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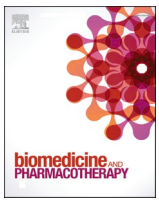

Metabolic stress-response in female rats: β-oxidation-induced vulnerability vs adaptive resilience

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ABSTRACT

Major depressive disorder (MDD) is characterized by substantial sex differences in its prevalence, with women having double the risk to experience MDD compared to men. One of the major goals of research is to understand the sex-specific factors that confer vulnerability or resilience to stress, which represents one of the most prominent risk factors for MDD. In this study, we performed a targeted metabolomic analysis in the ventral hippocampus of adult vulnerable or resilient female rats to unravel metabolic differences underlying the different susceptibility to stress. From a behavioral standpoint, female rats started to develop anhedonic-like symptoms after 2 weeks of stress. Interestingly, we observed that the anhedonic like behavior, an indicator of vulnerability, was associated to several metabolic pathways, among which it is evident clearly the enhancement of fatty acid β-oxidation, a feature previously observed in vulnerable male rats. Conversely, resilient female rats seem to set in motion a metabolic adaptation, since they do not activate any specific metabolic pathway to sustain resilience, although enrichment pathway analysis highlighted the modulation of antioxidant and neuroprotective mechanisms specifically for the maintenance of resilience. Taken together, our findings indicate that the mechanisms underlying vulnerability in female rats are similar with those we previously observed in males, while those underlying resilience clearly differ. These results reinforce the importance of pursuing the study of sex-specific molecular mechanisms characterizing the gender gap in developing depression.

1. Introduction

Major depressive disorder (MDD) is considered the leading cause of disability and the burdensome disease worldwide, affecting around 20 % of people in their lifetime. The prevalence of depression is higher in women than in men in developed countries and globally [1], however the reason for the sex-specific incidence remains poorly understood.

Indeed, to date most of the preclinical studies in this field have been focused on male rodents [2,3] consequently contributing to the failure of novel pharmacotherapies in clinical research for females [4]. Actually, in the last 10 years, the research has been extended with a trans-diagnostic approach, to address sex as a fundamental variable to be considered in the preclinical studies on MDD and psychiatric disorders at large [5]. To explore this aspect, we recently started including female rats in our preclinical studies, proving evidence that sex influences not only brain functions but also the development of pathological phenotypes in animal models based on genetic and pharmacological modulation of the serotonergic system [6,7].

Among the mechanisms investigated in the field, changes in brain energy metabolism, associated with specific metabolic signatures in plasma and urine, have been observed in patients with MDD [8]. Moreover, sex differences in metabolism have also been associated with treatment outcomes [9], thus revealing the importance of providing deeper metabolic insight through the role of bioenergetics in the sex-related susceptibility to develop MDD.

By employing the well-established animal model of depression, represented by the chronic mild stress (CMS) [10], which allows us to recapitulate anhedonia as an advantageous approach in translational

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research in MDD, we can discriminate the adverse stress effects by dissecting vulnerable and resilient phenotypes and investigate how sex differences may influence brain functions.

In particular, recent studies have demonstrated that the ventral hippocampus (vHip) plays a crucial role in modulating vulnerability and resilience to stress [11–13] in terms of hedonic phenotype, thus highlighting the importance of conducting subregion-specific studies to better define its role in regulating distinct aspects of mood-related behavior.

Accordingly, in adult male rats, we have recently demonstrated a specific metabolic signature in vHip that underpins the different susceptibility to develop a depressive-like behavior after 2 [14] and 6 [15, 16] weeks of CMS. In addition, we demonstrated that the antidepressant venlafaxine restored resilience to stress by shifting the balance between glycolysis and fatty acid β-oxidation [15]. Moreover, we proved that the exposure of resilient animals to an unfamiliar acute stressor unmasked a trait of vulnerability by enhancing β-oxidation [16], an effect that characterized vulnerable rats.

Here, for the first time, we start dissecting in adult female rats the mechanistic insight into the susceptibility to stress, by investigating the contribution of brain metabolism in the vulnerability and resilience by conducting a targeted metabolomic analysis in the vHip.

Our results show that the different susceptibility to develop an anhedonic-like behaviour in female rats was associated with several metabolic changes, including the enhancement of fatty acid β-oxidation, whereas resilience is sustained by an overall metabolic adaptation.

These findings reveal critical metabolic differences in female vs. male rats that highlight the importance of pursuing the study of sex-specific molecular mechanisms underpinning the gender gap in developing depression.

2. Material and methods

2.1. Animals

Female Wistar Han rats (Charles River, Germany) were brought into the laboratory one month before the start of the experiment. The animals were singly housed with food and water freely available and were maintained on a 12-h light/dark cycle (lights on at 08.00) and in a constant temperature (22 ± 2 °C) and humidity (50 ± 5 %) conditions. All procedures used in this study have conformed to the rules and principles of the 86/609/EEC Directive and have been approved by the Local Bioethical Committee at the Institute of Pharmacology, Polish Academy of Sciences, Krakow, Poland.

2.2. Stress procedure and sucrose consumption test

All procedures applied in this study are consistent with those previously implemented in male subjects [15]

After the period of adaptation to the housing conditions, the animals (6 rats for groups) were trained to consume 1 % sucrose solution (see [16] for details). Sucrose consumption test (SCT) was conducted at weekly intervals, and the sucrose intake was measured by weighing pre-weighed bottles containing the sucrose solution at the end of the test.

