Original Research

Stress causes cognitive impairment by affecting cholesterol efflux and reuptake leading to abnormalities in lipid metabolism of rats

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Depression is a common mental health disorder that can impair normal functions, cause distress, and adversely affect the quality of life. Cognitive impairment is considered one of the characteristics of major depression disorders-related dysfunction, and it has received attention in the treatment of major depressive disorders. To investigated the mechanisms underlying depression-induced cognitive disorders, we selected a rodent model of chronic unpredictable mild stress and used liquid chromatography/mass spectrometry-based metabolomics of sera. Behavioral tests, including the sucrose preference test and open field test, revealed that model rats developed depression-like symptoms in the sixth week of the chronic unpredictable mild stress period. Rats of the model group exhibited significant cognitive changes in the Morris water maze test in the tenth week of the period. Tau phosphorylation and decreased levels of postsynaptic density-95 and synaptophysin were observed in the rodent brains by the tenth week. These results suggest that rodents developed cognitive impairment in the tenth week of the period, while serum metabonomic showed that glycerophospholipid metabolism is the most relevant pathway to reveal the mechanism of depression-induced cognitive impairment. The disorders of lipid metabolism caused by the increased cholesterol efflux and reduced reuptake could be one of the mechanisms of depression-induced cognitive disorders. However, the relationship between cholesterol efflux in the brain and elevated serum cholesterol needs further research.

Keywords

Chronic unpredictable mild stress; depression; cognitive disorders; metabolomics; metabolites; cholesterol metabolism; rat model

1. Introduction

Depression is a common mental illness with symptoms of anhedonia, weight loss, and decreased activity (Nelson et al., 2018; Rahim and Rashid, 2017; Skinner, 2014). Major depression disorders (MDD) is a highly prevalent disabling disease that causes severe and often chronic dysfunction (Rosenblat et al., 2016). Recently, cognitive impairment is considered one of the characteristics of MDD-related dysfunction, and it has received much attention in the treatment of MDD (Castaneda et al., 2008; Darcet et al., 2016; Geerlings et al., 2000). Impaired synaptic plasticity and increased tau phosphorylation may provide the pathological basis of cognitive impairment (Buffington et al., 2014; Chung et al., 2015; Yanamandra et al., 2013). Synaptophysin (SYN) is a vesicular protein of the presynaptic membrane which plays a vital role in the regulation of synaptic plasticity and synaptic structure and is widely expressed in the synaptic membrane of neurons (Kwon and Chapman, 2011). Postsynaptic density-95 (PSD-95) is one of the postsynaptic density proteins which is closely related to signal transduction and the regulation of synaptic plasticity (Rapp et al., 2004; Scannevin and Huganir, 2000). Therefore, decreased levels of SYN and PSD-95 may impair synaptic plasticity in the hippocampus, leading to cognitive disorders. However, the pathophysiology of depression-induced cognitive impairment is complex, and its pathogenesis remains unclear.

Clinical diagnosis of cognitive impairment (McKhann et al., 2011) and depression (Darcet et al., 2016) is often evaluated by a manually set scale. This method is subjective and can easily lead to misdiagnosis or missed diagnosis. Therefore, an objective indicator is required for the early diagnosis of depression or cognitive impairment and to study the mechanism of depression-induced cognitive impairment. Metabolomics is a systems biology method used for detecting endogenous metabolites in organisms, particularly for investigating the downstream products of genes or proteins. Compared with genomes or proteomes, metabolomes change rapidly and thus represent the ultimate response of an organism to all stimuli. Therefore, metabolomics is essential for
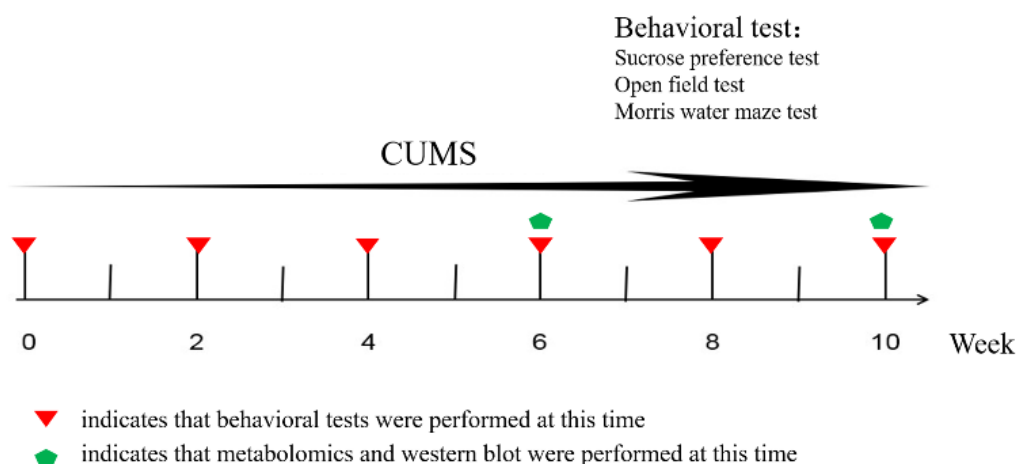
Figure 1. The schedule of animal experimentation.

identifying pathophysiological processes (Johnson and Gonzalez, 2012).

To better understand the mechanism of depression-induced cognitive disorders, a classical rodent depression model, chronic unpredictable mild stress (CUMS) (Katz, 1982; Willner, 2017), was used to detect depression and cognitive disorders through behavioral and pathological studies. Furthermore, liquid chromatography/mass spectrometry (LC-MS)-based metabolomics was conducted to obtain a rat serum metabolite database and investigate the influence of depression on cognitive disorders.

2. Materials and methods

2.1 Animals and treatment

The Twenty male adult Wistar rats (180 ± 20 g, two months old) were received from the Experimental Animal Center of Guangzhou University of Chinese Medicine. The rats were housed in individual cages under a reverse 12 h light/12 h dark cycle (lights on at 18: 00 h) and conventional situations (21 ± 1 °C, 55% ± 5% relative humidity). Food and water were provided ad libitum. Rats were housed undisturbed for 1 week and allowed to adapt to the environment before the start of the experiment. Then, the rats were randomly divided into two groups, either a control group or a model group (n = 10/group). Rats in the model group were exposed to CUMS until the rats exhibited decreased cognitive function, while the rats in the control group were fed regularly and did not performed any experimental procedures.

The CUMS process was based on the chronic mild stress (CMS) model established by Katz (1982) and Willner et al. (1987) (see also (Willner, 2017)). The rats were exposed to random mild stressors every day, and the same stressor was not scheduled for 3 consecutive days. The stressors included food or water deprivation (24 h), cage tilting (24 h), wet bedding (24 h), cold water bath (4 °C, 5 min), hot water bath (45 °C, 5 min), crowding (10 rats within one cage, 8 h), light/dark cycle inversion (24 h), and longtime illumination (24 h). In the CUMS period, body weight was measured weekly. In the sixth and tenth week of the CUMS period, five rats in control and model groups were sacrificed using an overdose of anesthesia. Serum was used for LC-MS-based metabolomics, and hippocampal tissue was used for Western blot. The overall sched-

ule for animal experimentation is shown in Fig. 1.

2.2 Sucrose preference test (SPT)

GenomicSPT was performed every 2 weeks during the CUMS period. On the first day, rats were provided access to two bottles of 1% sucrose solution for 24 h; one bottle was replaced with pure water on the next day, and the positions of the two bottles were changed after 12 h. On the third day, no food or water was provided to the rats within 24 h. On day four, the rats were provided with a bottle of pure water and a bottle of 1% sucrose solution. After 2 h, the weight reduction of each bottle was recorded to determine intake.

