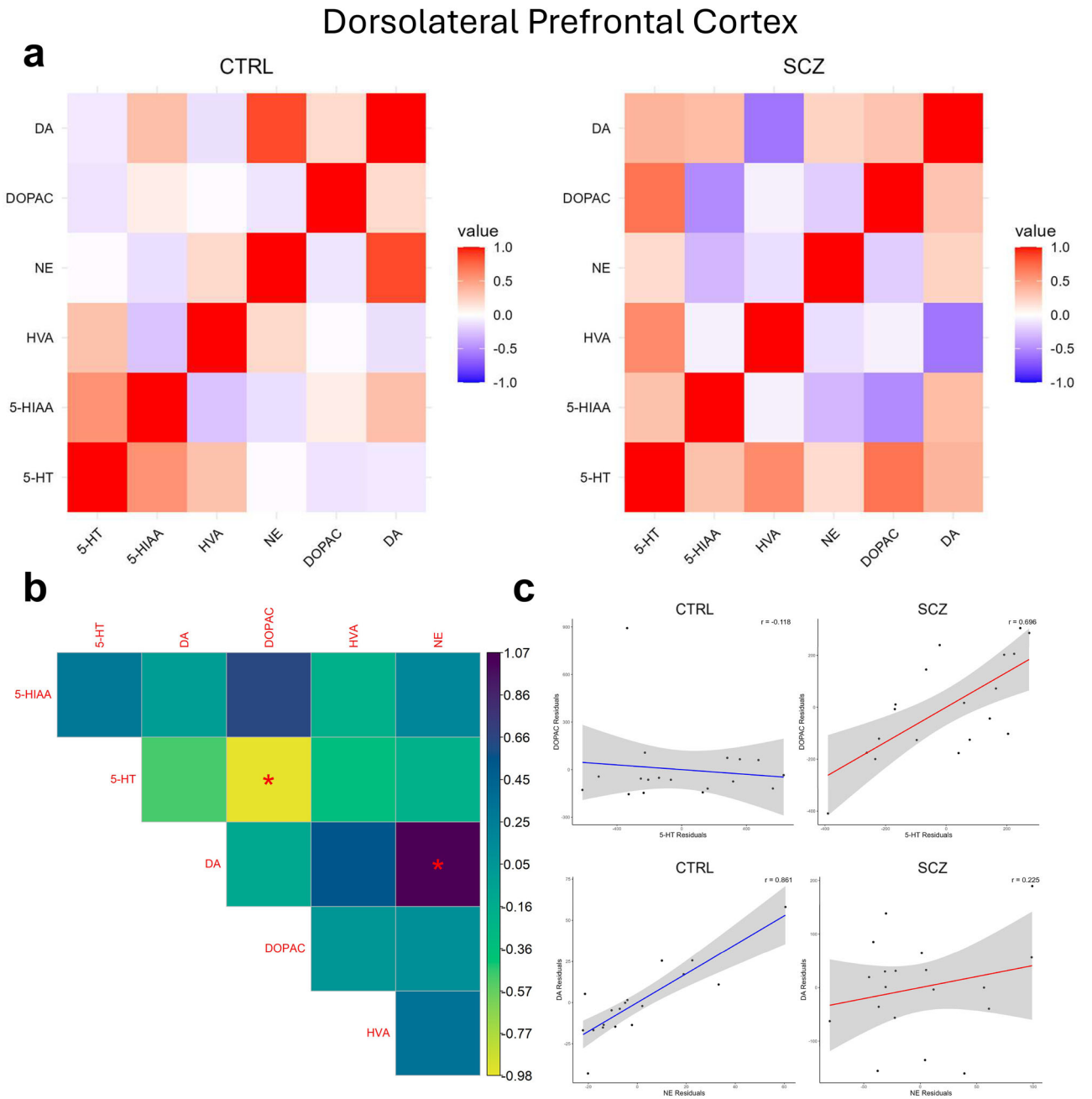

Fig. 4 Partial correlation analysis of monoamines and metabolites in the DLPFC. a Partial correlation matrices for control subjects (left) and patients with schizophrenia (right). Each cell represents the strength and direction of the partial correlation across monoamines and their metabolites, with colors ranging from blue (-1) to red (+1). **b** Triangular matrix illustrating the results of Fisher's z-test comparing partial correlations between schizophrenia patients and controls. The color scale reflects the effect size of the differences (Cohen's q), with blue indicating greater correlations in controls and yellow representing greater correlations in patients. Asterisks highlight statistically significant differences after multiple testing corrections. Significant differences emerged in the correlation between 5-HT and DOPAC (p-adjusted=0.042) and between DA and NE (p-adjusted=0.018). **c** Scatter plots depicting significant correlations in each group. Each dot represents an individual data point, and regression lines (blue for controls, red for patients) indicate the correlation trends.

well as adjusted/non-adjusted p-values, for the hippocampus is provided in Supplementary Table 5.

