Original Paper

Depressive-Like Behavior and Liver Damage Generate Behavioral and Cortical Microglial Morphological Differences in Mice

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ABSTRACT: Introduction: The correlation between nonalcoholic fatty liver disease (NAFLD) and depression has already been established, but the relation between the two is insufficiently studied. Various murine models have proven effective in evaluating the mechanisms involved in these pathologies. Material and Methods: In this study we aimed to assess how the chronic unpredictable mild stress (CUMS) protocol impacts the behavior of mice with liver damage induced by a methionine choline deficient (MCD) diet, and also to evaluate the changes in microglial morphology in the cortex of animals with depressive-like behavior and liver injury. Thus, the cortical region was analyzed using confocal microscopy. Results: Sucrose preference test and open field test revealed induced anhedonia and anxiety-like behavior, but short-term memory was not impaired, as assessed by novel object recognition test. Administration of the MCD diet led to an increase in total branch length and the number of terminal branches, revealing a hyperactivated microglia. The CUMS protocol in combination with MCD diet induced a reduced branching complexity, with fewer tertiary and terminal branches. Conclusion: Our study highlights the importance of microglial morphology at the cortical level in coexisting depression and liver injury.

KEYWORDS: *Microglia, morphology, cortex, depression, NAFLD.*

Introduction

The interaction between psychiatric disorders and liver diseases, such as nonalcoholic fatty liver disease (NAFLD) has become increasingly taken into account [1].

It has been shown that NAFLD can exacerbate symptoms of depression and anxiety, while also causing inflammation [2,3].

The inflammation and lipid accumulation in the liver exert significant effects on the central nervous system (CNS) [4,5], where microglia have an important role in mediating the brain's response to internal and external stressors [6,7].

These cells have a remarkable ability to be dynamic in response to various stimuli, manifesting morphological changes [8].

Neuroinflammatory and neurodegenerative disease cause significant changes in the complexity of microglia arborization [9,10].

To fully understand the role of microglia in CNS pathologies, it is important to analyze its response in the context of systemic diseases, such as liver damage.

In this context, the cumulative effect of chronic stress and liver injury on microglial morphology is insufficiently studied.

A suitable murine model for NAFLD has been shown to be the methionine choline deficient (MCD) diet [11,12], while the chronic unpredictable stress (CUMS) protocol proves to be effective in inducing depressive-like behavior in rodents [13-15].

In this study we aimed to assess the cumulative effect of the two pathologies on the behavior and cortical microglial morphology, in order to bring new perspectives on the interaction between depression and liver disease.

Materials and Methods

Experimental Animals

For this study were used male C57BL/6N mice, aged 16-18 weeks (n=12). The animals were obtained from the Animal Facility of the University of Medicine and Pharmacy of Craiova.

During the experiment, the mice were housed under controlled conditions and constant temperature.

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All experimental procedures and protocols were reviewed and approved by the Committee for Experimental Animals Wellbeing of the University of Medicine and Pharmacy of Craiova (2.1, 10 November 2022) and the Sanitary, Veterinary, and Food Safety Directorates (27, 18 October 2024).

Non-alcoholic fatty liver disease/non-alcoholic steatohepatitis and Depressive-and anxiety-like behavior induction

NAFLD was induced by substituting the standard food pellets with MCD diet (MP Biomedicals, Germany) [16-18].

The animals were allowed to consume the MCD diet ad libitum for 4 weeks, while the control groups were provided with standard food pellets.

Depressive- and anxiety-like behaviors were induced using the CUMS protocol, as previously described [18].

Briefly, mice in the CUMS group were exposed to 28 days of daily mild, unpredictable stressors, ensuring no repetition of the same stressor within a 3-day period. The stressors included water or food deprivation (24 hours), restraint stress in a tube (2 hours), continuous illumination (24 hours), 45° cage tilt (24 hours), tail suspension (10 minutes), and wet bedding (24 hours) [18].

The animals were randomly allocated into four groups: WT (control group, n=3), MCD (no stress protocol, n=3), CUMS (n=3), and CUMS+MCD (n=3).

During the induction of depressive-and anxiety-like behaviors, mice subjected solely to the MCD diet and those in the WT group were pair-housed (3 mice per cage). Conversely, mice undergoing the CUMS protocol were housed individually in accordance with widely accepted practices [19].

No disturbances, such as visitors or independent experimental activities, were allowed in the animal housing area to minimize external influences and ensure consistency.

Behavior testing

Mice were tested at the beginning of the experiment in order to establish a baseline, after a week of normal habituation.

Then, the animals were exposed to the MCD diet and CUMS protocol for 4 weeks, and retested to assess the impact of the procedures on their behavior.

The sucrose preference test (SPT) was used to assess anhedonia-like behavior [20,21], and was performed as described [18].

Shortly the test involved a habituation period of 4 days, with animals having free access to two drinking bottles (2% sucrose solution and tap water).

Before the testing phase, mice were deprived of food and water for 12h, and then were allowed access to both bottles for 24h. To eliminate any side preference, the positions of the bottles were changed every 12h. At the end of the test, the volumes consumed by each mouse were recorded, and the sucrose preference was calculated as the percentage of sucrose solution consumed relative to total fluid intake during the test period.

The open field test (OFT) was performed following established protocols [17,18].

The tests were conducted in an open arena measuring 50cm long, 33cm wide and 15cm high.

Each mouse was placed in the center of the arena and observed for 10 min. Anxiogenic behavior was assessed based on the time spent in the central squares compared to the peripheral squares [22].

All parameters measured during the test were recorded and automatically analyzed using EthoVision XT 17 software (Noldus Technology).

The novel object recognition test (NORT) was performed according to previous protocols [16,18] to assess short-term memory [20].

At the beginning of the test mice were allowed to explore an arena with two identical objects for 6 minutes, then were placed in their home cages for 1h. In the second session, one of the objects was replaced by a novel one and the animals were placed in the same arena for another 6 minutes [23].

Short term memory was measured by the preference index, representing the proportion of time spent exploring the new object compared to the total time spent exploring both. For the analysis EthoVision XT 17 was also used.

Histopathology and Immunohistochemistry

After anesthesia, the animals were intracardially perfused using 5ml of saline and 5ml of 4% paraformaldehyde (PFA), then the brains were placed in 4% PFA overnight at 4 degrees C, to prevent microglia activation [24].