Sucrose preference rate (%) = sucrose solution consumption/(sucrose solution consumption + water consumption) 100%.

2.3 Open field test (OFT)

All OFT was performed every 2 weeks during the CUMS period after SPT. The rats were placed in the center of a 100 cm × 100 cm × 60 cm black OFT box. The activity of each rat in the box was automatically recorded by a tracking analysis system for 3 min. The central area activity time, rest time, and total distance were used as indicators of activity and anxiety. Rats with anxiety-like behavior have reduced time and distance of activity, and the rest time is increased.

2.4 Morris water maze (MWM) test

The MWM test was conducted every 2 weeks during the CUMS period. It was performed for 6 days. The training period for rats is 5 days, with three training sessions per day. Every rat was released into the water by facing the wall in one of four water inlets. They need to find the hidden platform within 60 seconds. When the rat stayed on the platform for 3 seconds, the software automatically stopped. If the rat failed to reach the platform, it was directed to the platform and stayed there for 20 seconds. Exploratory tests were performed the next day after the training period. At this point, the platform was removed, and each rat was allowed 60 seconds for free swimming. The recorded data were analyzed by MWM test software (Feidi, Guangzhou, P. R. China).

2.5 Western blot

Hippocampal tissues were lysed on ice with Cell Protein extraction reagent (Beyotime, Jiangsu, P. R. China) for the preparation of total protein fractions, and then they were centrifuged for 10 min at 10,000 rpm at 4 °C. The supernatants were transferred to new tubes and preserved at -80 °C. A Bradford protein assay kit measured protein concentrations. The proteins were separated by electrophoresis and transferred to polyvinylidene fluoride (PVDF) membranes. The membranes were blocked for 2 hours in Tris-buffered saline, and Tween20 (1 × TBST, pH 7.6) containing 5% skim milk powder before they were incubated overnight at 4 °C with antibodies against Tau (1 : 1000, Affinity, USA), p-Tau (ser396; 1 : 750, Affinity, USA), PSD-95 (1 : 1000, Affinity, USA), SYN (1 : 1000, Affinity, USA), ABCA1 (1 : 1000, Abcam, UK), ApoE (1 : 1000, Affinity, USA), LRP (1 : 1000, Affinity, USA), Tubulin (1 : 5000, Affinity, USA), and GAPDH (1 : 5000, Affinity, USA). The membranes were then washed with 1 × TBST and subsequently incubated with the corresponding secondary antibodies (1 : 5000 dilutions) at 25 °C for 1 hour. Then, the membranes were washed with 1 × TBST, and the protein bands were detected with a BIO-RAD imaging system (BIO-RAD, Hercules, CA, USA) and quantified by Image Lab (Millipore, USA).

2.6 Metabolomics analysis

The sample preparation and LC-MS procedures were set up regarding relevant studies (Jia et al., 2016; Liu et al., 2016; Tang et al., 2014). For multivariate statistical analysis, Compound Discoverer 2.0 (Thermo Fisher, USA) was used for obtaining matched and aligned peak data, which were converted into Excel format. After peak area normalization, the data in Excel format was imported from SIMCA-P (version 14.0; Umetrics, Umea, Sweden).

A principal component analysis (PCA) was performed to observe sample distributions, and then an orthogonal partial least-squares discriminant analysis (OPLS-DA) was conducted to distinguish control group and model group in the 6 weeks of CUMS period, and it was also used for the differentiation of control group and model group in the 10 weeks of CUMS period. Then, we used 200-iteration permutation tests to validate the model of OPLS-DA. The criteria for identifying potential significant differential metabolites were the VIP > 1 in the V + S graph. A P value of < 0.05 between the groups was required before the metabolites were considered as differential metabolites.

2.7 Statistical analysis

The analyses were performed by IBM SPSS Statistics (version 22.0). All data are presented as the mean ± the standard error of the mean (S.E.M.). Most of the results of tests from different groups were analyzed by two-way ANOVA, using CUMS and increasing age as between-subject factors. The latency results of the MWM test were analyzed by a separate two-way ANOVA for each result, using CUMS and day as between-subject factors. Following significant analyses of variance, we used the independent t-test as the post hoc test for CUMS. A probability level of $P < 0.05$ was considered statistically significant.

3. Results
3.1 Effect of CUMS on depression-like behavior in rats

At baseline, no significant differences in body weight, SPT, and OFT tests were found between model and control groups (Fig. 2). For bodyweight, two-way ANOVA revealed significant main effects of CUMS (F(1,88) = 143.473, $P < 0.01$) and increasing age (F(5,88) = 85.380, $P < 0.01$), and their interaction (F(5,88) = 11.494, $P < 0.01$). Post hoc t-test analysis, compared with control group, the weight gain of CUMS rats was significantly slower after 2, 4, 6, 8, 10 weeks of modeling (2w: t(18) =3.175, $P = 0.018$; 4w: t(18) = 4.968, $P < 0.01$; 6w: t(18) = 5.252, $P < 0.01$; 8w: t(8) = 4.902, $P < 0.01$; 10w: t(8) = 6.446, $P < 0.01$; Fig. 2). For sucrose preference rates, the results also showed significant main effects of CUMS (F(1,88) = 213.940, $P < 0.01$), increasing age (F(5,88) = 34.518, $P < 0.01$), and their interaction(F(5,88) = 34.983, $P < 0.01$). Compared with control group, the sucrose preference rate of CUMS rats significantly decreased after 6, 8, 10 weeks of modeling (6w: t(18) = 10.260, $P < 0.01$; 8w: t(8) = 7.497, $P = 0.01$; 10w: t(8) = 10.777, $P < 0.01$; Fig. 2). For total distance and rest time, the results revealed significant main effects of CUMS (Total distance: F(1,88) = 22.129, $P < 0.01$; Rest time: F(1,88) = 47.244, $P < 0.01$) and increasing age (Total distance: F(5,88) = 33.538, $P < 0.01$; Rest time: F(5,88) = 30.918, $P < 0.01$), and their interaction (Total distance: F(5,88) = 4.590, $P < 0.01$; Rest time: F(5,88) = 10.469, $P < 0.01$). Compared with control group, the rest time of CUMS rats after 6, 8, 10 weeks of modeling was significantly increased, and the total distance was significantly reduced (Total distance: 6w: t(18) = 8.392, $P < 0.01$; 8w: t(8) = 2.722, $P = 0.026$; 10w: t(8) = 9.521, $P < 0.01$; Rest time: 6w: t(18) = -3.708, $P < 0.01$; 8w: t(8) = -3.697, $P < 0.01$; 10w: t(8) = -4.405, $P < 0.01$; Fig. 2).

