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Research article

Light and classical music therapies attenuate chronic unpredictable mild stress-induced depression via BDNF signaling pathway in mice

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ABSTRACT

Depression, a pervasive mental health issue, often necessitates innovative therapeutic interventions. This study explores the efficacy of music therapy, a non-pharmacological approach, in ameliorating depression symptoms in a murine model. Employing a chronic unpredictable mild stress (CUMS) model to induce depressionlike behaviors in mice, we investigated the therapeutic potential of four distinct music genres: light, classical, atonal composition, and rock music. Behavioral assessments, including sucrose preference and immobility time, were conducted to evaluate the impact of music therapy. Additionally, we measured the levels of brain-derived neurotrophic factor (BDNF), synaptic proteins and neurogenesis to elucidate the underlying biological mechanisms. Our findings indicated that light and classical music significantly alleviated depression-like behaviors in mice, evidenced by increased sucrose preference and reduced immobility time. Conversely, atonal composition and rock music did not yield similar therapeutic benefits. Biochemically, light and classical music were associated with decreased levels of corticosterone and increased levels of glucocorticoid receptor, alongside enhanced BDNF signaling, synaptic proteins and neurogenesis. In conclusion, the study demonstrates that specific genres of music, notably light and classical music, may contribute to alleviating depression-like symptoms, potentially through mechanisms associated with BDNF signaling and neurogenesis. These results highlight the potential of targeted music therapy as a complementary approach in treating depression, with implications for its incorporation into broader therapeutic regimes. Further re-search is warranted to translate these findings into clinical practice.

1. Introduction

Depression is a pervasive mental health disorder that significantly impacts the lives of millions worldwide. Characterized by

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persistent sadness, loss of interest in enjoyable activities, and a range of physical and psychological impairments, it's a leading cause of disability. The complexity of its symptoms, which can vary widely among individuals, makes it a challenging condition to treat effectively. Currently, the primary treatments for depression include pharmacotherapy, notably antidepressants, and various forms of psychotherapy [1]. Antidepressants, such as selective serotonin reuptake inhibitors and tricyclic antidepressants, work by altering the brain's chemical neurotransmitters. While effective for some, these medications are not without their drawbacks. A significant proportion of patients do not respond adequately to first-line antidepressant treatments, leading to a condition known as treatment-resistant depression. Moreover, the onset of the therapeutic effect of these drugs is typically delayed, often taking several weeks to manifest [2]. Additionally, the side effects of antidepressants can be substantial, deterring adherence to treatment regimes [3]. Given these challenges, there is a growing interest in alternative and complementary treatments for depression. These alternatives seek to address the shortcomings of conventional treatments, offering options that might be more effective for certain individuals or have fewer side effects.

As the quest for effective depression treatments continues, music therapy emerges as a promising non-pharmacological intervention. Music therapy, an established health profession, utilizes music within a therapeutic relationship to address physical, emotional, cognitive, and social needs of individuals [4]. It involves a range of activities such as listening to music, singing, and playing musical instruments. The American Music Therapy Association highlights its efficacy in promoting wellness, managing stress, alleviating pain, and enhancing memory. The therapeutic potential of music for mental health is rooted in its ability to evoke emotional responses and facilitate communication and expression, often transcending the barriers posed by traditional verbal communication. Research indicates that music can directly influence brain chemistry and structure [5]. It affects the limbic system, notably the amygdala, which is involved in emotional processing, and can modulate neurotransmitters like dopamine and serotonin, closely linked to mood regulation [6]. More importantly, several previous clinical studies indicated that the effectiveness of music as a non-pharmacologic approach for depression. For example, chronic kidney disease patients who listened to music exhibited alleviated depression than those who did not during hemodialysis [7,8]. Music therapy attenuated depressive symptoms in older patients with mild cognitive impairment [9]. Music therapy showed positive neurophysiological and psychological effects against depressive symptoms in for in attention-deficit hyperactivity disorder children and adolescents [10]. However, there is little study demonstrating the mechanism of music therapy.