After the sucrose training, rats were randomly divided into two groups. One group of animals was subjected to the CMS procedure for a period of 6 consecutive weeks (Fig. 1A).

Each week of the stress regime consisted of: two periods of food or water deprivation, two periods of 45-degree cage tilt, two periods of intermittent illumination (light on and off every 2 h), two periods of soiled cage (250 ml water in sawdust bedding), one period of paired housing, two periods of low intensity stroboscopic illumination (150 flashes/min), and three periods of no stress. All stressors were 10–14 h of duration and were applied individually and continuously, day and night, in a semi-random order.

Animals of the No stress group were housed in separate rooms and had no contact with the stressed animals. They were deprived of food and water for 14 h preceding each sucrose test, but otherwise, food and water were freely available in the home cage. On the bases of the results of the SCT, we separated stressed rats that consumed the sucrose solution around the 40 % of pre-stress values, named CMS vulnerable (CMS vul) from rats showing a similar hedonic phenotype to non-stressed animals, named CMS resilient (CMS res). During the last day of the

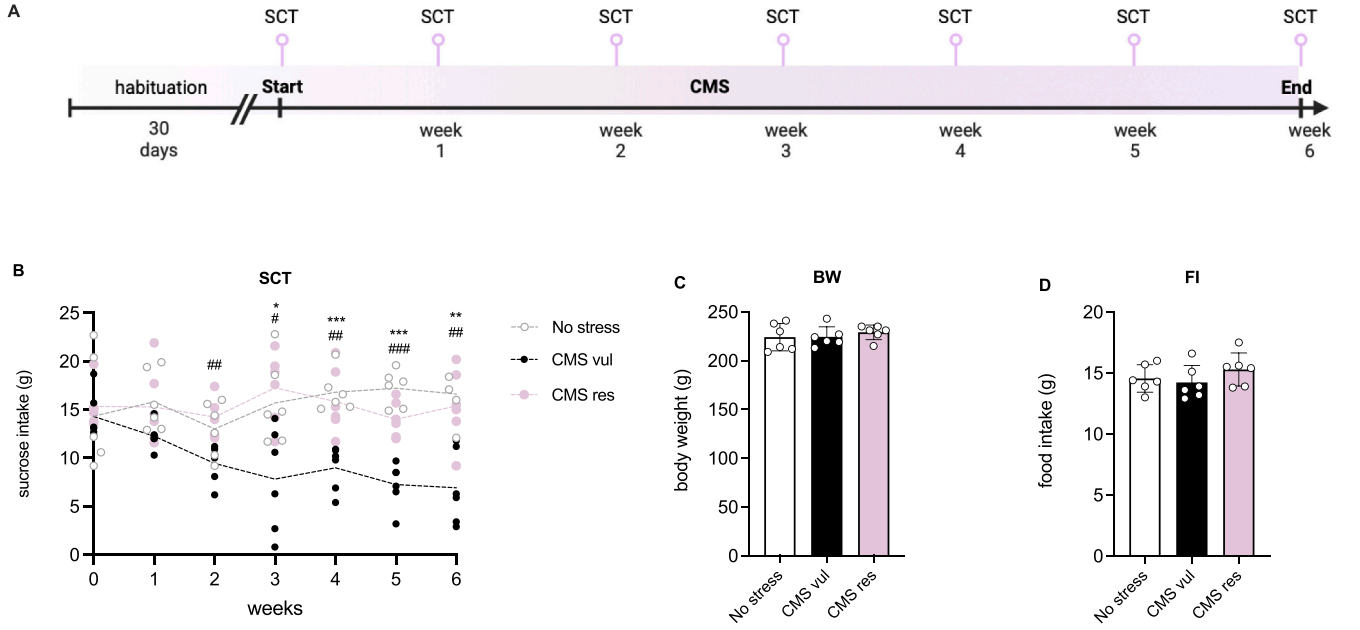

Fig. 1. Sucrose consumption test (SCT), body weight (BW) and food intake (FI) in adult female rats exposed to six weeks of chronic mild stress (CMS). Panel A shows the experimental design. SCT (panel B) was assessed at weekly intervals, while BW (panel C) and FI (panel D) were measured at the end of the sixth week of CMS. The data are the mean ± SEM of 6 independent determinations. Two-way ANOVA with repeated measures (panel B) and one-way ANOVA followed by Tukey multiple comparison test (panels C-D). * $p < 0.05$, ** $p < 0.01$ *** $p < 0.001$ vs No stress; # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$ vs CMS res.

entire CMS protocol, we measured the body weight (BW) and food intake (FI) (g/day).

24 h after the final stressor of the CMS protocol, the animals were decapitated and ventral hippocampus, which corresponds to Bregma levels −4.8 to −6.3 mm according to the Paxinos and Watson atlas [17], was collected and stored at −80°C.

2.3. Metabolomic analysis

Metabolomic data were obtained by liquid chromatography coupled to tandem mass spectrometry, by using an API-3500 triple quadrupole mass spectrometer (AB Sciex, Framingham, MA, USA) coupled with an ExionLC™ AC System (AB Sciex, Framingham, MA, USA). For the analysis, 10 mg of ventral hippocampus was used. Tissue was homogenized in 250 µL of ice-cold methanol/acetonitrile/water (v/v, 55/25/20) containing 1 ng/µL of [U-$^{13}C_6$]-Glucose (cat.no. 389374, Sigma-Aldrich) and [U-$^{13}C_5$]-Glutamine (cat.no. 605166, Sigma-Aldrich), 0.2 ng/µL of [U-$^{13}C_2$, ^{15}N]-GSH (cat.no. 683620, Sigma Aldrich) as internal standards. Lysates were spun at 15,000 g for 15 min at 4°C and supernatants were then dried under N_2 flow at 40°C. Dried samples were then resuspended in 110 µl of methanol/water (v/v 70:30) for subsequent analysis.