3.2 Effect of CUMS on cognitive disorders behavior in rats

At the same time, there were no significant differences in the MWM test between control and model groups before the CUMS period (Fig. 3). For latency, we performed a separate two-way analysis of variance for each result of MWM test, and the results showed that there was no significant main effect of CUMS (2w: F(1,90) = 0.194, $P = 0.661$; 4w: F(1,90) = 0.276, $P = 0.601$; 6w: F(1,90) = 0.780, $P = 0.379$) after 2, 4 and 6 weeks of CUMS period; But the two-way ANOVA revealed significant main effect of CUMS (8w: F(1,40) = 24.107, $P < 0.01$; 10w: F(1,40) = 76.686, $P < 0.01$) and day (8w: F(4,40) = 6.584, $P < 0.01$; 10w: F(4,40) = 70.006, $P < 0.01$), and their interaction (8w: F(4,40) = 2.095, $P = 0.099$; 10w: F(4,40) = 3.792, $P < 0.05$) in the 10th week of modeling. Post hoc t-test analysis, compared with control group, the latency of the MWM test of CUMS rats on days 2, 3, 4, and 5 were significantly increased after 10 weeks of modeling (Day2: t(8) = -3.142, $P = 0.014$; Day3: t(8) = -6.480, $P < 0.01$; Day4: t(8) = -3.049, $P = 0.016$; Day5: t(8) = -6.952, $P < 0.01$; Fig. 3). For crossing time of hidden platform, the results showed significant difference of CUMS (F(1,88) = 4.433, $P < 0.05$) and their interaction (F(5,88) = 2.863, $P < 0.05$). Compared with the control group, the crossing time of the platform of CUMS rats was significantly decreased in the 10th week of modeling (t(8) = 7.603, $P = 0.02$; Fig. 3). These results indicate that CUMS rats exhibited cognitive impairment in the tenth week of the CUMS period when compared with control rats. The results of the MWM test revealed that rats exposed to CUMS for 10 consecutive weeks experienced decreased learning and memory, which may eventually lead to cognitive impairment.
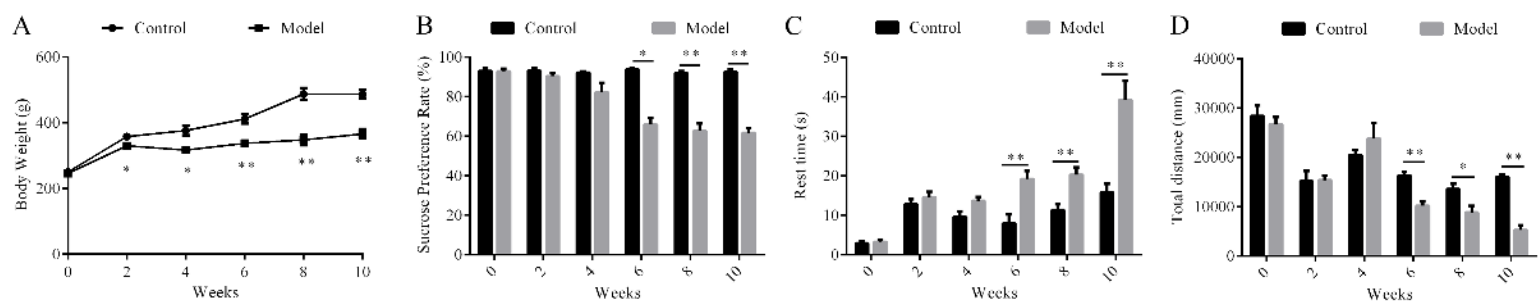
Figure 2. Changes in body weight (A), sucrose preference rate (B) in SPT, rest time (C), and total distance (D) in OFT. The results are expressed as the mean ± S.E.M. of the different groups (n = 10/group in the 2nd, 4th, 6th weeks of CUMS period and before CUMS period; n = 5/group in the 8th, 10th weeks of CUMS period). (compared with control rats, *P < 0.05; **P < 0.01)
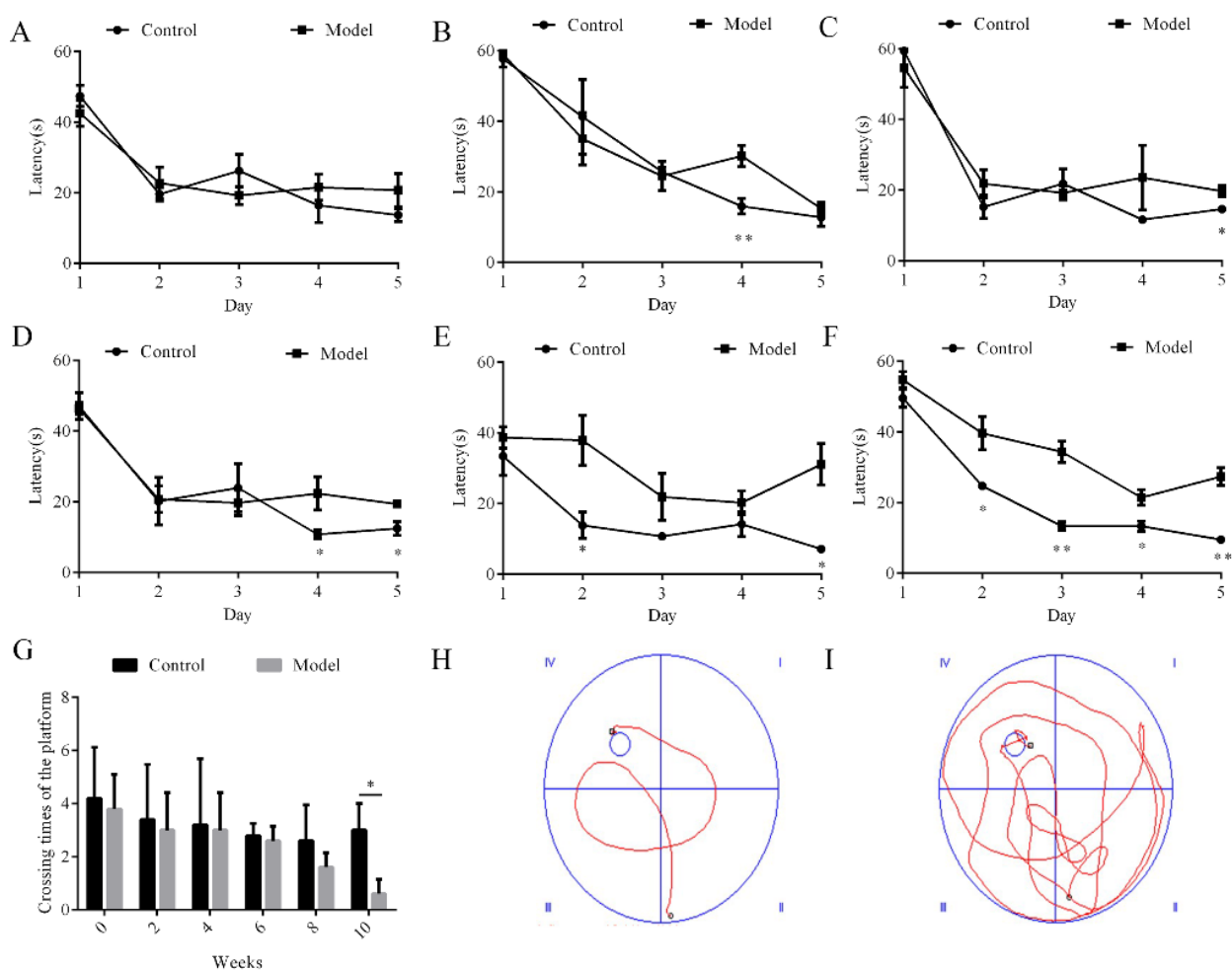.