DISCUSSION

Although extensive pre-clinical and clinical evidence highlight a critical contribution of monoaminergic neurotransmission to the pathophysiology of SCZ and to antipsychotic treatment responses, the composition and interaction of monoamines within non-striatal cortical and limbic regions, such as the DLPFC and hippocampus of chronic patients, remain debated.

Our findings revealed no significant differences in the levels of DA, 5-HT, NE, or their major metabolites, HVA, DOPAC and 5-HIAA, in the DLPFC and hippocampus of patients with chronic SCZ compared with matched healthy controls. Consistent with these neurochemical results, we also observed no significant differences in the expression levels of key enzymes responsible for monoamine metabolism, such as MAO-A, MAO-B, and COMT, in either brain region examined. These results align with previous post-

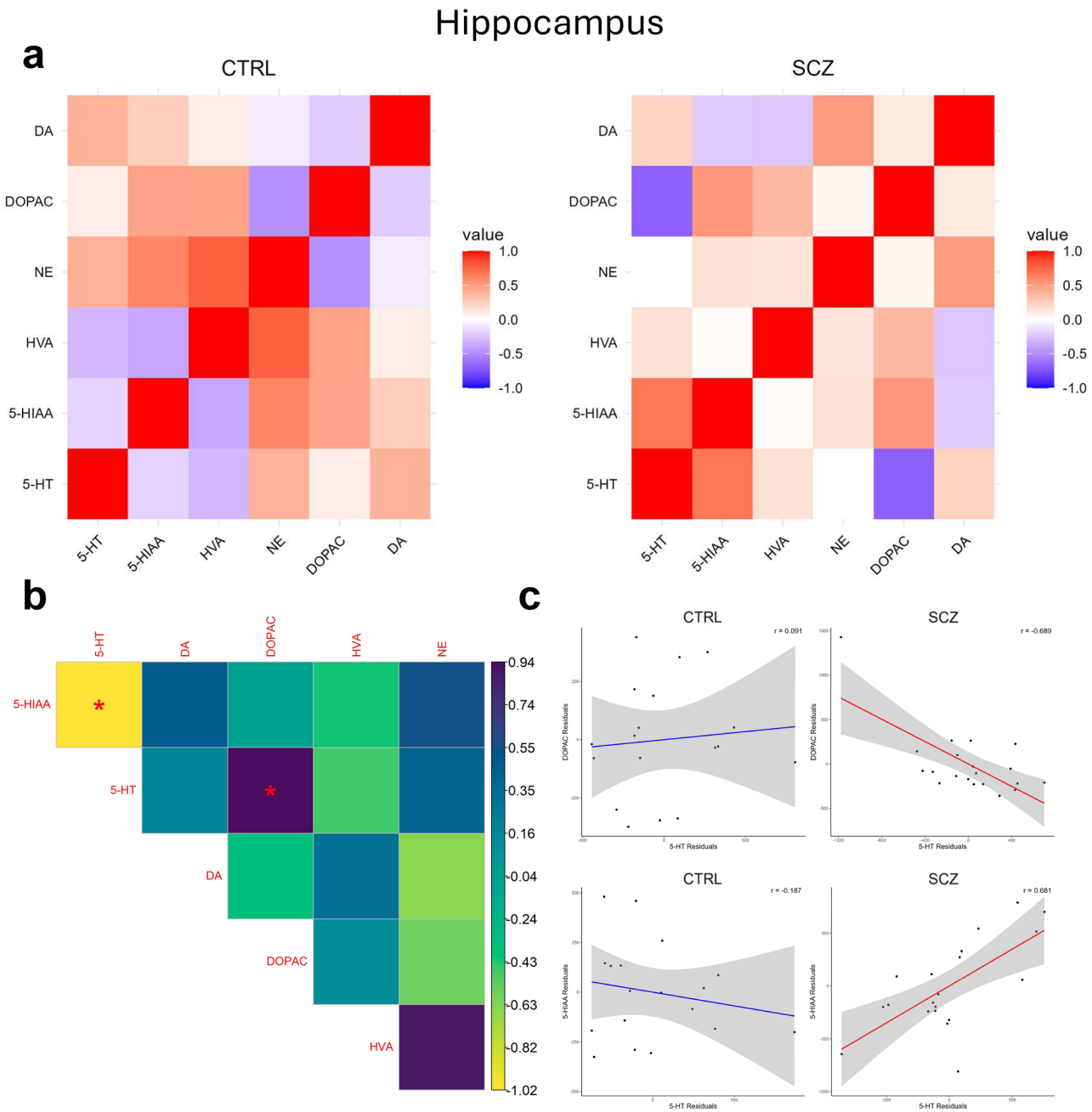

Fig. 5 Partial correlation analysis of monoamines and metabolites in the hippocampus. a Partial correlation matrices for control subjects (left) and patients with schizophrenia (right). Each cell represents the strength and direction of the partial correlation across monoamines and their metabolites, with colors ranging from blue (-1) to red (+1). **b** Triangular matrix illustrating the results of Fisher's z-test comparing partial correlations between schizophrenia patients and controls. The color scale reflects the effect size of the differences (Cohen's *q*), with blue indicating greater correlations in controls and yellow representing greater correlations in patients. Asterisks highlight statistically significant differences after multiple testing corrections. Significant differences emerged in the correlation between 5-HIAA and 5-HT (p-adjusted=0.024) and between 5-HT and DOPAC (p-adjusted=0.048). **c** Scatter plots depicting significant correlations in each group. Each dot represents an individual data point, and regression lines (blue for controls, red for patients) indicate the correlation trends.

mortem studies reporting comparable expression levels or enzymatic activity of MAO-A, MAO-B and COMT[45,51], as well as comparable cerebral concentrations of monoamines[44,50]. However, other studies reported alterations in DA[45,48], 5-HT[45,59,60] and NE[45,49] in SCZ brains, likely reflecting differences in antipsychotic exposure or brain regions investigated[44,61,62]. Most of these studies reported significant increases in monoamines in striatal regions. While we observed no significant differences in the expression of monoamine-catabolizing enzymes - although enzymatic activity was not directly measured - the absence of detectable changes in MAO-A, MAO-B or COMT expression does not rule out monoaminergic dysregulation, as neurotransmitter signaling depends on multiple regulatory levels, including synthesis, intracellular processing by membrane organelles (i.e Golgi apparatus), vesicular release, reuptake, and receptor signaling[63–66].

Despite the lack of quantitative differences in monoamine and metabolite levels, our partial correlation analyses highlighted distinct patterns of monoamine interplay in SCZ patients compared to controls, supporting the view that interaction-level