A recent preclinical study showed that listening to music alleviated depressive symptoms in chronic unpredictable mild stress (CUMS)-induced mice [11]. The study also indicated that music therapy inhibited hypothalamus-pituitary-adrenal axis hyperactivity and alleviated inflammation in the CUMS-induced depression mice. However, the study used a mixed music collection including light music, classical, atonal composition, flute and so on. It is still unclear which type of music is effective in response to depression treatment. In the present study, we evaluated the effects of four different music including light music, classical, atonal composition, and rock in CUMS-induced depressive mice. Furthermore, the effects of music therapy on brain-derived neurotrophic factor (BDNF) signaling related neurogenesis were also investigated.

2. Materials and methods

2.1. Animals

Male ICR mice aged 8 weeks, each weighing approximately 25 ± 1 g, were procured from Shanghai Slac Animal Center (Shanghai, China). The animals were accommodated in an environment with stringent regulation of both temperature (22 ± 2 °C) and humidity (55 ± 5 %). During the entire duration of the study, we ensured that the mice had unrestricted access to water and a standard diet formulated for rodents. Prior to commencing any experimental protocols, there was a crucial acclimatization period of one week. This phase involved habituating the mice to their new surroundings and gradually introducing human contact to reduce stress and facilitate their adjustment. The experimental protocol received approval from the Animal Ethics Committee of the Jiangxi University of Chinese Medicine (JZLLSC20230462, approval on 03/24/2023). Furthermore, our methodology adhered rigorously to the protocols and ethical guidelines set forth by this committee, ensuring compliance with regulations governing the treatment and management of laboratory animals used in experimental settings.

2.2. Biological reagents

Rabbit BDNF antibody (47808 for immunofluorescence), Mouse PSD95 antibody (36233), Rabbit synaptophysin antibody (36406) and Rabbit DCX antibody (4604) were purchased from Cell signaling Technology (Danvers, USA). Corticosterone (501320) kit was purchased from Cayman Chemical (Ann Arbor, USA). Fluoxetine hydrochloride (F844356) was purchased from Macklin (Shanghai, China). HRP-conjugated Alpha Tubulin antibody (HRP-66031) and Rabbit BDNF antibody (25699-1-AP for Western blot) were purchased from Proteintech (Wuhan, China).

2.3. Therapies

Mice were randomly divided into seven groups (n = 11): Normal-vehicle, CUMS-vehicle, CUMS-classical music, CUMS-light music, CUMS-atonal composition music, CUMS-rock music, CUMS-fluoxetine. In our experimental setup, the mice were exposed to their respective genres of music for a duration of 2 h every evening, specifically between 21:00 and 23:00, coinciding with their dark cycle from Week 5-Week 8 (Fig. 1A). The sound system used for playing the music was strategically positioned at a distance of approximately

2 m from the mice's enclosure. This setup was designed to replicate a natural listening environment. The playback of the music pieces was randomized, mirroring the typical listening habits of the general population. As for the music selection, a meticulously curated playlist was developed for each genre under investigation. This compilation comprised 20 distinct musical pieces per genre, as detailed in Tables 1, 2, 3 and 4. This diverse selection was employed to provide auditory stimulation in our murine subjects, ensuring a broad representation of each musical style under consideration. Fluoxetine was orally administrated at 20 mg/kg once a day for 4 weeks. Open-field test, sucrose preference test and forced swimming test were performed after the last music therapy, respectively.

Our selection was strategically designed to cover a broad spectrum of musical characteristics, including rhythm, melody, harmony, and tonality, which are believed to differentially influence psychological and neurobiological processes. (1) Light Music: Often characterized by its simple melodies, easy listening quality, and soothing nature, light music was chosen to investigate its potential calming and stress-reducing effects. (2) Classical Music: Known for its complex structure and emotional depth, classical music has been widely studied for its effects on cognitive function, stress reduction, and emotional regulation. Its inclusion in our study was aimed at examining whether these well-documented cognitive and emotional benefits could translate into therapeutic effects in a murine model of depression. (3) Atonal Music: Atonal music, which lacks a clear key or tonal center, presents a unique auditory experience that can be dissonant and challenging to interpret. We included atonal music to explore whether its less predictable structure and potential to induce stress or cognitive dissonance would have different effects on depressive behaviors compared to more harmonious and structured musical forms. (4) Rock Music: With its high energy, strong beat, and often loud dynamics, rock music was selected to assess whether stimulation of this intensity could have a positive or negative impact on depressive symptoms. Rock music's potential to evoke strong emotional and physiological responses made it a compelling genre to study in the context of depression and stress. Therefore, the diversity of these music genres allowed us to investigate the spectrum of emotional and neurophysiological responses they may elicit and to determine their varying therapeutic potentials in the context of depression-like behaviors in mice.