Figure 3. The Morris water maze (MWM) test of control and CUMS rats. The escape latencies in MWM were conducted over 5 consecutive days test before the CUMS period (A), in the second week (B), fourth week (C), sixth week (D), eighth week (E), and tenth week (F) of the CUMS period. Crossing times of the hidden platform were detected within 60 s (G) every 2 weeks during the CUMS period. Representative tracks of control (H) and CUMS (I) rats on the fifth day of the tenth week of the CUMS period. Bars represent mean ± S.E.M. of the different groups (n = 10/group in the 2nd, 4th, 6th weeks of CUMS period and before CUMS period; n = 5/group in the 8th, 10th weeks of CUMS period). (compared with control rats, *P < 0.05; **P < 0.01)
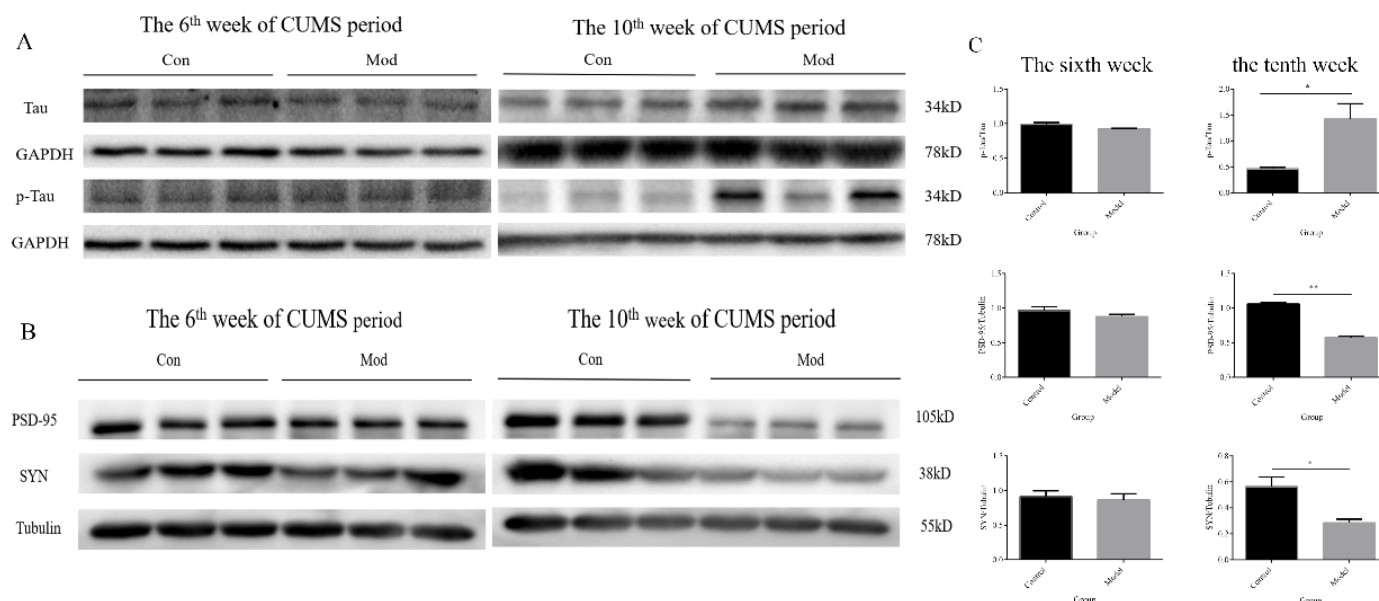.

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Figure 4. Tau protein phosphorylation and impairment of synaptic plasticity in CUMS rats. (A) The expression of p-Tau and Tau in control rats and CUMS rats in the sixth week and tenth week of the CUMS period. (B) The expression of PSD-95 and SYN in control rats and CUMS rats in the sixth week and tenth week of the CUMS period. Bars represent mean $\pm$ S.E.M of the different groups (n = 3/group). (compared with control rats, *$P < 0.05$; **$P < 0.01$).

3.3 Effects of CUMS on Tau phosphorylation and synaptic plasticity in rat hippocampus

To verify whether cognitive impairment occurred in CUMS rats, Western blot experiments were used for the detection of Tau protein phosphorylation and a decrease in synaptic plasticity proteins (PSD-95 and SYN) in the hippocampus of rats. By comparing the ratio of phosphorylated Tau (p-Tau) and GAPDH to the ratio of Tau and GAPDH, we calculated the ratio of p-Tau/Tau. For p-Tau, two-way ANOVA revealed a significant main effect of CUMS ($F_{(1,8)} = 15.751$, $P < 0.01$) and increasing age ($F_{(1,8)} = 202.214$, $P < 0.01$), and their interaction ($F_{(1,8)} = 28.511$, $P < 0.01$). Post hoc t-test analysis, compared with the control group, the p-Tau level of CUMS rats was significantly increased in the 10th week of modeling ($t(4) = -3.238$, $P = 0.032$; Fig. 4). Furthermore, for PSD-95 and SYN, the results displayed significant difference in CUMS (PSD-95: $F_{(1,8)} = 32.002$, $P < 0.01$; SYN: $F_{(1,8)} = 8.592$, $P < 0.05$), increasing age (PSD-95: $F_{(1,8)} = 8.259$, $P < 0.05$; SYN: $F_{(1,8)} = 59.356$, $P < 0.01$), or their interaction (PSD-95: $F_{(1,8)} = 64.136$, $P < 0.01$; SYN: $F_{(1,8)} = 2.156$, $P > 0.05$). Compared with a control group, the PSD-95 and SYN levels of CUMS rats was significantly decreased in the 10th week of CUMS period (PSD-95: $t(4) = 18.489$, $P < 0.01$; SYN: $t(4) = 3.489$, $P = 0.025$; Fig. 4).

3.4 Effect of CUMS on serum metabolomics

The measured body weights, SPT, and OFT results showed depression-like and anxiety behaviors in CUMS rats during the sixth week of the CUMS period. The MWM test results in CUMS rats demonstrated significant changes in the tenth week of the CUMS period, indicating that CUMS rats exhibited cognitive impairment after 10 weeks of CUMS. CUMS rats in the tenth week showed a significant increase in Tau phosphorylation and decreased synaptic plasticity protein expression in Western blot, demonstrating that neurological damage occurred in CUMS rats in the tenth week. Therefore, serum differential metabolites in CUMS rats in the sixth and tenth weeks may be potential biomarkers for disease progression.

More than 600 metabolites were detected by LC-MS metabolomics analysis in the serum samples from the control and CUMS rats and used for multivariate analysis. The OPLS-DA score plots in the positive and negative ion mode showed that the model group was clearly distinguished from the control group in the sixth week of the CUMS period, indicating the high quality of the data and good predictive ability of the model (Fig. 5). The results of the permutation tests showed that there is no overfitting of data, and the model of OPLS-DA was valid. Moreover, based on the OPLS-DA results, 30 significantly different metabolites were identified between the model group and the control group in the sixth week of the CUMS period by screening VIP > 1. Most of the metabolites were primarily involved in amino acid metabolism.

The OPLS-DA score plots in the positive and negative ion mode showed that the model group was different from the control group in the tenth week of the CUMS period (Fig. 5), and the OPLS-DA model was valid. Furthermore, based on the OPLS-DA, 35 significantly different metabolites were identified between the model group and the control group by screening VIP > 1. According to the results, most of these metabolites were primarily involved in amino acid and lipid metabolism.