For immunostaining were used 35 um coronal sections, cut using a vibrotome, and placed in 0.1M phosphate buffered saline (PBS). The sections were incubated for 1h at room temperature with blocking solution (0,5% Triton X 100 and 5% horse serum in PBS), then overnight at 4 degrees C with primary antibody rabbit anti-Iba 1 (Wako, 01919741, 1:1000).

The next day, the sections were washed and incubated for 2h at room temperature with secondary

antibody Alexa Fluor 647 donkey anti-rabbit (Invitrogen, A31573, 1:1000), and mounted with Fluoromount G with DAPI (ThermoScientific, 00495952).

Image analysis

For the analysis, Z-stack images of the cortical region were obtained using a 20× objective on a Zeiss LSM 900 Airyscan 2 confocal microscope, with image acquisition facilitated by Zen 3.5 software (Figure 1A).

Microglial morphology was assessed through a semi-manual quantification method as described previously [25].

Specifically, from each complete Z-stack, microglia exhibiting fully intact arborization were manually isolated for analysis (Figure 1B, C).

Cells with processes extending beyond the boundaries of the Z-stack were excluded from the study to ensure consistent and accurate measurements. A total of 10 microglial cells per animal were analyzed.

Following the isolation of individual cells, skeletization was applied to facilitate quantitative morphological analysis (Figure 1D, E).

For each microglial cell, key structural parameters were measured, including the total length of the branching tree, the number of branches, the average branch length, and the frequency of branches at each hierarchical order [26, 27].

These metrics were used to identify and compare differences in microglial morphology across experimental groups.

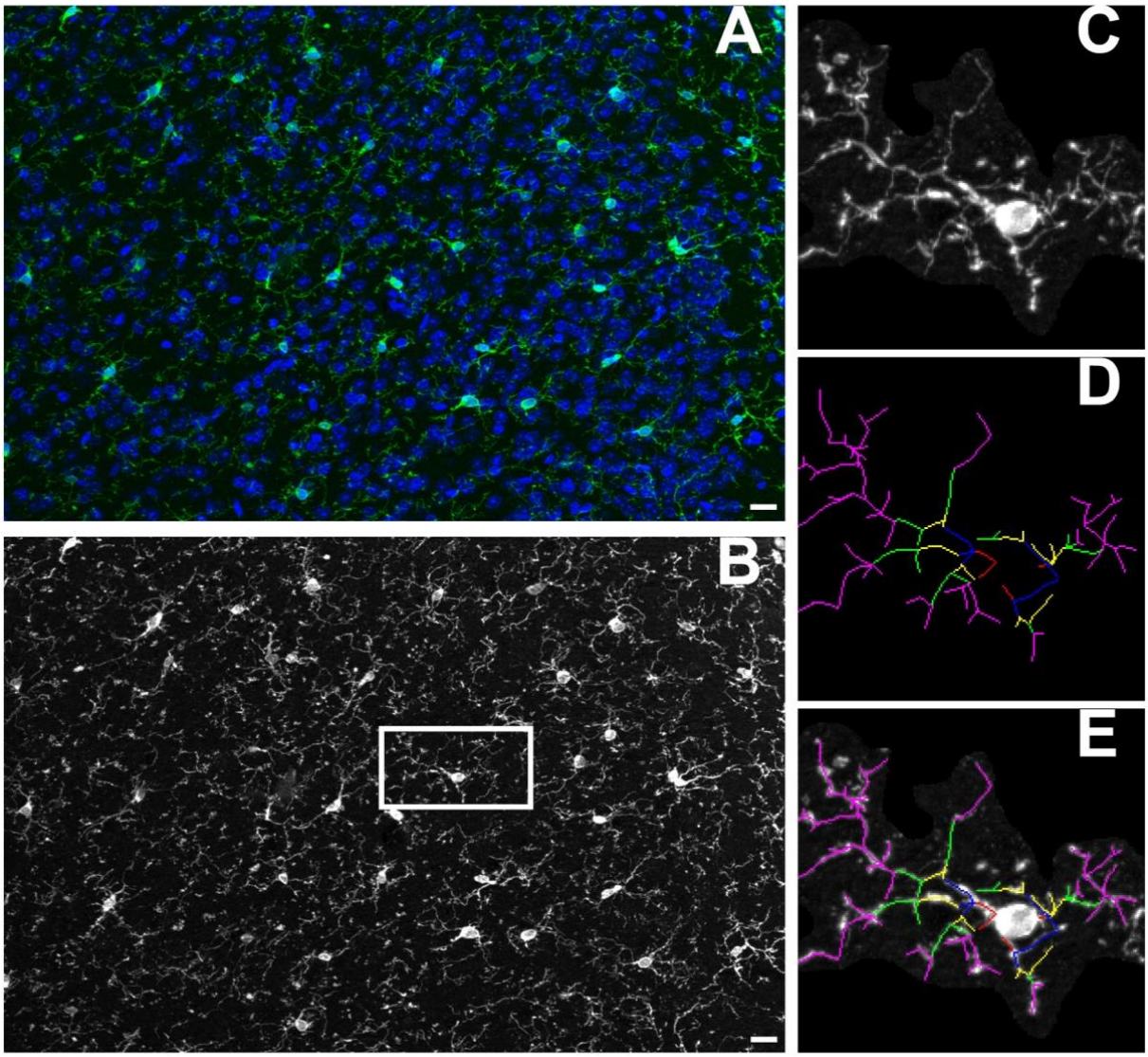

Figure 1. Schematic representation of the methodology.
(A) Visualization of microglial cells in the mouse cortex. (B) An example of a microglial cell (highlighted within the white square), displaying both the soma and fine processes. Each analyzed cell underwent isolation (C), manual tracing (D), and subsequent verification (E). Scale bar: 20µm.

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Statistical analysis

Statistical analysis was conducted using GraphPad Prism 10 and Microsoft Excel.

Data normality was assessed using the Shapiro-Wilk and Kolmogorov-Smirnov tests to confirm the suitability of parametric methods.

Differences in group means were analyzed using one- or two-way repetitive ANOVA (Tukey's multiple comparisons test), and Geisser-Greenhouse correction applied.

In the ANOVA analysis, Sessions (Baseline and Post-Exposure) were treated as a within-factor, while Protocols (WT, MCD, CUMS, CUMS+MCD) were considered a between-factor.