For the quantification of metabolites, cofactors and nucleotides, a cyano-phase LUNA column (50 mm×4.6 mm, 5 µm; Phenomenex, Torrance, CA, USA) was used in a 5 min run in negative ion mode (ESI-negative). The mobile phases were phase A: Water and B: 2 mM ammonium acetate in MeOH. The gradient was 50 %B for all the analysis with a flow rate of 600 µL/min.

Samples were also analyzed by different runs in positive to quantify amino acids and acyl-carnitine levels.

Additional analyses in positive ion mode were performed to quantify amino acids and acyl-carnitines. Acyl-Carnitine were separated using a ZORBAX Stable Bond CN (2.1 ×150 mm, 5µm; Agilent). Amino acid and biogenic amine were quantified following derivatization of the samples. Briefly, 50 µL of 5 % phenyl isothiocyanate (PITC) in 31.5 % EtOH and 31.5 % pyridine in water were added to 10 µL of each sample. The mixtures were incubated for 20 min at room temperature, dried under N_2 flow, and resuspended in 100 µL of 5 mM ammonium acetate in MeOH/H_2O (v/v, 1:1). Quantification was performed using a C18 column (Biocrates, Innsbruck, Austria) maintained at 50°C.

For positive ion mode analysis, the mobile phases were phase A: 0.2 % formic acid in water and phase B: 0.2 % formic acid in acetonitrile. The gradient was T0: 100 %A, T5.5: 5 %A, T7: 100 %A with a flow rate of 500 µl/min for amino acids and biogenic amine, and 300 µl/min for acyl-carnitine.

All metabolites included in this targeted panel were previously validated using pure analytical standards. Each analyte was characterized by direct infusion of the standard solution to define the precursor ion (Q1), product ion (Q3), and fragmentation pattern, as well as its specific retention time under our chromatographic conditions.

Stable isotope-labeled internal standards were used to monitor extraction efficiency, instrument performance, and sample handling reproducibility, but not for normalization.

MultiQuant™ software (version 3.0.3, AB Sciex, Framingham, MA, USA) was used for data analysis and peak review of chromatograms. Raw peak areas (Supplementary tables 1) were exported and uploaded into MetaboAnalyst 6.0 for data processing. Within MetaboAnalyst, data were normalized to the median of all detected metabolites in each sample, log-transformed, and Pareto scaled to correct for heteroscedasticity and minimize the influence of highly abundant metabolites [18,19].

Metabolic pathway enrichment analysis was performed using MetaboAnalyst 6.0 web tool. Pathways were defined based on the human Small Molecule Pathway Database (SMPDB). Significantly enriched pathways were identified based on a false discovery rate (FDR) or p-value < 0.05.

2.4. Statistical analysis

Graph Pad Prism 10 (GraphPad Software Inc., CA, USA) was used for graphical representations and statistical analysis.

The normality of residuals was assessed using the Shapiro-Wilk test, and no discernible variation in homogeneity was discovered.

The SCT results were analyzed with the two-way analysis of variance (ANOVA) with repeated measures, followed by Tukey multiple comparison test, whereas BW, FI and metabolites levels were analyzed with one-way ANOVA followed by Tukey's multiple comparison test. For the analysis of metabolite ratios, one-way ANOVA was followed by Fisher's LSD test. Each experimental group consisted of 6 rats.

Significance for all tests was assumed for $p < 0.05$. Behavioral data are presented as means ± standard error of means (SEM), whereas metabolomic data are shown as means ± standard deviation (SD).

3. Results

3.1. Six weeks of CMS induced anhedonic-like behavior in adult female rats

As reported in Fig. 1B, two-way ANOVA with repeated measures showed a statistically significant effect of CMS ($F_{2–16}$ =14.85, $p < 0.001$) and of CMS X week ($F_{12–6}$ =3.99, $p < 0.001$) on sucrose intake. Accordingly, Tukey multiple comparison test indicated that two weeks of CMS were necessary to identify two subpopulations of stressed animals, i.e., vulnerable (CMS vul) and resilient (CMS res). Indeed, the former (black dashed line) had a reduction of sucrose intake in comparison to both No stress and resilient while the latter (pink dashed line) showed a similar sucrose intake to No stress group, effects that were maintained for the whole duration of the stress protocol (CMS vul - week2: −3.5 g $p > 0.05$ vs No stress, −4.8 g $p < 0.01$vs CMS res; week3: −7.9 g $p < 0.05$ vs No stress, −9.4 g $p < 0.05$ vs CMS res; week4: −7.8 g $p < 0.001$ vs No stress, −6.8 g $p < 0.01$vs CMS res; week5: −9.9 g $p < 0.001$ vs No stress, −6.7 g $p < 0.001$vs CMS res; week6: −9.7 g $p < 0.01$ vs No stress, −8.4 g $p < 0.01$vs CMS res).

In addition, one-way ANOVA did not reveal a statistically significant effect of CMS or in body weight (Fig. 1B) either in food intake (Fig. 1C).