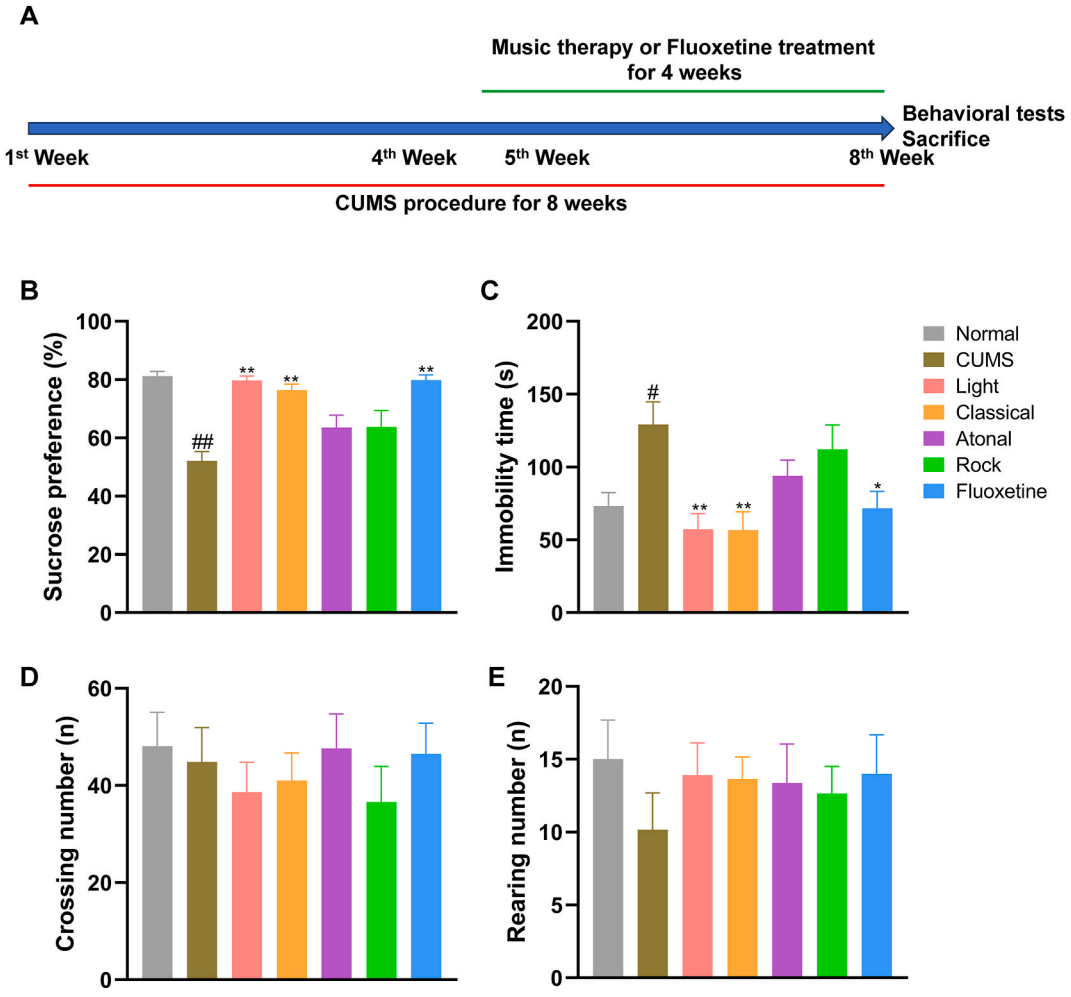

Fig. 1. The effects of different music on the behaviors in mice exposed to CUMS. (A) Timeline of the present study. (B) Sucrose preference in sucrose preference test. (C) Immobility time in forced swimming test. (D) Crossing number in open-field test. (E) Rearing number in open-field test. N = 11 in each group. #p < 0.05 and ##p < 0.01 vs Normal group. *p < 0.05 and **p < 0.01 vs CUMS group.