All data are presented as mean±standard deviation (SD) in the figures.

Statistical significance is indicated as follows: *p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001.

Results

CUMS protocol increases anhedonia-like behavior in mice with liver damage

The two-way ANOVA used to evaluate the anhedonia-like behavior in SPT (Figure 2A) revealed differences between Sessions ($F_{1,8}$=51.68, p<0.0001).

Although no differences were observed in the utilized Protocols ($F_{3,8}$=2.444, p=0.1389), significant Interaction was noticed between Sessions and Protocols ($F_{3,8}$=33.86, p<0.0001). Post-hoc analysis revealed that animals exposed to CUMS, irrespective of dietary condition, exhibited a reduction in sucrose preference from the baseline to the post-exposure session.

Animals subjected to CUMS protocol exhibited a sucrose preference of 74.69±1.85%, lower than their baseline value of 86.57±3.76% (p<0.0001) (Figure 2B).

In the CUMS+MCD group, sucrose preference decreased from 88.88±1.70% at baseline to 81.36±0.87% (p=0.0008).

No differences were observed in the WT and MCD groups (p>0.05).

In post-exposure session, mice from CUMS group displayed increased anhedonia compared to WT (p=0.0016) and MCD (p=0.0013) animals (Figure 2B).

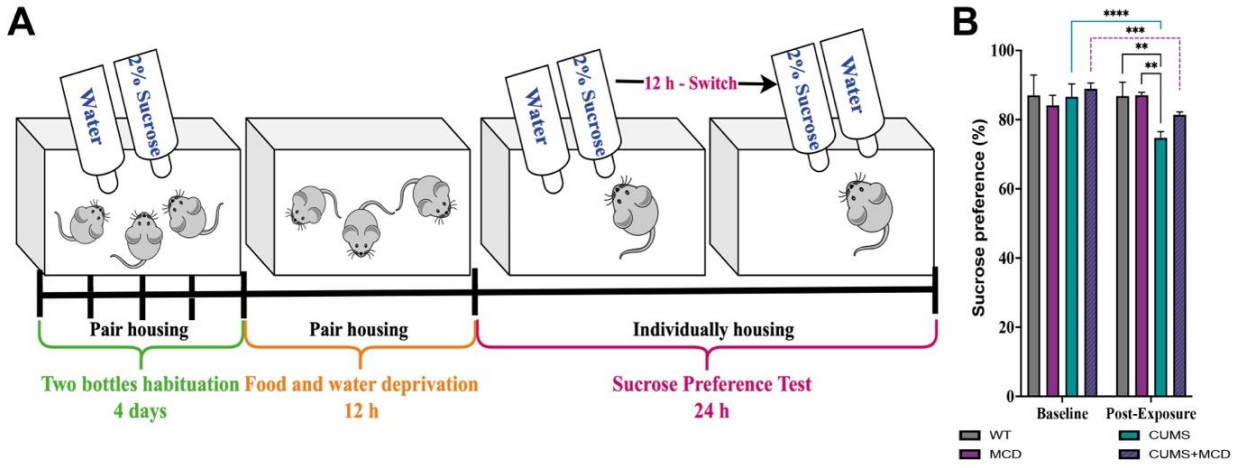

Figure 2. Anhedonia-like behavior assessed by SPT.
(A) Schematic illustration of the test protocol, detailing the habituation phase and the testing phase.
(B) Data obtained from the SPT showed increased anhedonia-like behavior in CUMS and CUMS+MCD mice. The graph shows mean values±SD, **p<0.01, ***p<0.001, and ****p<0.0001.

CUMS protocol amplifies anxiety-like behavior in mice with liver injury

The use of ANOVA in order to evaluate the anxiety-like behavior revealed differences between Sessions ($F_{1,8}$=33.90, p=0.0004), Protocols ($F_{3,8}$=8.426, p=0.0074), and Interaction ($F_{3,8}$=5.25, p=0.0270).

Post-hoc test showed a significant reduction in the time spent in the center of the arena for CUMS animals, decreasing from 89.76±16.85 seconds at baseline to 50.55±14.83 seconds in the post-exposure session (p=0.0078).

Similarly, CUMS+MCD animals showed a decline from 96.04±17.96 seconds to 56.69±4.42 seconds (p=0.0076) (Figure 3A).

No differences were observed in the WT and MCD mice (p>0.05).

In the post-exposure session, mice in the CUMS+MCD group (56.69±4.42 seconds) spent less time in the center of the arena compared to the WT group (109.28±15.90 seconds) (p=0.0008).

Similarly, mice subjected to the CUMS protocol (50.55±14.83 seconds) also spent less time in the center compared to both the WT group (109.28±15.90 seconds) (p=0.0003) and the MCD group (86.35±11.04 seconds) (p=0.0191).

Anxiety-like behavior, evaluated based on the time spent in the center of the arena, is represented by the track paths of a single mouse from each experimental group: WT (Figure 3B), MCD (Figure 3C), CUMS (Figure 3D), and CUMS+MCD (Figure 3E).