3.2. Ventral hippocampus metabolomic profile was differently affected by six weeks of CMS in vulnerable and resilient female rats

Targeted metabolomic analysis was performed in the ventral hippocampus to investigate metabolic alterations associated with the behavioral phenotype observed following CMS exposure.

As showed in Fig. 2A, Partial Least Squares Discriminant Analysis (PLS-DA) revealed a different metabolic profile between unstressed and stressed animals, with further separation among CMS vul and CMS res groups. To identify the key metabolites driving group discrimination, we examined Variable Importance in Projection (VIP) scores (Fig. 2B). In this context, VIP scores > 1 indicate variables/metabolites that significantly contribute to group separation. The corresponding regression coefficients (represented by colored squares to the right of each metabolites' row) provide information on the direction (increase or decrease) of each metabolite's association with the phenotype.

To complement the multivariate analysis, univariate statistical testing was performed using one-way ANOVA followed by Tukey's HSD post hoc test, identifying 28 metabolites that were significantly altered among the experimental groups ($p < 0.05$; Fig. 2C). Notably, these included several amino acids, acyl-carnitines, and nucleotides.

To provide a more comprehensive overview of the metabolic landscape associated with stress vulnerability and resilience, a heatmap was generated to visualize the relative abundance (expressed as z-scores) of all 96 detected metabolites across the groups (Fig. 2D).

Based on the analyses presented in Fig. 2, we next performed pairwise comparisons (no stress vs CMS vul, no stress vs CMS res, and CMS

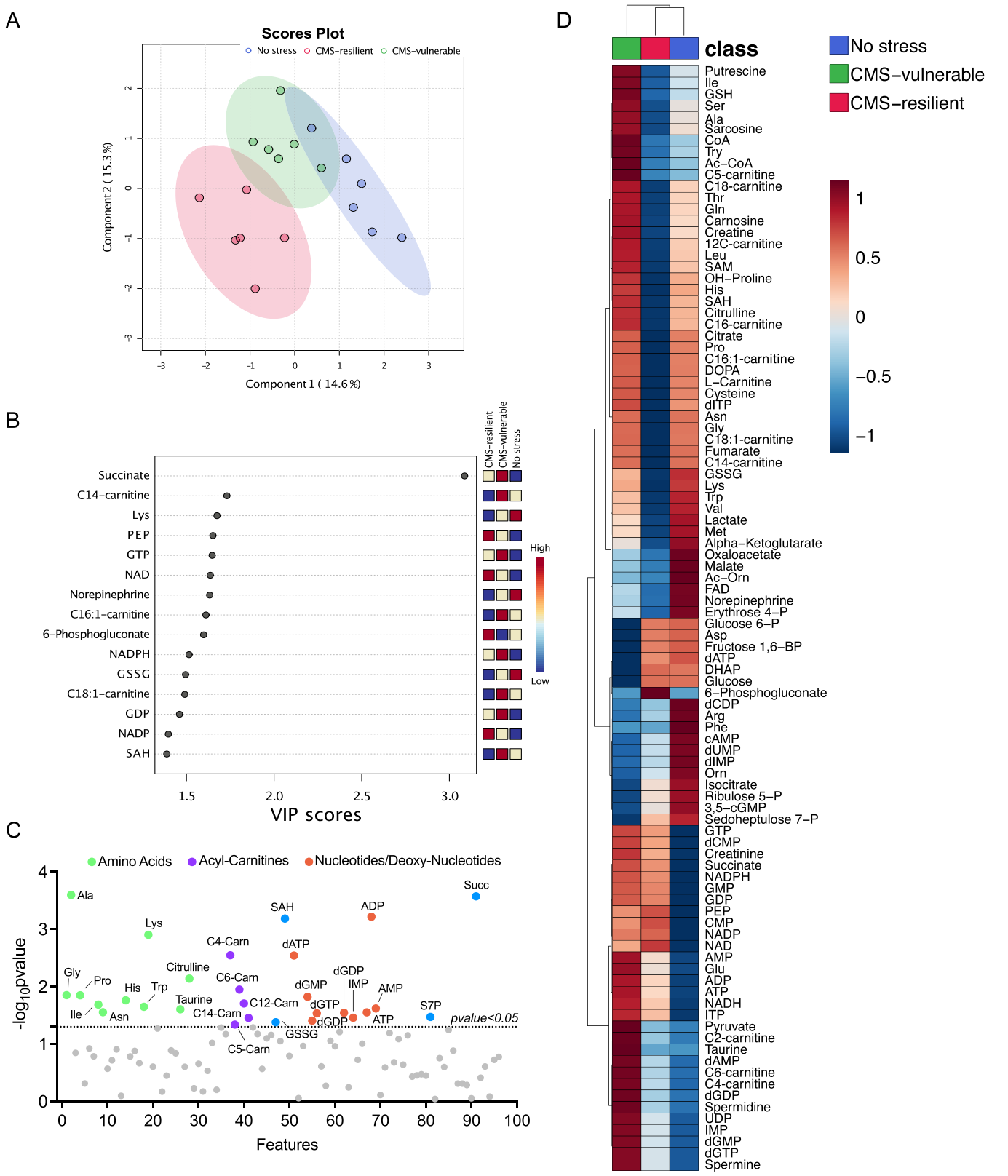

Fig. 2. Metabolic profile of the ventral hippocampus in adult female rats following six weeks of chronic mild stress (CMS). Panel A: PLS-DA analysis reveals distinct metabolic profiles among no stress, CMS vul, and CMS res animals. Panel B: VIP score plot identifying key metabolites contributing to group separation. C: univariate ANOVA analysis. Gray dots represent non-significant metabolites (p value>0.05); green dots indicate significantly altered amino acids, violet dots represent acyl-carnitines, orange dots correspond to nucleotides or deoxynucleotides, and light blue dots are other metabolites. D: Heatmap displaying z-score of all 96 detected metabolites, presented as the mean per group.

vul vs CMS res) to further dissect the specific metabolic alterations associated with stress vulnerability and resilience.