Based on the correction for multiple comparisons, and the student's t-test ($P < 0.05$), these differential metabolites were selected for further analysis in MetaboAnalyst 4.0 (http://www.metaboanalyst.ca/). The relevant contents of the heatmap of these specific metabolites are shown in Fig. 6. Significantly
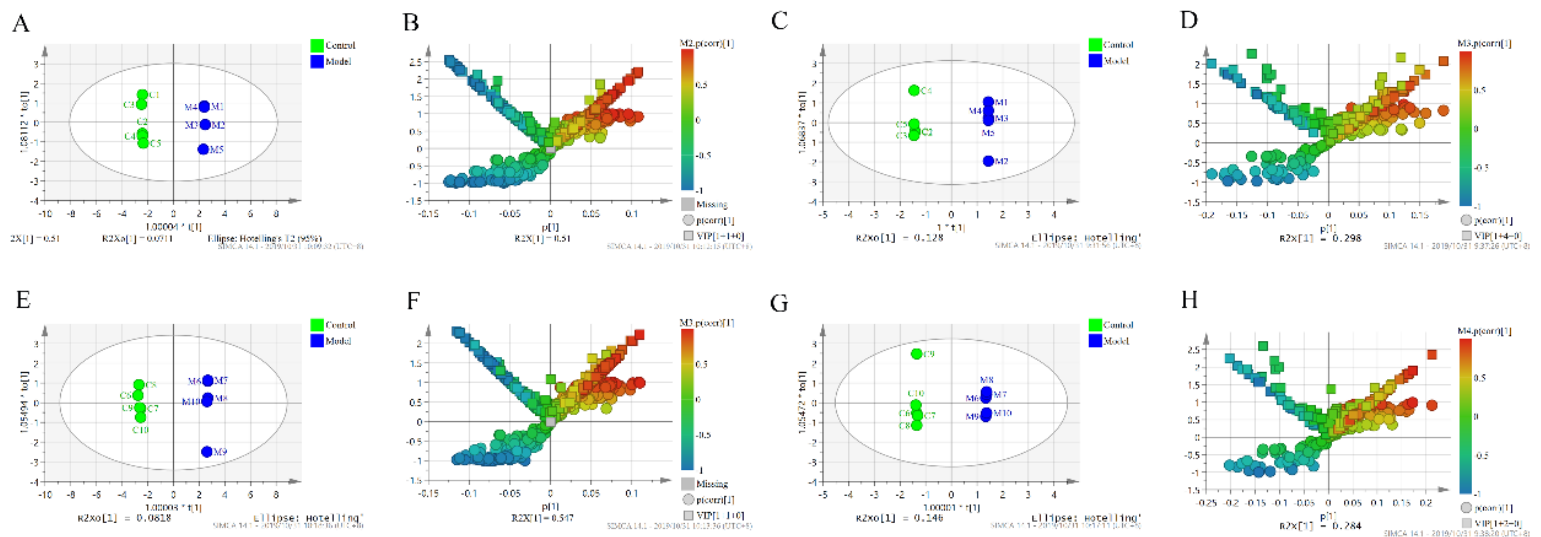
Figure 5. The analysis in SIMCA-P of control and CUMS rats. (A) The OPLS-DA score plot of the sixth week of the CUMS period in the positive ion. (B) The (V + S)-plot of the sixth week of the CUMS period in the positive ion. (C) The OPLS-DA score plot of the sixth week of the CUMS period in the negative ion. (D) The (V + S)-plot of the sixth week of the CUMS period in the negative ion. (E) The OPLS-DA score plot of the tenth week of the CUMS period in the positive ion. (F) The (V + S)-plot of the tenth week of the CUMS period in the positive ion. (G) The OPLS-DA score plot of the tenth week of the CUMS period in the negative ion. (H) The (V + S)-plot of the tenth week of the CUMS period in the negative ion.

altered metabolites were those associated with glycerophospholipid, fatty acid, amino acid, and bile acid metabolism. Based on the results, we considered nine metabolic pathways to an impact value > 0.05 as the most pertinent pathways between control and model group in the sixth week of CUMS period (Fig. 6), including aminoacyl-tRNA biosynthesis; glutathione metabolism; nitrogen metabolism; histidine metabolism; valine, leucine and isoleucine biosynthesis; valine, leucine, and isoleucine degradation; D-glutamine and D-glutamate metabolism; arginine and proline metabolism and alanine, aspartate, and glutamate metabolism. Moreover, glycerophospholipid metabolism; aminoacyl-tRNA biosynthesis; valine, leucine, and isoleucine biosynthesis; histidine metabolism; and valine, leucine, and isoleucine degradation were also selected as the most relevant metabolic pathways between control and model group in the tenth week of CUMS period.

By comparing the different metabolites of the depression model and cognitive disorder model, cholesterol and some glycerophospholipids were detected. The most different metabolic pathway between cognitive impairment and depression models was glycerophospholipid metabolism. These results indicated that cholesterol and glycerophospholipids might be the most promising biomarkers to reveal a transition from depression to cognitive impairment in rats.

3.5 Effects of CUMS on the expression of cholesterol efflux and reuptake proteins in rat hippocampus

Through metabolomics analysis, we observed that serum levels of cholesterol in CUMS rats increased significantly in the tenth week of the CUMS period, suggesting that cholesterol metabolism dysfunction may cause CUMS-induced cognitive impairment. Western blot was used also for the detection of ATP-binding

cassette (ABC) transporter A1 (ABCA1), low-density lipoprotein (LDL) receptor-related protein 1 (LRP1), and apolipoprotein (apo)E (ApoE) in the rat hippocampus. For LRP1 and ApoE, the results displayed significant difference of CUMS (LRP1: $F_{(1,8)} = 19.789$, $P < 0.01$; ApoE: $F_{(1,8)} = 15.889$, $P < 0.01$), and ApoE also showed significant effect in the interaction (LRP1: $F_{(1.8)} = 0.126$, $P = 0.732$; ApoE: $F_{(1.8)} = 8.673$, $P < 0.05$). Compared with the control group, the LRP1 and ApoE levels of CUMS rats was significantly decreased in the 10th week of CUMS period (LRP1: $t(4) = 2.836$, $P = 0.047$; ApoE: $t(4) = 5.021$, $P = 0.007$; Fig. 7). For ABCA1, there were revealed significant main effect of CUMS (ABCA1: $F_{(1,8)} = 75.700$, $P < 0.01$), increasing age (ABCA1: $F_{(1,8)} = 5.970$, $P < 0.05$), and their interaction(ABCA1: $F_{(1,8)} = 9.081$, $P < 0.05$). Compared with the control group, and the ABCA1 level of CUMS rats was significantly increased in the 10th week of the CUMS period ($t(4) = 8.787$, $P < 0.01$; Fig. 7).