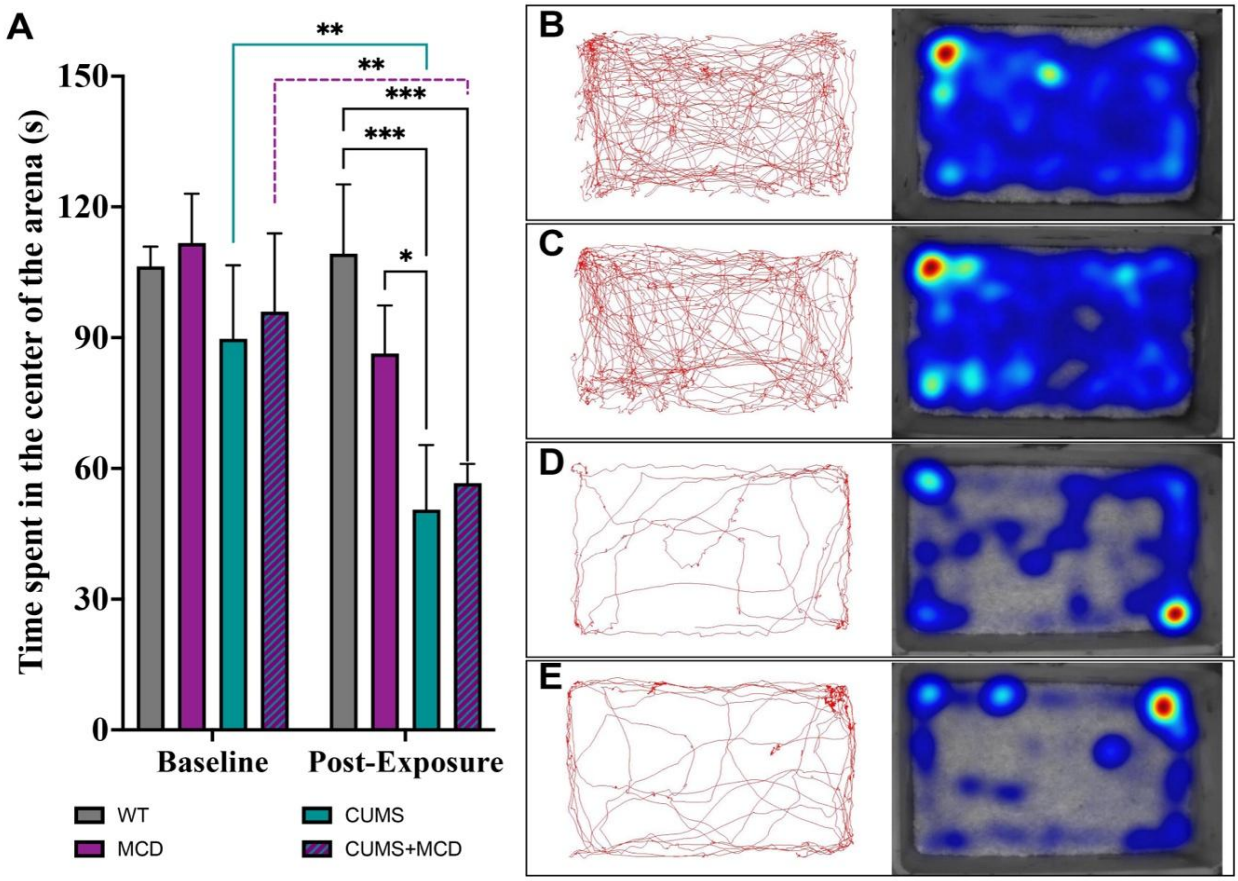

Figure 3. Anxiety-like behavior, as measured by the OFT, was (A) elevated in the CUMS and CUMS+MCD groups. Representative exploratory track paths from each experimental group are shown: (B) WT, (C) MCD, (D) CUMS, and (E) CUMS+MCD. Data are presented as mean±SD, with statistical significance indicated as *p<0.05, **p<0.01, ***p<0.001.

Short-term memory remains unaffected by liver injury or depressive-like behavior

When evaluating the short-term memory, two-way ANOVA revealed variances between Sessions ($F_{1,8}$=5.79, p=0.0427), and Protocols ($F_{3,8}$=4.501, p=0.0395), but no differences in Interaction ($F_{3,8}$=0.9270, p=0.4708). Post-hoc test showed no differences between the analyzed groups (Figure 4A).

The preference for the novel object within the arena was illustrated by a representative mouse from each experimental group: WT (Figure 4B), MCD (Figure 4C), CUMS (Figure 4D), and CUMS+MCD (Figure 4E).

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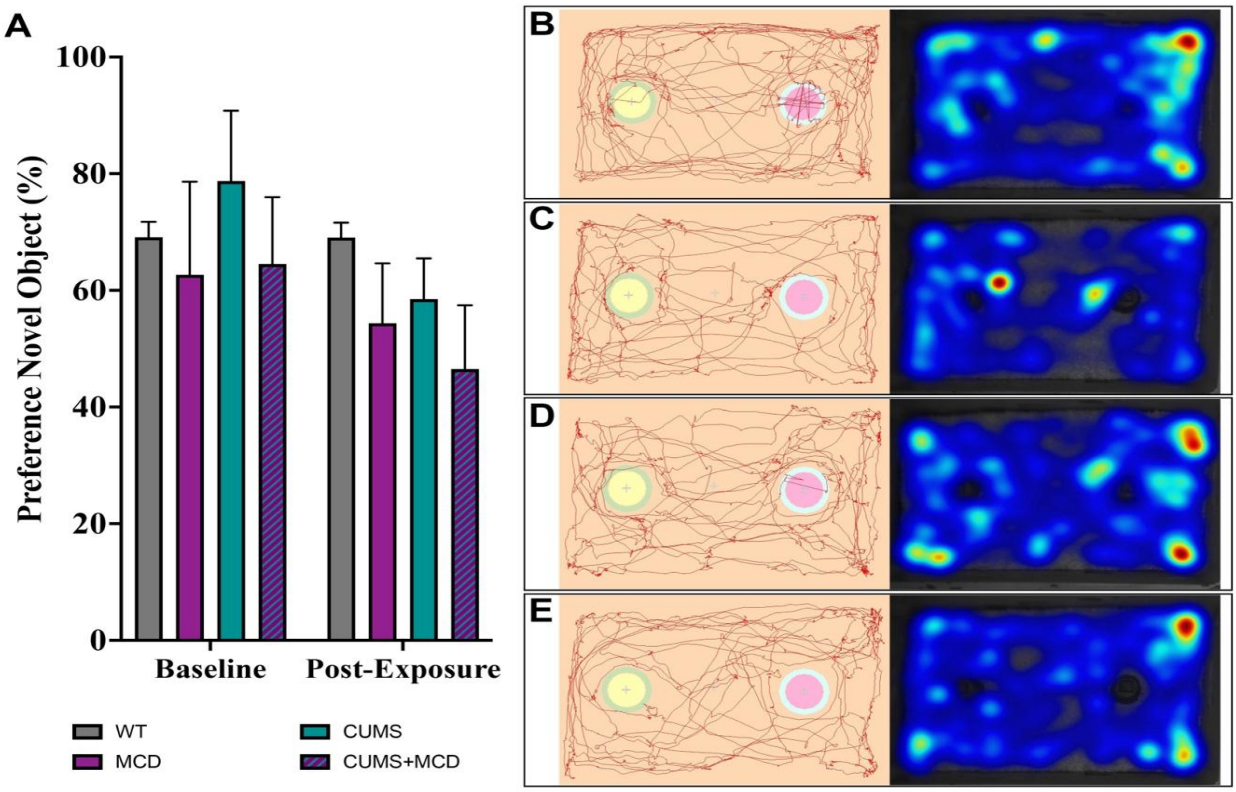

Figure 4. Short-term memory evaluation using NORT. (A) Short-term memory was not impacted by either liver injury or depressive-like behavior. The exploratory track paths are displayed by a representative mouse from each group: (B) WT, (C) MCD, (D) CUMS, and (E) CUMS+MCD. Data are presented as mean±SD.