As shown in Fig. 3A-B, volcano plots were used for a comparative analysis of metabolites in the experimental groups relative to the no stress condition. By setting the significance threshold to $p < 0.05$, we identified 13 dysregulated metabolites in the CMS res vs No stress comparison, including 2 increased and 11 decreased metabolites (Fig. 3A) and 25 altered metabolites in the CMS vul vs No stress comparison, comprising 21 increased and 4 decreased (Fig. 3B).

Moreover, correlation heatmaps generated from the comparisons CMS-vulnerable vs. no stress (Fig. 3C) and CMS-resilient vs. no stress (Fig. 3D) reveal distinct patterns of metabolite co-regulation. These differences suggest phenotype-specific metabolic reprogramming, potentially reflecting adaptive (resilient) vs. unfavorable (vulnerable) responses to chronic stress. While volcano plot analysis highlights changes in individual metabolite levels, correlation analysis provides insight into alterations in the relationships between metabolites, further supporting divergent biochemical network dynamics across phenotypes.

Finally, we performed Metabolite Set Enrichment Analysis (MSEA) to categorize the identified metabolites using the human Small Molecule Pathway Database (SMPDB).

All significantly enriched pathways (FDR < 0.05) are reported in Supplementary tables 2 and 3. To further explore the metabolic basis of vulnerability or resilience to CMS, we conducted a Venn diagram analysis, which revealed a total of 79 pathways uniquely enriched in the vulnerable group. Redundant or overlapping pathways were clustered, and representative pathways are shown in Fig. 3E, providing a clearer overview of the major metabolic processes affected. Among these, fatty acid β-oxidation and branched-chain amino acid metabolism emerged as enriched pathways in the vulnerable phenotype.

Prompted by these results, we next focused on the direct comparison between CMS res vs CMS vul animals. As shown in Fig. 4A, a volcano plot analysis identified 29 significantly altered metabolites in the CMS-res vs. CMS-vul comparison ($p < 0.05$), including 1 upregulated and 28 downregulated metabolites. Interestingly, many of the discriminant metabolites belonged to the acylcarnitine and amino acid classes. MSEA revealed several significantly enriched pathways ($p < 0.05$), which are presented in Fig. 4B. These pathways collectively suggest a metabolic reprogramming in stress-vulnerable female rats, particularly involving amino acid metabolism, lipid catabolism (fatty acid β-oxidation), neurotransmitter biosynthesis, and redox homeostasis.

Notably, the C2/C0 ratio, a commonly used marker of fatty acid β-oxidation activity, was significantly increased in CMS vul compared to No stress group, and was also elevated compared to the CMS res group, although this difference did not reach statistical significance. We also observed an increase in the sum of short-chain acylcarnitines compared to both No stress and CMS res groups, whereas medium- and long-chain acylcarnitines were decreased specifically in CMS-res compared to CMS-vul (Fig. 4C).

Although the total branched-chain amino acids (BCAAs, leucine, isoleucine, and valine) were not significantly different across the three conditions, isoleucine levels were elevated in CMS vul compared to CMS res (with no change relative to No stress). This was accompanied by an increased C5/(leu+ile+val) ratio in CMS vul animals, suggesting alterations in BCAA catabolism (Fig. 4D).

We also observed a reduction in the sum of essential amino acids (EAAs) in CMS res compared to both No stress and CMS vul groups, along with a decrease in non-essential amino acids (NEAAs) in CMS vul relative to CMS res (Fig. 4E). Among glucogenic amino acids, alanine was increased in CMS vul compared to both No stress and CMS res, while glycine and proline were reduced in CMS res relative to the other two conditions (Fig. 4F). Consequently, the combined abundance of these three glucogenic amino acids was significantly lower in CMS res animals (Fig. 4F).

Regarding energy metabolism, although ATP energy charge remained unchanged, ATP levels were elevated in CMS vul animals compared to both No stress and CMS res groups (Fig. 4G). We also detected higher levels in the total nucleotide pool in CMS vul compared to No stress condition, suggesting enhanced nucleotide turnover (Fig. 4H). Additionally, levels of deoxyribonucleotides were elevated, indicating greater DNA precursor availability and reflecting global changes in deoxynucleotide metabolism and potential DNA biosynthetic activity (Fig. 4H). Finally, the $NAD^+/NADH$ ratio was not significantly altered, although a trend toward increased NADH was observed in CMS vul animals compared to controls ($p = 0.0569$) (Fig. 4I).

4. Discussion

In this study we provide evidence that, in female rats, vulnerability to CMS, measured as anhedonic-like phenotype, is associated to specific metabolic changes whereas resilience with an overall metabolic adaptation. Taking into account our previous results in male rats exposed to the same paradigm [15] it appears that vulnerability occurs through the same mechanism observed in males, i.e., increased fatty catabolism through fatty acid β-oxidation, whereas resilience mechanisms diverge between sexes.