4. Discussion

To explore the impact of CUMS on cognitive impairment, we used a serum metabolomics analysis and evaluated depression and cognitive impairment with the well-recognized classical behavior approaches. These methods are superior to the clinical scale evaluation, more objective, and combined with the neurological damage-related proteins (p-Tau, PSD-95, SYN), which may contribute to reflect the emergence of cognitive impairment indirectly from pathological changes. Compared with the control rats, CUMS rats exhibited significant depressive-like symptoms based on the results of depression-like behavioral tests, such as weight loss, anhedonia, and decreased activity, and the situation did not change until the tenth week of the CUMS period. Using two indi-
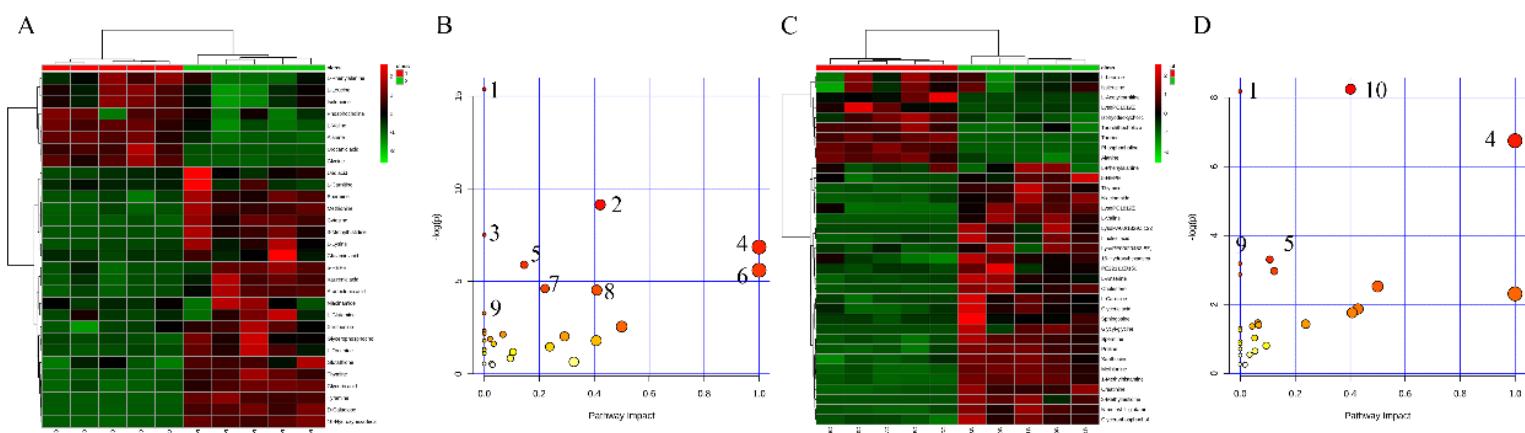
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Figure 6. The heatmaps and pathway enrichment of differential metabolites. (A) The heatmap of control and CUMS rats in the sixth week of the CUMS period. (B) Pathway enrichment of control and CUMS rats in the sixth week of the CUMS period. (C) The heatmap of control and CUMS rats in the tenth week of the CUMS period. (D) Pathway enrichment of control and CUMS rats in the tenth week of the CUMS period. For heatmaps, Class 1: control group. Class 2: Model group. The ribbon-2~2 represents the relative content of the differential metabolites from low to high. For pathway enrichment, The size and color of the dots are positively correlated with the effects of metabolic pathways. The explanation of the numbers in the figures: 1. Aminoacyl-tRNA biosynthesis; 2. Glutathione metabolism; 3. Nitrogen metabolism; 4. Valine, leucine, and isoleucine biosynthesis; 5. Histidine metabolism; 6. D-Glutamine and glutamate metabolism; 7. Arginine and proline metabolism; 8. Alanine, aspartate, and glutamate metabolism; 9. Valine, leucine, and isoleucine degradation; 10. Glycerophospholipid metabolism.
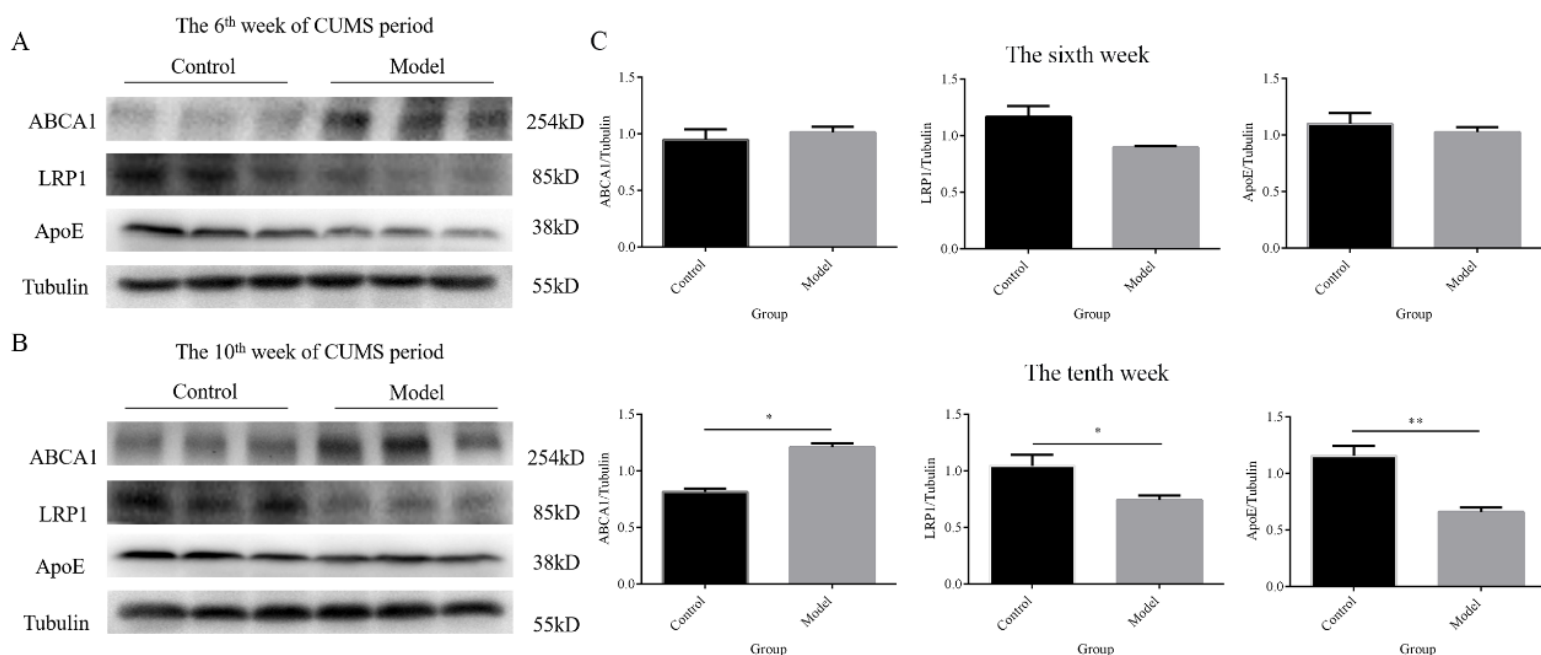

Figure 7. The levels of efflux and reuptake proteins of cholesterol in CUMS rats. (A) The expression of ABCA1, LRP1, and ApoE in control and CUMS rats in the sixth week of the CUMS period. (B) The expression of ABCA1, LRP1, and ApoE in control and CUMS rats in the tenth week of the CUMS period. Data are shown as the mean $\pm$ S.E.M. of different groups (n = 3/group). (compared with control rats, *$P < 0.05$; **$P < 0.01$)

cators of escape latency and platform crossing times in the MWM test, we observed that CUMS rats had a longer latency than that of control rats and fewer platform crossing times in the tenth week of the CUMS period. These results indicated that CUMS rats devel-oped cognitive impairment in the tenth week of the CUMS phase.

Furthermore, in the tenth week of the CUMS period, p-Tau in-creased, PSD-95 and SYN levels decreased in model rats, which are indicative of neurological damage. Based on the results of the

above behavioral tests, we can determine the time scales of depression and cognitive impairment and start to develop more effective drugs. Therefore, we collected the sera from control rats and CUMS rats in the sixth and tenth weeks of the CUMS period to perform LC-MS analysis.

Metabolites are the ultimate products of all stimuli. Based on metabolomics analysis, model rats developed depression in the sixth week of the CUMS period. These extensive changes in metabolites and enrichment pathways persisted in the tenth week of the CUMS period. Most of these differential metabolites were amino acids. Enrichment pathways indicate that they are involved in the biosynthesis and metabolism of amino acids, suggesting that the pathogenesis of anxiety and depression may be closely related to these amino acids. CUMS rats manifested cognitive dysfunction in the tenth week of the CUMS period. At this time scale, in addition to the amino acid, the changed differential metabolites also include lipids, including cholesterol, phosphatidylcholine (PC), and phosphatidylethanolamine (PE), which may demonstrate that the pathogenesis of cognitive impairment. Enrichment pathways increase glycerophospholipid metabolism, suggesting that lipid metabolism is vital in the development of cognitive impairment and may underpin the mechanisms of cognitive impairment caused by depression. Correlation analysis of some altered lipids in the serum and synaptic plasticity protein (PSD-95) found that there is a high correlation between them, and a decrease in synaptic plasticity may cause changes in lipids (Fig. 8).