dysregulation may reveal disease-relevant alterations that remain invisible to traditional single-analyte comparisons. In both regions examined, SCZ patients displayed a significant correlation between 5-HT and DOPAC levels, a pattern that was absent in the controls. Specifically, this correlation was positive in the DLPFC and negative in the hippocampus of SCZ patients, pointing to regional-specific monoaminergic metabolism dysregulation. These findings suggest a disruption in the dynamic interaction of serotonergic and dopaminergic systems in the SCZ brain. In this context, DA catabolism — primarily assessed through DOPAC accumulation — appears more closely linked to 5-HT signaling, and vice versa, than under physiological conditions, as measured in control brains. In agreement with this hypothesis, correlation analyses conducted in rats revealed no significant correlation between 5-HT and DOPAC in the prelimbic cortex[67], a region considered a rodent proxy of the human PFC[68]. This cross-species consistency supports the idea that the observed abnormal monoamine correlations in the SCZ brain likely reflect a disease-specific disruption of monoamine metabolism interactions that are not present in healthy controls.

Although DA and 5-HT biosynthesis are regulated by distinct enzyme pathways, their catabolism converges on MAO enzymes, which oxidize 5-HT into 5-HIAA and DA into DOPAC[69]. Thus, the altered 5-HT–DOPAC correlations detected in the postmortem DLPFC and hippocampus of SCZ patients likely reflect a common disruption in the balance between serotonergic and dopaminergic catabolism. This is particularly relevant given the extensive evidence showing that serotonergic and dopaminergic systems reciprocally interact to modulate emotional reactivity[70] and contribute to the regulation of negative[71–73] and cognitive symptoms of SCZ[74–76]. Therefore, a dysregulation of the reciprocal interaction of these two neurotransmitter systems may represent a mechanistic contributor to symptom severity across these domains.

In the DLPFC of patients with SCZ, we observed a reduced positive DA-NE correlation compared to controls. This finding is noteworthy as DA and NE share the same biosynthetic pathway, with DA serving as the immediate NE precursor[77] and their terminals converge on the dendritic spines of prefrontal glutamatergic neurons, a major post-synaptic target for both neurotransmitters[78]. Experimental evidence shows that selective NE depletion in the PFC impairs amphetamine-induced DA release and influences salience attribution[79,80]. Given that aberrant salience driven by dopaminergic dysfunction is considered a key neurobiological mechanism underlying psychotic symptoms in SCZ[81], a disruption in DA-NE coupling may have clinical relevance. Moreover, optimal and balanced NE and DA signaling at postsynaptic receptors is crucial for PFC-mediated cognitive functions[82]. Microdialysis studies in rats further support this neurotransmitter interplay, showing that NE and DA levels increase in a sustained, coordinated manner during cognitive task engagement[83]. Taken together, the weakened DA-NE correlation observed in the postmortem DLPFC of SCZ patients compared to controls may reflect a reduction in the functional integration of these two catecholaminergic systems. Such a disruption could contribute to the impairment of salience processing and cognitive functions, both of which represent core phenotypic domains in SCZ.