Accordingly, we showed differences also at behavioral levels, since, in females, the separation between vulnerable and resilient rats induced by CMS appears evident starting from the second week of stress, i.e., one week later compared to male rats.

As mentioned, at the level of vHip metabolism, vulnerability to CMS was associated with alterations in a higher number of metabolites compared to resilience, indicating a broader metabolic remodeling rather than a direct causal link. Indeed, 21 metabolites showed elevated levels and 4 were reduced in CMS-vulnerable rats compared to unstressed controls, while only 2 were elevated and 11 diminished in the resilient group. This led to 81 pathways modulated by chronic stress with 79 specific metabolic pathways underlying vulnerability, but none involved in resilience. Of note, only 2 pathways were modulated by stress per sè, i.e., the carnitine synthesis and phosphatidylcholine biosynthesis. Among the major metabolic processes affected in vulnerable animals, fatty acid β-oxidation and branched-chain amino acid metabolism clearly emerged.

Indeed, considering the acylcarnitine class, we observed that vulnerability to CMS was associated to an enhanced fatty acid catabolism, as highlighted by the increased C2-Carnitine/C0-Carnitine ratio as well as by the increased sum of short-chain acylcarnitines, and by enhanced levels of medium- and long-chain in CMS vul compared to CMS res. In addition, we observed an alteration in the BCAA catabolism in vulnerable animals, suggesting an overall degradation to produce short chain acylcarnitines, particularly C5-Carnitines, and in turn more substrates for energy production via β-oxidation, specifically in female rats which developed the depressive-like phenotype.

These results are in line with our previous findings reporting that, in the ventral hippocampus of adult male rats, vulnerables preferentially use fatty acids as primary energy source, an effect that was normalized by chronic venlafaxine treatment [15]. Accordingly, it has been demonstrated that adult female rats with brain damage showed elevated fatty acid β-oxidation to meet energy demand [20].

This evidence converges into a cohesive picture that highlights the potential implication of this metabolic signaling in the vulnerability to stress.

Actually, alterations in BCAA levels have been observed in plasma of patients with MDD, [21,22] supporting the implication of abnormalities in β-oxidation and BCAA catabolism in the pathology.

Furthermore, we found a reduction of both EAA, NEAA as well as of gluconeogenic amino acids specifically in resilient animals indicating a potential utilization of this class of metabolites to produce glucose as compensatory mechanisms to meet the energy need caused by prolonged stress exposure. However, we did not observe changes in glycolysis nor in vulnerable neither in resilient animals. Indeed, contrary to the shift to glycolysis as mechanism to sustain the resilience to chronic