Analysis of microglial morphology reveals that liver damage and depressive-like behavior result in distinct changes

Liver damage led to an increase in total branch length in the MCD group (264.8±87.40µm) compared to the WT group (157.3±26.78µm, p=0.0105), CUMS group (169.1±33.90µm, p=0.0048), and CUMS+MCD group (169.5±51.16µm, p=0.0050) (Figure 5A).

One-way ANOVA confirmed significant differences among Protocols ($F_{3,31}$=6.763, p=0.0012).

Analysis of the number of branches revealed significant differences among Protocols, as indicated by ANOVA ($F_{3,31}$=27.11, p<0.0001).

MCD mice exhibited the highest number of branches (97.90±29.44), which was greater than WT mice (58.80±10.13) (p=0.0012), CUMS mice (38.40±8.51) (p<0.0001), and CUMS+MCD mice (38.50±3.65) (p<0.0001) (Figure 5B).

One-way ANOVA used to assess the mean branch length showed differences between Protocols ($F_{3,31}$=11.88, p<0.0001). Mice subjected to the CUMS protocol showed increased mean branch length (4.47±0.73µm), compared to WT (2.68±0.23µm) (p=0.0032) and MCD (2.71±0.37µm) (p=0.0004) groups. Similarly, CUMS+MCD animals displayed increased mean branch length (4.41±1.34µm), compared to WT (p=0.0044) and MCD (p=0.0006) mice (Figure 5C).

Evaluation of branch order revealed a decrease in the number of primary branches in animals subjected to CUMS protocol (4.40±0.69), compared to MCD animals (5.60±1.07) (p=0.0490) (Figure 5D).

One-way ANOVA indicated significant differences among Protocols ($F_{3,31}$=3.242, p=0.0353).

While there was no difference between Protocols when looking at the number of secondary branches ($F_{3,31}$=2.088, p=0.1220) (Figure 5E), the number of tertiary branches decreased in CUMS (7.40±2.87) (p=0.0017) and CUMS+MCD (8.90±2.68) (p=0.0306) groups, compared to MCD animals (12.80±3.29) (Figure 5F), with one-way ANOVA showing differences between Protocols ($F_{3,31}$=6.114, p=0.0022).

The number of quaternary branches were also decreased in CUMS (8.70±3.23) (p=0.0109) and CUMS+MCD (8.90±3.17) (p=0.0150) mice, compared to MCD group (13.90±3.87), with

variances between Protocols in one-way ANOVA ($F_{3,31}$=5.949, p=0.0025) (Figure 5G).

The number of terminal branches was reduced in CUMS (9.50±5.75, p<0.0001) and CUMS+MCD mice (8.20±3.39, p<0.0001) compared to the MCD group (55.00±26.21).

Additionally, the MCD group showed a higher number of terminal branches compared to the WT group (17.40±8.41, p=0.0004) (Figure 5H).

One-way ANOVA confirmed significant differences among Protocols ($F_{3,31}$=21.67, p<0.0001).