Table 1
Light music list.

No.	Composition	Composer	Time (s)
1.	Heaven On Earth	Bandari	250
2.	Moonglow	Bandari	229
3.	Xixing Peach Blossom Garden	Li zhihui	313
4.	Ink Orchid Pavilion	Li zhihui	336
5.	The Truth That You Leave	Pianoboy	223
6.	Windy Street	Yukiko Isomura	286
7.	Komorebi	M-taku	212
8.	Summer	Joe Hisaishi	199
9.	Melody of The Night	Shi Jin	217
10.	Through the Arbor	Kevin Kern	225
11.	Canon in D Major	The O'Neill Brothers	300
12.	Theme From Limelight	Gheorghe Zamfir	204
13.	Be Still My Soul	Kevin Kern	225
14.	Souvenirs D'Enfance	Paul De Senneville	174
15.	Mariage D'Amour	Paul De Senneville	175
16.	Ballade Pour Adeline	Paul De Senneville	157
17.	Le Cygne	Saint-Saens	185
18.	Bamboo	Y·Watanabe	257
19.	Por Una Cabeza	Carlos Gardel	134
20.	Playing Love from The Legend of 1900	Ennio Morricone	109

Table 2
Classical music list.

No.	Composition	Composer	Time (s)
1.	Mass in B Minor	Johann Sebastian Bach	322
2.	Symphony No.9 in D Minor, Op.125	Ludwig Van Beethoven	899
3.	Messiah, Hwv 56	George Frideric Handel	217
4.	Piano Sonata No.8 in C minor: 2	Ludwig Van Beethoven	343
5.	Piano Concerto No.2 in B-flat Major, Op.83	Johannes Brahms	1067
6.	Piano Concerto in A Minor, Op.54	Robert Schumann	304
7.	String Quintet in C Major, Posth.163	Franz Schubert	863
8.	The Nutcracker, Op.71	Pyotr Llyich Techaikovsky	248
9.	The Nutcracker: Dance of the Sugar Plum Fairy	Pyotr Llyich Techaikovsky	134
10.	A Midsummer Night's Dream Overture, Op.21	Felix Mendelssohn	778
11.	Prelude to The Afternoon of a Faun	Claude Debussy	377
12.	In A-flat major, Hob.XVI:46	Franz Joseph Haydn	341
13.	Appalachian Spring	Aaron Copland	335
14.	Sonata no. 3 in C major	Franz Joseph Haydn	151
15.	Peer Gynt Suite No.1, Op.23	Edvard Grieg	168
16.	Guantanamera	Joseito Fernandez	188
17.	II. Andante cantabile	Wolfgang Amadeus Mozart	614
18.	Traumerei	Robert Schumann	156
19.	24 Studies in African Rhythms	Fred Onovwerosuoke	160
20.	Spanish Romance	Anonymous	137

2.4. CUMS

To induce a state mimicking depression in mice, we utilized the CUMS model. This model is widely recognized and extensively employed in research to mimic the effects of depression, as it closely replicates the unpredictable and prolonged nature of stress factors often observed in human depressive conditions [12]. The CUMS regimen involved a varied array of stress-inducing factors to facilitate chronic stress in our animal subjects. These factors included alternating periods of food and water deprivation, disruptions of the light/dark cycle, exposure to wet bedding, strobe lighting, inversion of the light cycle, exposure to loud noise, reduction of living space, and tilting of the cage. The implementation of the CUMS protocol spanned an eight-week duration. At the end of CUMS, open-field test, sucrose preference test, and forced swimming test were performed.