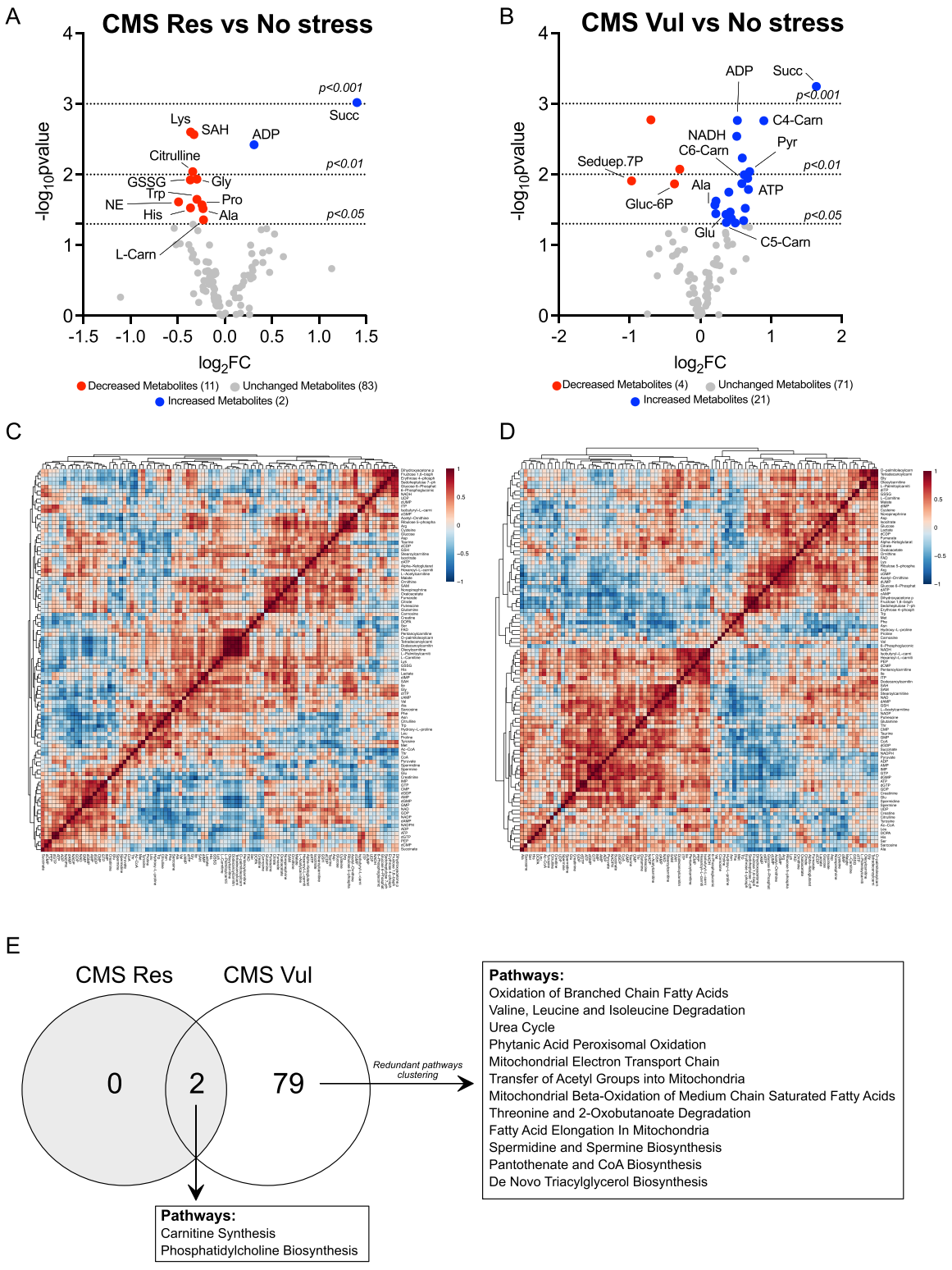

Fig. 3. Pathway and enrichment analysis in vulnerable and resilient female rats exposed to six weeks of CMS. Panel A-B: volcano plots showing the significantly altered metabolite in CMS res and No stress (A), and CMS vul and No stress (B) comparisons. Panel D: Heatmaps displaying Pearson correlation coefficients of metabolites in the same comparisons: CMS-vul vs. No Stress (C) and CMS-res vs. No Stress (D), indicating distinct correlation patterns between groups. Panel E: Venn diagram illustrating the overlap of significantly enriched metabolic pathways (FDR < 0.05) identified in each comparison. Notably, 79 pathways were uniquely enriched in the vulnerable group. Redundant or overlapping pathways were clustered to emphasize key nodes of metabolic rewiring specific to stress vulnerability.