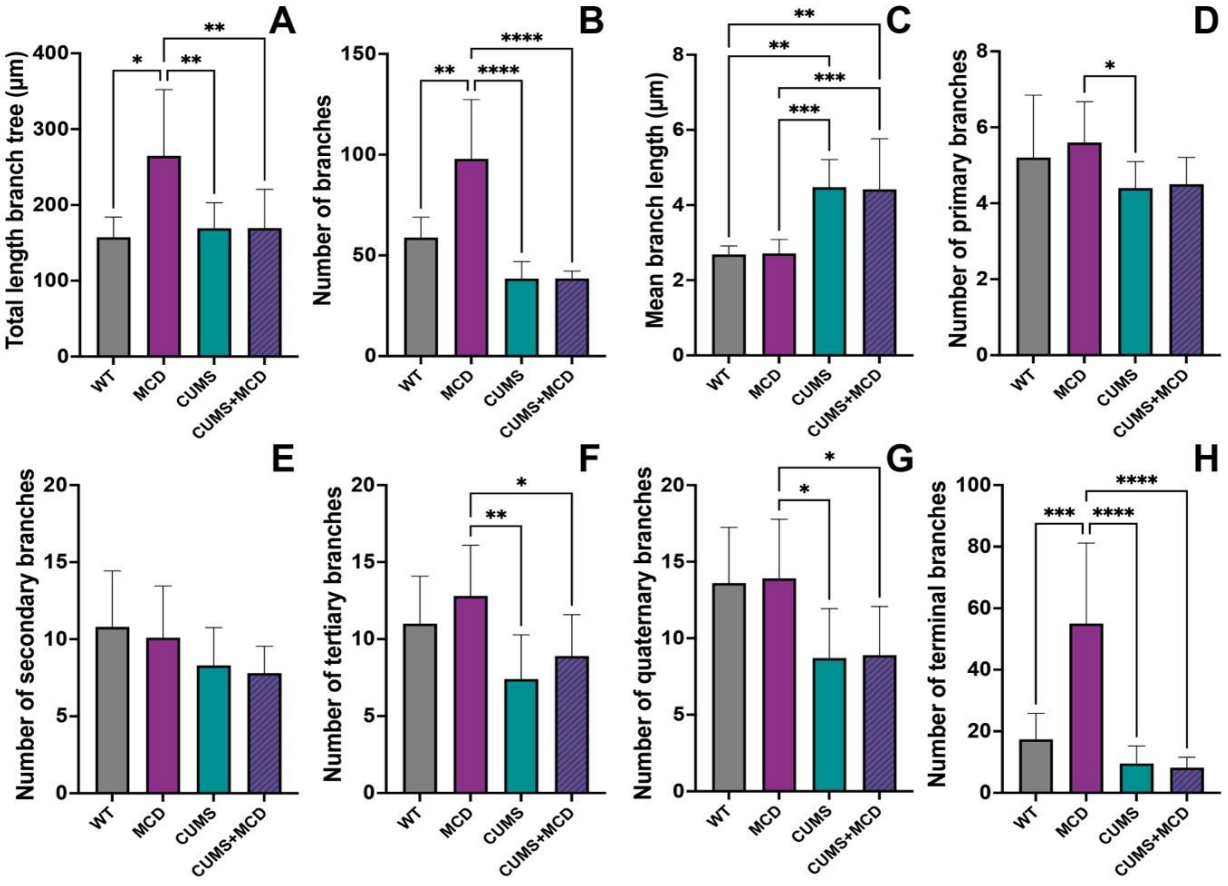

Figure 5. Microglial Arbor Morphology: Detailed microglial morphology was characterized through the systematic analysis of individual branches. This included the evaluation of basic parameters such as (A) total arbor length, (B) the number of branches, and (C) mean branch length. Additionally, a comprehensive morphological analysis revealed differences in the number of (D) primary, (E) secondary, (F) tertiary, (G) quaternary, and (H) terminal branches. Data are presented as mean±SD, with statistical significance indicated as *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001.

Discussion

As populations are aging and medical care improves, individuals experience extended lifespans. While this is a net positive, this also means that medical professionals will start treating increased amounts of patients that present associations between chronic and acute illnesses [28].

As such, reports investigating the molecular and cellular implications of acute and chronic associations have started to show that the presence of a chronic pathology can induce changes in the way our body can cope with acute lesions. While there are examples of such associations between heart and brain [29-31], sepsis and brain [32], or liver and brain [16-18], the long-term effects on the CNS, particularly during healthy or pathological aging, remain largely unexplored [33-35].

With a concerning incidence increase, chronic liver conditions, such as NAFLD, are strongly associated with higher risks of anxiety and depression [3,36-38].

These effects are thought to be mediated by inflammatory pathways involving the immune activity of microglia within the brain [25,26,39].

In the present study, we were able to show that systemic liver damage directly impacts microglial morphology, thus highlighting a potential involvement of liver-induced systemic inflammation of neuropsychiatric conditions,

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confirming microglia involvement in depressive disorders [40-42].

The CUMS protocol effectively induced depressive-and anxiety-like behaviors, as evidenced by reduced sucrose preference and altered exploratory patterns in the OFT.

Behavioral findings in this study are consistent with existing literature demonstrating that the CUMS protocol induces significant behavioral changes in mice, serving as a robust model for depressive- and anxiety-like behaviors [43-45].

The observed reduction in sucrose preference aligns with well-documented evidence of stress-induced anhedonia [18,46], a hallmark of depression characterized by diminished interest or pleasure in rewarding activities [47].

Similarly, the altered exploratory behavior in the OFT reflects heightened anxiety-like responses, frequently reported in rodents subjected to chronic stress paradigms [18,48].

At the morphological level, cortical microglia exhibited significant alterations in response to both liver damage and depressive-like behaviors.

Notably, the MCD diet alone increased total branch length, suggesting an activated microglial state potentially linked to systemic inflammation associated with liver pathology, aligning with previous research.

Conversely, the combination of CUMS and liver damage resulted in distinct reductions in microglial branching complexity, including fewer tertiary and terminal branches. While studies have reported increases in systemic inflammatory markers in both MCD [49] and CUMS rodents [50,51], some studies have reported only minimum changes in the levels of this markers in MCD [52].

Our results suggest a potential shift in microglial function and morphology under conditions of combined systemic and psychological stress, which may contribute to synaptic and neuronal dysregulation.

However, the differential effects observed between experimental groups might be due to overlapping but distinct pathways to influence microglial morphology.

Future research should focus on delineating these pathways to better understand their contributions to the observed changes and their relevance to human neuropsychiatric disorders.

Conclusion

This study highlights the intricate interplay between systemic liver damage and depressive-like behaviors, providing valuable insights into their combined impact on behavior and microglial morphology. The CUMS protocol effectively increased anhedonia-like and anxiety-like behaviors. Notably, short-term memory was unaffected by either liver injury or depressive-like behaviors. At the cellular level, microglial morphology exhibited significant alterations in response to both liver damage and depressive-like behaviors.

Data Availability Statement

The corresponding author can provide the data described in this study upon request.

Authors' contribution

Gabriel Nedelea and Mădălina Iuliana Mușat contributed equally to the present manuscript, therefore share the first authorship.

Conflict of interests

The authors declare that they have no conflict of interests.

References

1. Gangopadhyay A, Ibrahim R, Theberge K, May M, Houseknecht KL. Non-alcoholic fatty liver disease (NAFLD) and mental illness: Mechanisms linking mood, metabolism and medicines. Front Neurosci, 2022, 16:1042442.
2. Shao Q, Wu Y, Ji J, Xu T, Yu Q, Ma C, Liao X, Cheng F, Wang X. Interaction Mechanisms Between Major Depressive Disorder and Non-alcoholic Fatty Liver Disease. Front Psychiatry, 2021, 12:711835.
3. Shea S, Lionis C, Kite C, Lagojda L, Uthman OA, Dallaway A, Atkinson L, Chaggar SS, Randeva HS, Kyrou I. Non-alcoholic fatty liver disease and coexisting depression, anxiety and/or stress in adults: a systematic review and meta-analysis. Front Endocrinol (Lausanne), 2024, 15:1357664.
4. Lin YK, Cai XR, Chen JZ, Hong HJ, Tu K, Chen YL, Du Q. Non-alcoholic fatty liver disease causally affects the brain cortical structure: a Mendelian randomization study. Front Neurosci, 2023, 17:1305624.
5. Ntona S, Papaefthymiou A, Kountouras J, Gialamprinou D, Kotronis G, Boziki M, Polyzos SA, Tzitiridou M, Chatzopoulos D, Thavayogarajah T, Gkolia I, Ntonas G, Vardaka E, Doulberis M. Impact of nonalcoholic fatty liver disease-related metabolic state on depression. Neurochem Int, 2023, 163:105484.
6. Perry VH, Teeling J. Microglia and macrophages of the central nervous system: the contribution of microglia priming and systemic inflammation to chronic neurodegeneration. Semin Immunopathol, 2013, 35(5):601-612.
7. Lannes N, Eppler E, Etemad S, Yotovski P, Filgueira L. Microglia at center stage: a comprehensive review about the versatile and unique residential macrophages of the central nervous system. Oncotarget, 2017, 8(69):114393-114413.