2.5. Sucrose preference test

Prior to initiating the experimental phase, we conducted a sucrose adaptation process over a period of three days to familiarize the mice with the sucrose solution. Initially, the mice's cages were equipped with two bottles, each containing a 1 % sucrose solution, for a full day (24 h). Subsequently, for the next 24 h, one bottle of the 1 % sucrose solution was replaced with a bottle of pure water. To ensure a balanced exposure, the positions of these bottles were alternated every 12 h. During the formal experiment, each cage was outfitted with one bottle of 1 % sucrose solution and one bottle of water. Similar to the adaptation phase, we rotated the position of

Table 3
Atonal composition music list.

No.	Composition	Composer	Time (s)
1.	Clusters (1–3)	Johanna Beyer	311
2.	At the still Point	Chester Biscardi	397
3.	Ghost Nets	Henry Brant	1482
4.	Synchronisms.No 7	Mario Davidovsky	596
5.	The Clothed Woman	Duck Ellington	175
6.	Time Cycle	Lukas Foss	1235
7.	The Hound of Heaven	Miriam Gideon	435
8.	Music for Twelve	Leon Kirchner	876
9.	Wozzeck	Alban Berg	321
10.	Piano Study in Mixed Accents	Ruth Crawford Seeger	78
11.	Caractères	Henri Pousseur	17
12.	Three Melodies	Dane Rudhyar	637
13.	Angles	Carl Ruggles	184
14.	Pierrot Lunaire	Arnold Schoenberg	100
15.	Peripetie	Arnold Schoenberg	129
16.	The Rite of Spring	Igor Stravinsky	2079
17.	Thirteen Ways of Looking at a Blackbird	Louise Talma	522
18.	Density 21.5	Edgard Varèse	258
19.	Six Bagatelles	Anton Webern	694
20.	Enactments	Stefan Wolpe	1905

Table 4
Rock music list.

No.	Composition	Composer	Time (s)
1.	Welcome To The Jungle	Guns N'Roses	273
2.	Back In Black	Brian Johnson	255
3.	Whole Lotta Love	James Oden	334
4.	Kashmir	Robert Plant	508
5.	Enter Sandman	Kirk Hammett	331
6.	Crazy Train	Ozzy Osbourne	291
7.	Smells Like Teen Spirit	Krist Novoselic	301
8.	In-A-Gadda-Da-Vida	Doug Ingle	1022
9.	Runnin' with the Devil	Roth, David Lee	214
10.	Ace of Spades	Eddie Clarke	166
11.	Blitzkrieg Bop	Colvin Douglas	131
12.	Breaking the Law	Kenneth Downing	153
13.	Everlong	Dave Grohl	251
14.	Run to the Hills	Steve Harris	234
15.	Dr. Feelgood	Nikki Sixx	291
16.	Bad Reputation	Joan Jett	169
17.	Dirty Deeds Done Dirt Cheap	Bon Scott	231
18.	Rock You Like a Hurricane	Rudolf Schenker	253
19.	Cat Scratch Fever	Ted Nugent	219
20.	You Give Love a Bad Name	Jon Bon Jovi	223

these bottles at 12-h intervals. The consumption of both the sucrose solution and the water was meticulously measured over a 24-h period. Based on these measurements, we calculated the sucrose preference of each mouse, which served as an indicator of anhedonic behavior, a key symptom of depression.

2.6. Open-field test

In our experimental protocol, each mouse was individually positioned at the center of an open-field apparatus, which was a square box measuring 40 cm × 40 cm x 30 cm, divided into 25 equal squares. We observed and recorded two key behavioral parameters: the number of squares traversed by the mice (indicative of their locomotor activity) and the frequency of rearing, which involved the mice standing on their hind limbs. To ensure the integrity of each test session and avoid any olfactory biases, the open-field apparatus was thoroughly cleansed with ethanol following each mouse's session.