Lipid metabolism is the most relevant metabolic pathway in the development of neurodegenerative diseases, including cognitive disorders (Gonzalez-Dominguez et al., 2014; Pan et al., 2017). Glycerophospholipids, which are the most common phospholipids *in vivo*, comprise PC, PE, phosphatidylserine (PS), phosphatidylglycerol (PG), and phosphatidylinositol (PI). It was known that patients with AD have higher serum levels of PC, indicating that elevated PC may contribute to cognitive impairment (Ademowo et al., 2017), and higher brain levels of PC may lead to the impairment of the brain synapses. The elevated PC causes a decrease in acetylcholine release, which affects synaptic plasticity(Ulus et al., 1989; Wurtman, 2015). PE, as a very high content of glycerophospholipids in eukaryotic cells, performs many cellular functions. Evidence has shown that PE metabolism is associated with AD (Calzada et al., 2016).

Cholesterol is a crucial molecule not just in the brain but in the periphery as well, and studies have shown that cholesterol levels in the serum of CUMS rats are significantly increased, which is consistent with our findings (Zhang et al., 2019). There is evidence that several neurodegenerative disorders, such as AD and HD, are relevant to the disruptions to cholesterol homeostasis (Segatto et al., 2012). Moreover, various studies have shown that synapses are rich in cholesterol (Deutsch and Kelly, 1981; Mitter et al., 2003; Pfrieger, 2003). As such, many researchers propose that disturbances in cholesterol metabolism could damage to synaptic plasticity, and lead to the impairment of learning and memory. Meantime, we tested the expression of cholesterol homeostasis-related proteins in the hippocampus, and the results suggest that brain cholesterol homeostasis may be disrupted (Fig. 7). Astrocytes mainly secrete brain cholesterol, and the primary apolipoprotein is ApoE. ABCA transporter, especially ABCA1, is also re-

quired for cholesterol to shuttle from astrocytes to neurons. The down-regulation of ABCA1 in neurons reduces the outward flow of cholesterol, while an increase in ABCA1 increases the release of lipids (Kim et al., 2008; Koldamova et al., 2003; Minagawa et al., 2009; Wellington et al., 2002). LRP1 mediates neuronal absorption, thereby supporting synapse generation and maintaining synaptic connections (Giudetti et al., 2016; Segatto et al., 2014; Zhang and Liu, 2015). When ApoE and LRP1 decrease, ABCA1 increases, suggesting cholesterol outflow, indicating that cholesterol homeostasis in the hippocampus is damaged, and the cholesterol provided to synapses is reduced.

The correlation analysis between cholesterol homeostasis-related proteins in the hippocampus and PSD-95, and the correlation analysis between cholesterol homeostasis-related proteins and serum cholesterol are highly correlated (Fig. 9). Thus, an increase in cholesterol efflux and decrease in reuptake promotes an imbalance in cholesterol homeostasis, affects synaptic plasticity and leads to cognitive impairment, and may be related to a significant increase in serum cholesterol levels. In summary, we believe that CUMS-induced cognitive impairment damage synaptic plasticity by impairing cholesterol homeostasis (Fig. 10). However, the causal relationship between serum cholesterol levels and cerebral cholesterol efflux is still unclear. We have not detected changes in brain cholesterol, and further research is needed. As well, serum metabolites may not show the same changes as brain metabolites, and brain samples should be obtained to perform LC-MS analysis in future studies.

5. Conclusions

We used a rodent model of CUMS to identify differential serum metabolites in depression and cognitive disorders to elucidate the mechanism of depression-induced cognitive dysfunction. Our results suggested that impairment of cholesterol homeostasis may be an important factor in depression that affects cognition.

Abbreviations

ABCA1, ATP-binding cassette transporter A1; ApoE, apolipoprotein E; AD, Alzheimer's disease; CMS, chronic mild stress; CUMS, chronic unpredictable mild stress; LC-MS, liquid chromatography/mass spectrometry; LDL, low-density lipoprotein; LRP1, LDL receptor-related protein 1; MDD, major depressive disorder; MWM, Morris water maze; OFT, open field test; OPLS-DA, orthogonal partial least-squares discriminant analysis; PCA, principal component analysis; PC, phosphatidylcholine; PD, Parkinson's disease; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PS, phosphatidylserine; PSD-95, Postsynaptic density-95; PVDF, polyvinylidene fluoride; SPT, sucrose preference test; SYN, Synaptophysin.

Author contributions

These should be presented as follows: LY and RZ designed the research study. RY performed the research. MZ, SZ provided help and advice on the metabolomic. SSB, ZYJ, QC provided support and information on the Western blot. RY, KRC, and CKS analyzed the data. LY, YFS, and RY wrote the manuscript.
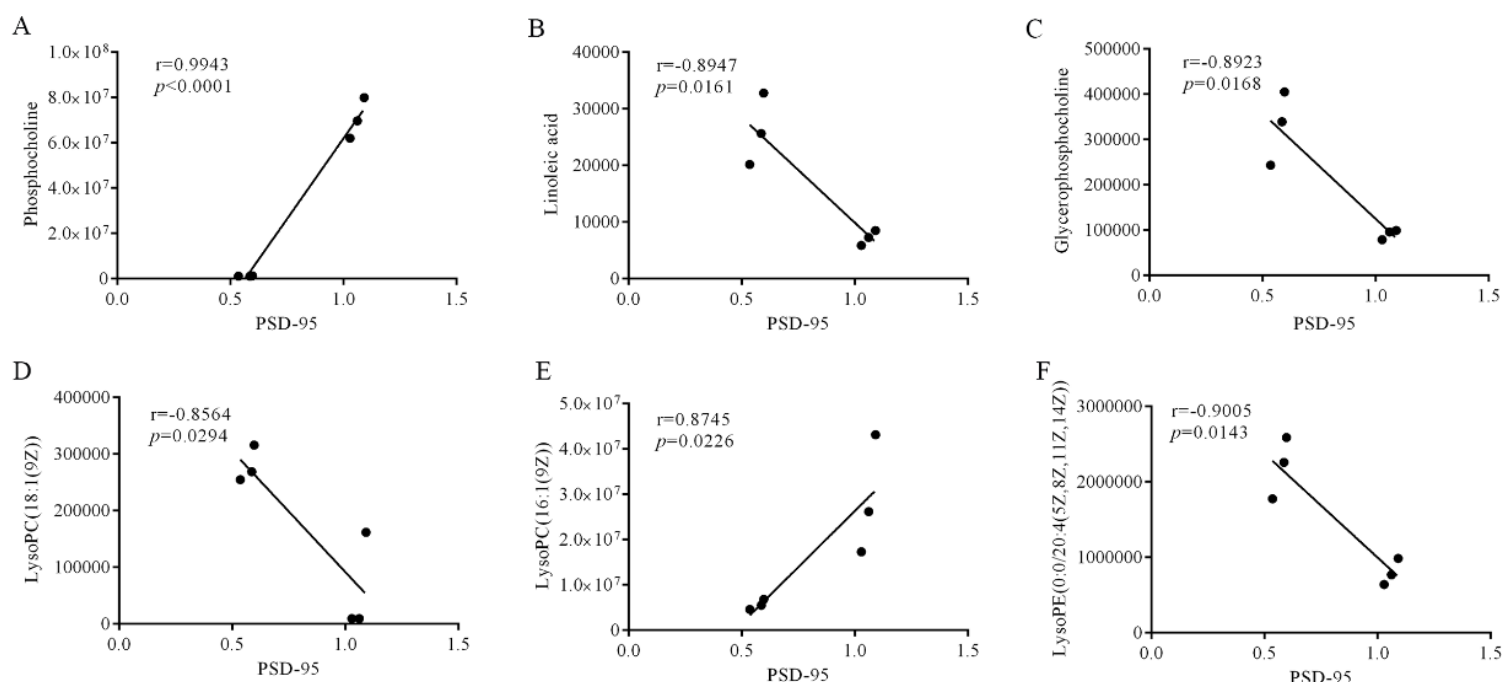
Figure 8. The correlation analysis between PSD-95 and some altered lipids in serum. (A) The correlation between PSD-95 and phosphocholine. (B) The correlation between PSD-95 and linoleic acid. (C) The correlation between PSD-95 and glycerophosphocholine. (D) The correlation between PSD-95 and LysoPC (18:1 (9Z)). (E) The correlation between PSD-95 and LysoPC (16:1 (9Z)). (F) The correlation between PSD-95 and LysoPE (0:0/20:4 (5Z,8Z,11Z,14Z)). Note: $r > 0.8$ is considered highly correlated.
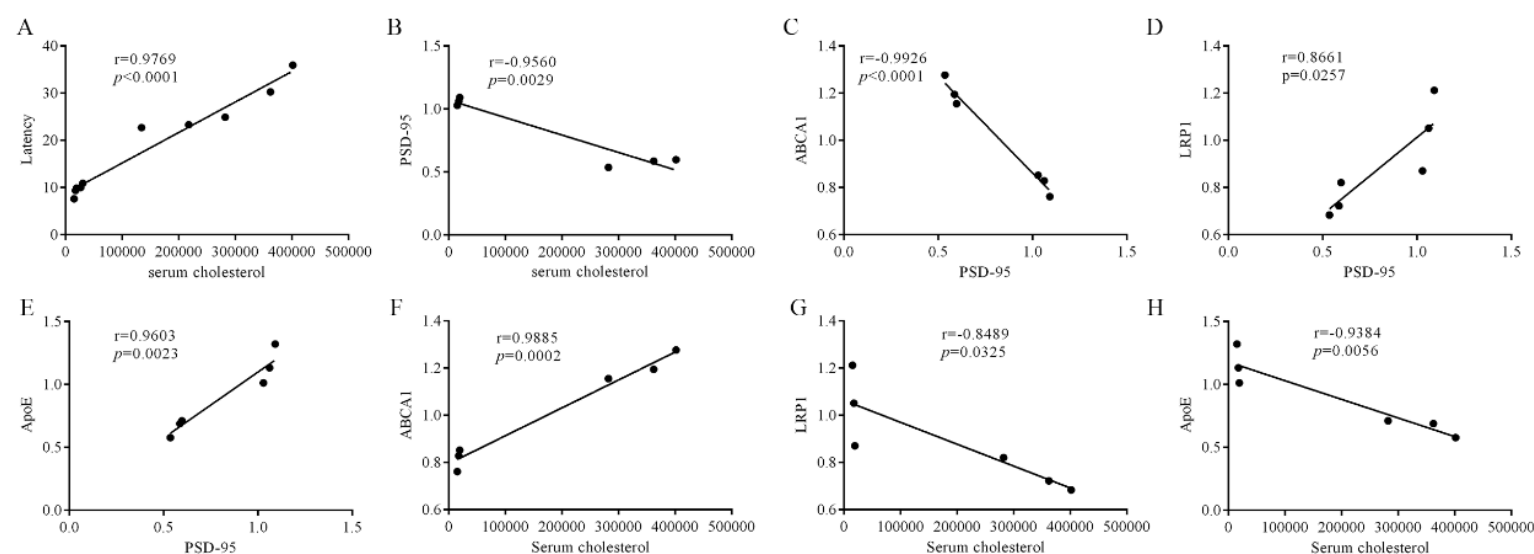