8. Ransohoff RM, Perry VH. Microglial physiology: unique stimuli, specialized responses. Annu Rev Immunol, 2009, 27:119-145.
9. Davies DS, Ma J, Jegathees T, Goldsbury C. Microglia show altered morphology and reduced arborization in human brain during aging and Alzheimer's disease. Brain Pathol, 2017, 27(6):795-808.
10. Vidal-Itriago A, Radford RAW, Aramideh JA, Maurel C, Scherer NM, Don EK, Lee A, Chung RS, Graeber MB, Morsch M. Microglia morphophysiological diversity and its implications for the CNS. Front Immunol, 2022, 13:997786.
11. Machado MV, Michelotti GA, Xie G, Almeida Pereira T, Boursier J, Bohnic B, Guy CD, Diehl AM. Mouse models of diet-induced nonalcoholic steatohepatitis reproduce the heterogeneity of the human disease. PLoS One, 2015, 10(5):e0127991.
12. Itagaki H, Shimizu K, Morikawa S, Ogawa K, Ezaki T. Morphological and functional characterization of non-alcoholic fatty liver disease induced by a methionine-choline-deficient diet in C57BL/6 mice. Int J Clin Exp Pathol, 2013, 6(12):2683-2696.
13. Burstein O, Doron R. The Unpredictable Chronic Mild Stress Protocol for Inducing Anhedonia in Mice. J Vis Exp, 2018, (140):58184.
14. Antoniuk S, Bijata M, Ponimaskin E, Wlodarczyk J. Chronic unpredictable mild stress for modeling depression in rodents: Meta-analysis of model reliability. Neurosci Biobehav Rev, 2019, 99:101-116.
15. Alqurashi GK, Hindi EA, Zayed MA, Abd El-Aziz GS, Alturkistani HA, Ibrahim RF, Al-Thepyani MA, Bakhlgi R, Alzahrani NA, Ashraf GM, Alghamdi BS. The Impact of Chronic Unpredictable Mild Stress-Induced Depression on Spatial, Recognition and Reference Memory Tasks in Mice: Behavioral and Histological Study. Behav Sci (Basel), 2022, 12(6):166.
16. Morega S, Gresita A, Mitran SI, Musat MI, Boboc IKS, Gheorman V, Udristoiu I, Albu CV, Streba CT, Catalin B, Rogoveanu I. Cerebrolysin Use in Patients with Liver Damage-A Translational Study. Life (Basel), 2022, 12(11):1791.
17. Morega S, Cătălin B, Simionescu CE, Sapalidis K, Rogoveanu I. Cerebrolysin Prevents Brain Injury in a Mouse Model of Liver Damage. Brain Sci, 2021, 11(12):1622.
18. Mușat MI, Mitran SI, Udriștoiu I, Albu CV, Cătălin B. The impact of stress on the behavior of C57BL/6 mice with liver injury: a comparative study. Front Behav Neurosci, 2024, 18:1358964.
19. Pałucha-Poniewiera A, Podkowa K, Rafało-Ulińska A. The group II mGlu receptor antagonist LY341495 induces a rapid antidepressant-like effect and enhances the effect of ketamine in the chronic unpredictable mild stress model of depression in C57BL/6J mice. Prog Neuropsychopharmacol Biol Psychiatry, 2021, 109:110239.
20. Mușat MI, Cătălin B, Hadjiargyrou M, Popa-Wagner A, Greșiță A. Advancing Post-Stroke Depression Research: Insights from Murine Models and Behavioral Analyses. Life (Basel), 2024, 14(9):1110.
21. Liu M-Y, Yin C-Y, Zhu L-J, Zhu X-H, Xu C, Luo C-X, Chen H, Zhu D-Y, Zhou Q-G. Sucrose preference test for measurement of stress-induced anhedonia in mice. Nat Protoc, 2018, 13(7):1686-1698.
22. Pitzer C, La Porta C, Treede RD, Tappe-Theodor A. Inflammatory and neuropathic pain conditions do not primarily evoke anxiety-like behaviours in C57BL/6 mice. Eur J Pain, 2019, 23(2):285-306.
23. Leger M, Quiedeville A, Bouet V, Haelewyn B, Boulouard M, Schumann-Bard P, Freret T. Object recognition test in mice. Nat Protoc, 2013, 8(12):2531-2537.
24. Cătălin B, Stopper L, Bălşeanu TA, Scheller A. The in situ morphology of microglia is highly sensitive to the mode of tissue fixation. J Chem Neuroanat, 2017, 86:59-66.
25. Surugiu R, Catalin B, Dumbrava D, Gresita A, Olaru DG, Hermann DM, Popa-Wagner A. Intracortical Administration of the Complement C3 Receptor Antagonist Trifluoroacetate Modulates Microglia Reaction after Brain Injury. Neural Plast, 2019, 2019:1071036.
26. Godeanu S, Clarke D, Stopper L, Deftu AF, Popa-Wagner A, Bălșeanu AT, Scheller A, Catalin B. Microglial morphology in the somatosensory cortex across lifespan. A quantitative study. Dev Dyn, 2023, 252(8):1113-1129.
27. Godeanu S, Mușat MI, Scheller A, Osiac E, Cătălin B. Minimal differences observed when comparing the morphological profiling of microglia obtained by confocal laser scanning and optical sectioning microscopy. Front Neuroanat, 2025, 18:1507140.
28. Furman D, Campisi J, Verdin E, Carrera-Bastos P, Targ S, Franceschi C, Ferrucci L, Gilroy DW, Fasano A, Miller GW, Miller AH, Mantovani A, Weyand CM, Barzilai N, Goronzy JJ, Rando TA, Effros RB, Lucia A, Kleinstreuer N, Slavich GM. Chronic inflammation in the etiology of disease across the life span. Nat Med, 2019, 25(12):1822-1832.
29. Scherbakov N, Doehner W. Heart-brain Interactions in Heart Failure. Card Fail Rev, 2018, 4(2):87-91.
30. Mueller K, Thiel F, Beutner F, Teren A, Frisch S, Ballarini T, Möller HE, Ihle K, Thiery J, Schuler G, Villringer A, Schroeter ML. Brain Damage With Heart Failure: Cardiac Biomarker Alterations and Gray Matter Decline. Circ Res, 2020, 126(6):750-764.
31. Afridi R, Suk K. Microglial Responses to Stress-Induced Depression: Causes and Consequences. Cells, 2023, 12(11):1521.
32. Rotaru-Zavaleanu AD, Neacșu AI, Neacșu AD, Pirici D, Osiac E, Cătălin B, Gheonea DI. Effects of Acute Sepsis on Cellular Dynamics and Amyloid Formation in a Mouse Model of Alzheimer's Disease. Curr Issues Mol Biol, 2022, 44(9):3822-3834.
33. Cojocaru A, Burada E, Bălșeanu AT, Deftu AF, Cătălin B, Popa-Wagner A, Osiac E. Roles of Microglial Ion Channel in Neurodegenerative Diseases. J Clin Med, 2021, 10(6):1239.
34. Popa-Wagner A, Catalin B, Buga AM. Novel putative mechanisms to link circadian clocks to healthy aging. J Neural Transm (Vienna), 2015, 122 Suppl 1:S75-82.