In the hippocampus, a positive correlation between 5-HIAA and 5-HT was observed in SCZ patients but not in controls. This finding may suggest an enhanced 5-HT turnover—namely a tighter interplay between 5-HT catabolism and synthesis, respectively indexed by 5-HIAA and 5-HT levels—which aligns with previously reported serotonergic alterations in SCZ[84]. In keeping with this, hippocampal serotonin depletion has been shown to exacerbate psychotic-like behaviors in phencyclidine (PCP)-treated rats[85]. Furthermore, inhibition of serotonin transmission significantly alters dendritic spine morphology across multiple brain regions, with the most pronounced effects in the hippocampal formation, closely resembling spine abnormalities described in the post-mortem SCZ brains[86].

Our findings suggest that the analysis of quantitative correlations among monoamines and their metabolites in post-mortem brains can reveal SCZ-related modifications that may remain undetectable when examining absolute metabolite levels alone. These interaction-level alterations could underlie disruptions in neurotransmitter pathway regulation and cross-system interplay. This interpretation is reinforced by in vivo PET studies showing altered amphetamine-induced neurotransmission responses in SCZ patients[87–89]. While absolute monoamine levels did not differ between diagnostic groups, our findings point to subtle yet biologically meaningful variations in the interactions among DA, 5-HT, NE, and their catabolites, HVA, DOPAC, and 5-HIAA. These alterations may represent a core component of the neurobiological changes contributing to SCZ symptoms.

Future research on larger cohorts should further investigate the covariation and functional interplay among monoaminergic molecules and their metabolic pathways in SCZ. A key limitation of the present study is that, although the patient cohort was well balanced, we were unable to control for the effects of antipsychotic medication on monoamine levels or the expression of related metabolizing enzymes. Consequently, it remains unclear whether the observed lack of changes in monoamine levels and their metabolites reflects intrinsic disease-related features or the influence of pharmacological treatments. Previous post-mortem investigations have yielded conflicting results regarding the impact of AP medication on monoamine levels in SCZ. For example, a previous study reported that patients receiving APs exhibit higher tyrosine hydroxylase activity[90], suggesting enhanced dopamine synthesis and turnover. Conversely, another study in rats showed that APs like clozapine and risperidone can increase extracellular serotonin concentration in the prefrontal cortex and nucleus accumbens[91]. More recently, Purves-Tyson and colleagues demonstrated that midbrain mRNA expression of key monoamine-metabolizing enzymes, including COMT, MAO-A and MAO-B, does not correlate with AP dosage, suggesting a limited effect of medication on these molecular pathways[92]. Finally, chronic haloperidol administration in rodents has been found to increase striatal DA turnover without consistent effects on cortical monoamines[93]. Taken together, these data indicate that a clear directional hypothesis on the relationship between AP exposure and post-mortem monoamine levels remains currently elusive, underscoring the need for controlled studies integrating detailed medication histories with molecular profiling across distinct brain regions.

The lack of specific information on age of onset or illness duration in SCZ patients, two variables that could influence monoamine brain levels either through illness-related brain modification or differences in antipsychotic treatment exposure, represents another limitation of our study. Unfortunately, we did not have any access to such clinical information. We also acknowledge that the intrinsic heterogeneity of the disorder and the relatively small sample size, which is a common limitation in post-mortem studies, make the detection of relevant biological changes particularly challenging.

In conclusion, our findings highlight the importance of investigating monoamine interactions rather than focusing solely on individual levels. The weaker DA-NE correlation observed in the DLPFC, and the increased 5-HIAA-5-HT correlation in the hippocampus of patients with SCZ suggest region-specific alterations in the combined turnover of monoamine and their metabolites. These results provide new insight into the molecular mechanisms underlying monoamine dysregulation in SCZ and may open avenues for pharmacological strategies targeting disease treatment.

DATA AVAILABILITY

Data will be made available on request.

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AUTHOR CONTRIBUTIONS

A.D.M. investigation; formal analysis; visualization; writing—review & editing; V.B. and A.H.: investigation; G.D.S.: formal analysis; visualization; writing—original draft; writing - review & editing; A.B., F.E., and A.R.: writing—review & editing; A.d.B.: supervision, writing—review & editing; funding acquisition; A.U.: conceptualization, funding acquisition, supervision, writing—review & editing. All authors approved the final version of the manuscript.

COMPETING INTERESTS

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ADDITIONAL INFORMATION

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