2.7. Forced swimming test

In the forced swimming test component of our study, we used a transparent cylindrical container measuring 32 cm in height and 10 cm in diameter. This container was filled with water to a depth of 15 cm, and the water was maintained at a constant temperature of 25 °C. Each mouse was individually placed in this container and required to swim for a total duration of 6 min. We specifically focused

on observing and recording the duration of immobility during the final 4 min of the test. Immobility in this context was characterized by the mouse passively floating in the water, with minimal movement necessary to keep its head above the surface for breathing. In this state, the forelimbs of the mice remained largely stationary, while the hind limbs executed minimal movements, just enough to maintain buoyancy. This behavior was considered indicative of a despair-like state, an important measure in assessing the efficacy of our interventions on depression-like symptoms in mice.

2.8. Enzyme-linked immunosorbent assays (ELISA) measurement

Post the forced swimming test, the animals were humanely euthanized for sample collection. We extracted blood samples, which were then left to coagulate at ambient temperature for a duration of 30 min. Following coagulation, these samples underwent centrifugation at 3000*g* for a period of 15 min. The supernatant, which constitutes the serum, was carefully extracted and deposited into microcentrifuge tubes. To preserve the integrity of the samples and prevent any biochemical degradation, the serum samples were stored in a deep freeze at −80 °C until the time of analysis. For the purpose of measuring the levels of serum corticosterone, we employed the ELISA technique. This quantification was carried out strictly in accordance with the procedural guidelines provided by the ELISA kit manufacturer.

2.9. Real-time PCR

Hippocampal tissues were carefully harvested and processed to extract total RNA. The extracted RNA was then reverse-transcribed using reverse transcriptase enzymes to generate cDNA. The synthesized cDNA served as a template for the ensuing process of PCR amplification. The real-time PCR reaction was executed in a thermal cycler, proceeding through a series of cycles consisting of denaturation (95 °C for 30 s), annealing (53 °C for 60 s), and extension (72 °C for 60 s). For relative gene expression analysis, the expression levels of target genes including glucocorticoid receptor (Forward primer: 5′-CAAAGCCGTTTCACTGTCC-3′ and Reverse primer: 5′-ACAATTTCACACTGCCACC-3′), BDNF (Forward primer: 5′-TTATTTCATACTTCGGTTGC-3′; Reverse primer: 5′-TGTCAGC-CAGTGATGTCG-3′), synaptophysin (Forward primer: 5′-CCACCTCCTTCTCCAATCAG-3′; Reverse primer: 5′-CAGCAAAGA-CAGGGTCTCCT-3′) and PSD95 (Forward primer: 5′-CGCTACCAAGATGAAGACACG-3′; Reverse primer: 5′-CAATCACAGGGGGAGAATTG-3′) were normalized against the housekeeping gene GAPDH (Forward primer: 5′-TGAGGCCGGTGCT-GAGTATGT-3′; Reverse primer: 5′-CAGTCTTCTGGGTGGCAGTGAT-3′). The 2-ΔΔCT method was subsequently employed, utilizing the Ct values to calculate the gene expression levels quantitatively.

2.10. Western blot

Hippocampal protein samples were first extracted and quantified by BCA method, followed by mixing with loading buffer. Subsequently, the protein samples were subjected to SDS-PAGE gel electrophoresis to separate proteins based on molecular weight, followed by transferring the electrophoresed proteins from the gel to a PVDF membrane. The portion of the membrane not occupied by proteins was blocked for 1 h using 5 % BSA to reduce non-specific binding. The membrane was then incubated overnight with primary antibodies against the target proteins (BDNF: 1:1000; synaptophysin: 1:1000; PSD95: 1:1000; α-tubulin: 1:5000). Afterwards, unbound primary antibody was removed using TBST and the membrane bound to the primary antibody was incubated with secondary antibody labeled with HRP for 1 h (1:2500 except to α-tubulin). The unbound secondary antibody was washed again with TBST. Finally, the signal on the membrane was detected using the ECL kit.