35. Pinosanu LR, Capitanescu B, Glavan D, Godeanu S, Cadenas IFN, Doeppner TR, Hermann DM, Balseanu AT, Bogdan C, Popa-Wagner A. Neuroglia Cells Transcriptomic in Brain Development, Aging and Neurodegenerative Diseases. Aging Dis, 2023, 14(1):63-83.
36. Mușat MI, Militaru F, Udriștoiu I, Mitran SI, Cătălin B. Alcohol Consumption Is a Coping Mechanism for Male Patients with Severe Anxiety Disorders Treated with Antidepressants Monotherapy. J Clin Med, 2024, 13(9):2723.
37. Mușat MI, Militaru F, Gheorman V, Udriștoiu I, Mitran SI, Cătălin B. Moderate Alcohol Consumption Increases the Risk of Clinical Relapse in Male Depressed Patients Treated with Serotonin-Norepinephrine Reuptake Inhibitors. Diagnostics, 2024, 14(11):1140.
38. Choi JM, Chung GE, Kang SJ, Kwak MS, Yang JI, Park B, Yim JY. Association Between Anxiety and Depression and Nonalcoholic Fatty Liver Disease. Front Med (Lausanne), 2020, 7:585618.
39. Anton R, Ghenghea M, Ristoiu V, Gattlen C, Suter MR, Cojocaru PA, Popa-Wagner A, Catalin B, Deftu AF. Potassium Channels Kv1.3 and Kir2.1 But Not Kv1.5 Contribute to BV2 Cell Line and Primary Microglial Migration. Int J Mol Sci, 2021, 22(4):2081.
40. Wang H, He Y, Sun Z, Ren S, Liu M, Wang G, Yang J. Microglia in depression: an overview of microglia in the pathogenesis and treatment of depression. J Neuroinflammation, 2022, 19(1):132.
41. Li B, Yang W, Ge T, Wang Y, Cui R. Stress induced microglial activation contributes to depression. Pharmacol Res, 2022, 179:106145.
42. Yirmiya R. Depressive Disorder Associated Microglia as a Target for a Personalized Antidepressant Approach. Biol Psychiatry, 2023, 94(8):602-604.
43. Wu J, Li J, Gaurav C, Muhammad U, Chen Y, Li X, Chen J, Wang Z. CUMS and dexamethasone induce depression-like phenotypes in mice by differentially altering gut microbiota and triggering macroglia activation. Gen Psychiatr, 2021, 34(6):e100529.
44. Gaignier F, Legrand-Frossi C, Stragier E, Mathiot J, Merlin J-L, Cohen-Salmon C, Lanfumey L, Frippiat J-P. A Model of Chronic Exposure to Unpredictable Mild Socio-Environmental Stressors Replicates Some Spaceflight-Induced Immunological Changes. Front Physiol, 2018, 9:514.
45. Zhang M, Li A, Yang Q, Li J, Zheng L, Wang G, Sun Y, Huang Y, Zhang M, Song Z, Liu L. Matrine alleviates depressive-like behaviors via modulating microbiota-gut-brain axis in CUMS-induced mice. J Transl Med, 2023, 21(1):145.
46. Berrio JP, Kalliokoski O. Rethinking data treatment: The sucrose preference threshold for anhedonia in stress-induced rat models of depression. J Neurosci Methods, 2023, 395:109910.
47. De Fruyt J, Sabbe B, Demyttenaere K. Anhedonia in Depressive Disorder: A Narrative Review. Psychopathology, 2020, 53(5-6):274-281.
48. Liu D, Hu H, Hong Y, Xiao Q, Tu J. Sugar Beverage Habitation Relieves Chronic Stress-Induced Anxiety-like Behavior but Elicits Compulsive Eating Phenotype via vLSGAD2 Neurons. Int J Mol Sci, 2022, 24:661.
49. Sahai A, Malladi P, Melin-Aldana H, Green RM, Whitington PF. Upregulation of osteopontin expression is involved in the development of nonalcoholic steatohepatitis in a dietary murine model. Am J Physiol Gastrointest Liver Physiol, 2004, 287(1):G264-273.
50. Gupta GL, Sharma L, Sharma M. 18β-Glycyrrhetinic Acid Ameliorates Neuroinflammation Linked Depressive Behavior Instigated by Chronic Unpredictable Mild Stress via Triggering BDNF/TrkB Signaling Pathway in Rats. Neurochem Res, 2023, 48(2):551-569.
51. Lu Y, Xu X, Jiang T, Jin L, Zhao X-D, Cheng J-H, Jin X-J, Ma J, Piao H-N, Piao L-X. Sertraline ameliorates inflammation in CUMS mice and inhibits TNF-α-induced inflammation in microglia cells. Int Immunopharmacol, 2019, 67:119-128.
52. Cho MH, Lee HS, Choe BH, Kwon SH, Chung KY, Koo JH, Ko CW. Interleukin-8 and tumor necrosis factor-alpha are increased in minimal change disease but do not alter albumin permeability. Am J Nephrol, 2003, 23(4):260-266.

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