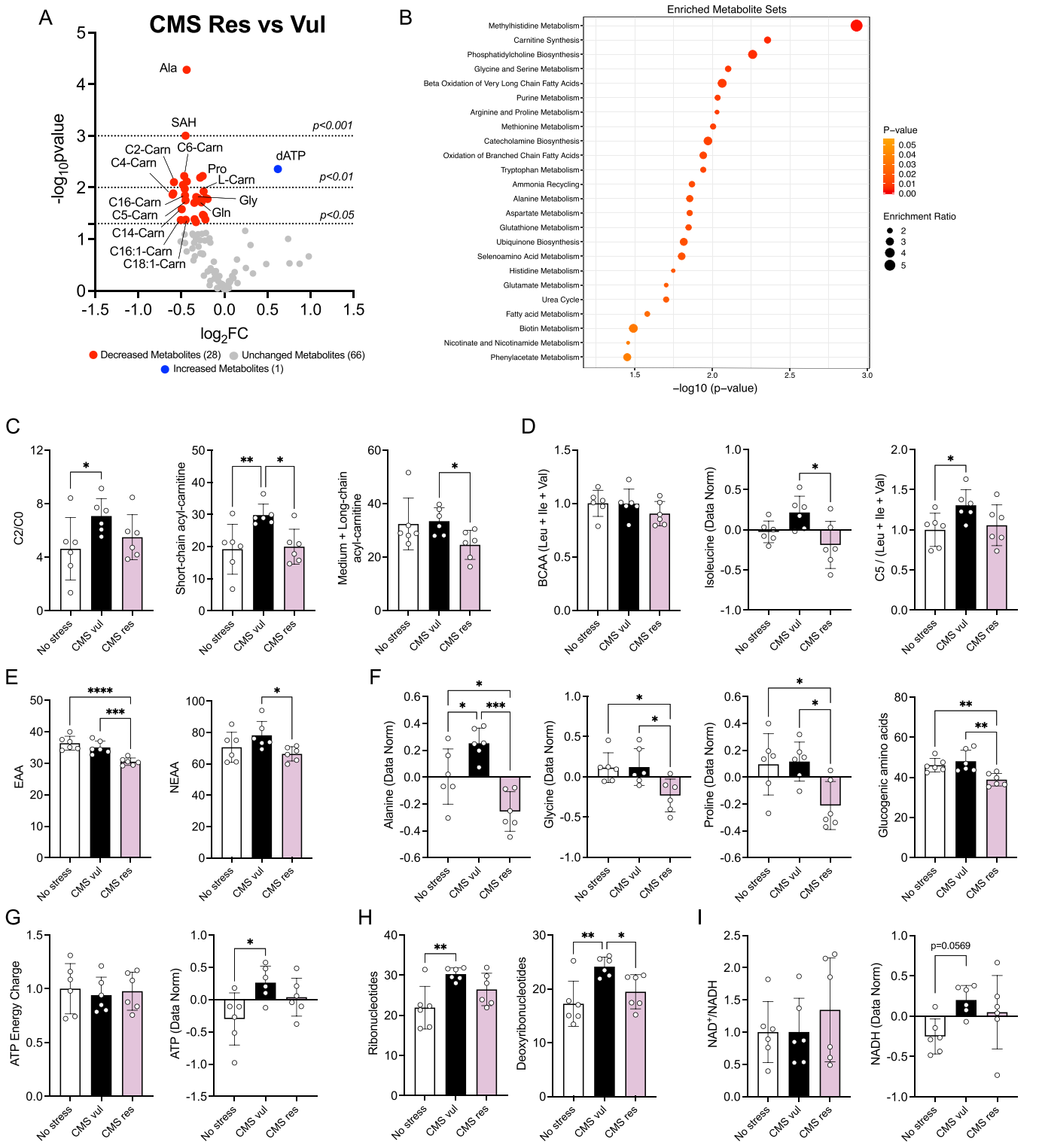

Fig. 4. Modulation of metabolite levels in vHip of vulnerable and resilient female rats. Panel A: volcano plot showing significantly altered metabolite between CMS res and CMS vul groups. Panel B: MSEA highlighting significantly enriched pathways (p value<0.05). Panel C: ratio C2/C0 (left), sum of short-chain acylcarnitine (center), sum of medium- and long-chain acylcarnitine (right). Panel D: sum of BCAAs (ile + leu + val) (left), normalized level of isoleucine (center), ratio C5/ sum of BCAAs (ile + leu + val) (right). Panel E: sum of essential (left) and non-essential amino acids (right). Panel F: normalized levels of alanine, glycine, proline (first three plots) and sum of gluconeogenic amino acids (right). Panel G: ATP energy charge (left) and normalized ATP level (left). Panel H: sum of ribonucleotides (left) and deoxyribonucleotides (right). Panel I: ratio NAD+ /NADH (left) and normalized level of NADH. The data are the mean ± SD of 6 independent determinations. *$p < 0.05$, **$p < 0.01$ vs No stress; #$p < 0.05$ vs CMS vul (One-way ANOVA with Tukey's multiple comparison test or Fisher LDS test).

stress in males [15], here we observed that female rats do not seem to activate this energy pathway to face stress, suggesting that sex-dependent different strategies exist to foster resilience.

Similarly to what we previously observed in males [15], we found an overall modulation of ribonucleotides and deoxyribonucleotides in vulnerable rats, but not in resilient ones, suggesting alterations in the nucleotide biosynthetic activity with possible impact on DNA. This finding is in line with the evidence that stress response is mediated by a critical regulation of hippocampal neurogenesis [23,24].

However, despite the stress-related modulation of nucleotides, the ATP energy charge resulted unchanged in vulnerable vs resilient female rats, in contrast with our previous evidence in males both after 2 [14] and 6 weeks of CMS [15], possibly due to the lack of effect in glycolysis in the two subpopulations of stressed female rats. Of note, looking at the overall redox state, the trend of increase of NADH in vulnerable rats may not enable the ventral hippocampal metabolic plasticity to properly adapt to the environment perturbation caused by chronic stress, suggesting that, on the opposite, resilient animals try to buffer the negative stress consequences, counteracting the reductive stress and the altered metabolic environment which may result in pathological conditions [25].

In conclusion, the metabolomic approach in the vHip of adult female rats subjected to 6 weeks of CMS captures the dynamics of the behavioral phenotype, confirming the role of the fatty acid β-oxidation as a common feature of the susceptibility to stress in male and female rats. Differently, resilience to develop the anhedonic phenotype seems to be sustained by different mechanisms in female and male rats. Indeed, resilient female rats came off as being more resistant to the adverse effects of stress, since they do not activate selective metabolic pathway to sustain the consequences of chronic stress exposure, as resilient males do by shifting their energy production toward glycolysis [15]. Accordingly, this represents a reliable explanation for the evidence that female rats took longer than males to become vulnerable, i.e., 2 vs. 1 week of CMS, respectively.

This is in apparent contrast with epidemiological data showing that females are more prone to develop depression than males [1]. However, while human studies showed increase severity of anhedonia in women, in rats it was reported exaggerated impairment in sucrose preference in male compared to females [3]. Moreover, since our model is based on stress exposure, and, in real life, women and men are sensitive to different types of stressors, it is difficult to make a generalization, but our data clearly highlighted differences between sexes after the application of the same stress protocol.

In this study we did not verify the estrous cycle, and this may be viewed as a potential limitation. However, since we are using a chronic model and therefore female rats are exposed during all phases of the cycle to various stressors and it has been shown estrous cycle is disrupted in a high percentage of female rats exposed to CMS, estrous cycle cannot be considered a variable influencing these effects [3,26,27].

Overall, we here show that fatty acid β-oxidation triggers vulnerability in the vHip of female rats exposed to CMS, whereas resilience appears to occur through different, not yet identified, mechanisms. Since these findings differ from our previous observation in male rats, our results support the importance of studying the sex differences in stress response, for the identification of new specific therapeutic targets as well as of new preventive strategies for paving the way to a more personalized form of medicine, which is particularly relevant not only for MDD but also in the field of stress-related disorders at large.

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CRediT authorship contribution statement

Magdalena Lason: Methodology. **Ewa Litwa:** Methodology. **Mariusz Papp:** Writing – review & editing, Methodology. **Nico Mitro:** Writing – review & editing, Data curation. **Paola Brivio:** Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization. **Dominika Biala:** Methodology. **Fabio Fumagalli:** Writing – review & editing. **Arianna Palumbo:** Formal analysis. **Marta Boccazzi:** Methodology. **Francesca Calabrese:** Writing – original draft, Visualization, Conceptualization. **Silvia Pedretti:** Methodology, Formal analysis, Data curation. **Maria Teresa Gallo:** Formal analysis. **Piotr Gruca:** Methodology.

Declaration of Competing Interest

All the authors have nothing to declare

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.biopha.2025.118747.

Data Availability

Data will be made available on request.

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