Figure 9. (A) The correlation analysis between serum cholesterol and MWM test. (B) The correlation analysis between serum cholesterol and PSD-95. (C) The correlation analysis between PSD-95 and ABCA1. (D) The correlation analysis between PSD-95 and LRP1. (E) The correlation analysis between PSD-95 and ApoE. (F)The correlation analysis between serum cholesterol and ABCA1. (G) The correlation analysis between serum cholesterol and LRP1. (H) The correlation analysis between serum cholesterol and ApoE. Note: $r > 0.8$ is considered highly correlated.

Ethics approval and consent to participate

All experiments were authorized by the Experimental Animal Ethics Committee of Guangzhou University of Chinese Medicine. And the experiments were performed according to the ethical standards in the 1964 Declaration of Helsinki and its later amendments and were reviewed and approved by Guangzhou University of Chinese Medicine.
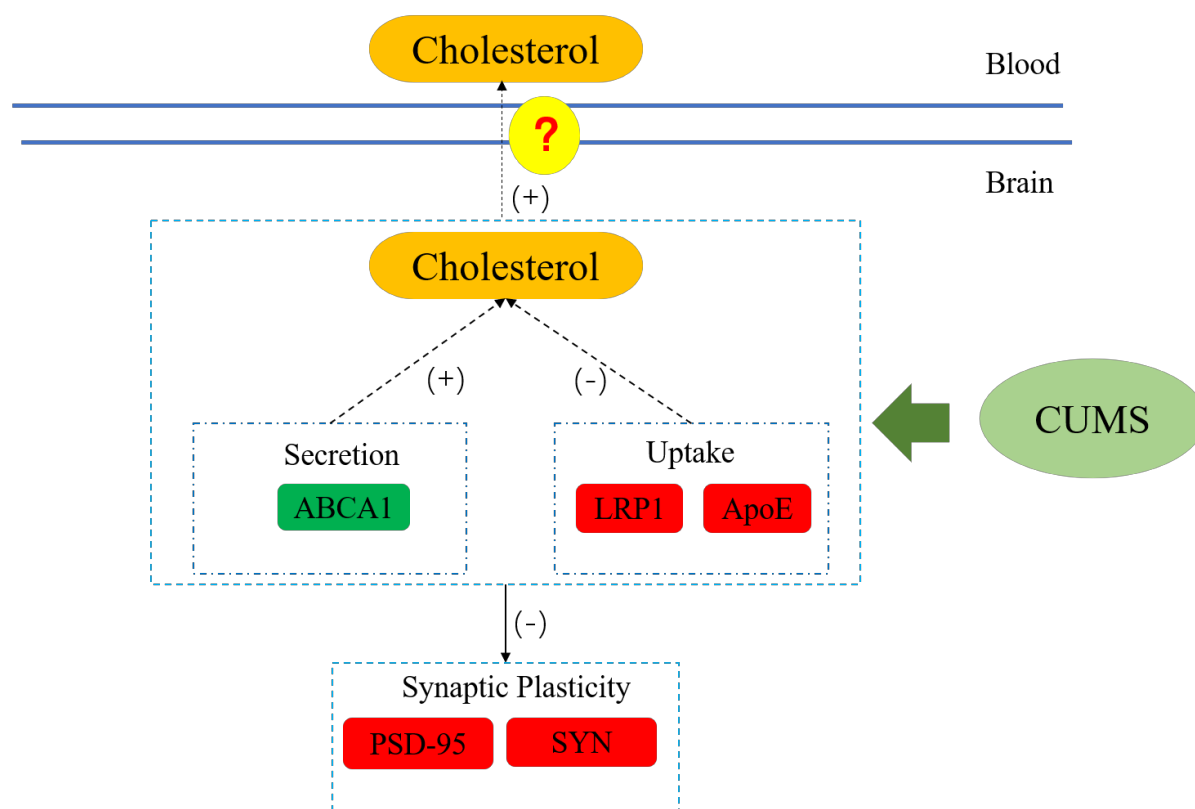
Figure 10. The suggested mechanism of CUMS acting on cholesterol efflux and re-uptake of proteins, resulting in increased expression of the efflux-related protein ABCA1 and decreased expression of re-uptake proteins LRP1 and ApoE, causing the imbalance of brain cholesterol, impairing synaptic plasticity, and leading to cognitive impairment. It may lead to an increase in serum cholesterol, but the relationship between cholesterol efflux in the brain and serum cholesterol levels is currently unknown.

Conflict of Interest

The authors declare no conflict of interest.

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