2.11. Immunofluorescence

Post-collection, the whole brain tissues were thoroughly fixed in a 4 % solution of paraformaldehyde for a 24-h period at a controlled temperature of 4 °C. Following fixation, these tissues were carefully prepared for preservation by embedding them in OCT. The OCT-embedded tissues were then rapidly frozen by exposure to liquid nitrogen and subsequently stored at a temperature of −80 °C to ensure preservation until the time of sectioning. The process of sectioning involved slicing the frozen brain tissue blocks into sections of 15 μm thickness. The obtained tissue sections were then mounted on slides coated with poly-L-lysine and stored at −20 °C for further processing. During the formal experiment, the sections were immersed in a 4 % paraformaldehyde solution for a duration of 10 min at ambient temperature. Post-fixation, the sections underwent a washing process using TBST-Triton solution to eliminate any remnants of the fixative. The sections were then subjected to a blocking procedure for 1 h at room temperature. The next phase involved the incubation of the sections with primary antibodies targeting BDNF (1:100) and DCX (1:200). This incubation occurred overnight at 4 °C within a humidified chamber. Following the primary antibody incubation, the sections were incubated with secondary antibodies that were fluorophore-conjugated and compatible with the primary antibodies. After the secondary antibody incubation, the final stage involved the visualization of nuclei, achieved through a brief staining process using DAPI-based antifade mounting medium. The completed sections were then analyzed and imaged using a confocal microscope (Leica TCS SP8). To ensure comparability across all samples, the microscope's acquisition settings were kept consistent throughout the imaging process.

2.12. Statistical analysis

The statistical representation of our data is provided as mean values accompanied by the SEM. For the purpose of comparing

between groups, we applied a one-way ANOVA. In instances where the ANOVA revealed significant disparities, we further conducted pairwise comparisons using Tukey's post hoc test to delineate these differences more clearly. We established the threshold for statistical significance at a p-value of less than 0.05.

3. Results

3.1. Light music and classical music therapy alleviated the depression-like symptoms in CUMS

Following a four-week period of music therapy in mice subjected to CUMS, we assessed indicators of depression-like behaviors such as anhedonia and despair. Additionally, assessments of locomotor activity were conducted. As shown in Fig. 1B, exposure to CUMS resulted in a notable reduction in sucrose preference among the mice. This change, indicative of anhedonia, was significantly counteracted by exposure to light music and classical music, as these genres markedly increased sucrose preference in the CUMS-exposed mice. Conversely, neither atonal composition nor rock music showed any significant impact on sucrose preference. Regarding despair-like behavior, as measured by immobility time in the forced swimming test, an increase was observed in CUMS-exposed mice (Fig. 1C). Notably, this immobility time was substantially reduced in mice that were treated with light music and classical music, in contrast to the CUMS-only group. However, this effect was not observed in groups exposed to atonal composition and rock music, as these genres did not influence immobility time. Furthermore, we evaluated locomotor activity through the open-field test, which was performed before the forced swimming test. The results from this test revealed no significant differences in the number of crossing or rearing among the various groups (Fig. 1D and E), suggesting that locomotor activity was not affected by the different music therapies.

3.2. Light music and classical music therapy attenuated the hyperactivity of hypothalamus-pituitary-adrenal axis

Subsequent to the behavioral analyses, we measured the levels of serum corticosterone and assess the expression of glucocorticoid receptor in the hippocampus of the mice undergoing music therapy. The results indicate that exposure to CUMS led to a significant elevation in serum corticosterone levels (Fig. 2A). However, this increase was notably attenuated in mice that were treated with light music and classical music, as these genres effectively reduced corticosterone levels in the CUMS-exposed mice. In contrast, exposure to atonal composition and rock music did not yield a similar reduction in corticosterone levels. Additionally, the investigation into hippocampal glucocorticoid receptor revealed that light music and classical music therapies had a reversing effect on their down-regulation in CUMS-exposed mice (Fig. 2B). This suggests a restorative impact of light music and classical music on stress-induced alterations in hypothalamus-pituitary-adrenal axis.

3.3. Light music and classical music therapy enhanced BDNF signaling in the hippocampus

In our exploration of the neurobiological underpinnings of depression-like symptoms in mice, we specifically examined the expression of BDNF, in the hippocampus. Our data revealed that exposure to CUMS led to a significant reduction in the expression of both BDNF, indicating a potential disruption in neurotrophic support and signaling pathways critical for neuronal health and synaptic plasticity. Interestingly, upon assessing the impact of music therapies, we observed a remarkable shift. Mice subjected to four weeks of listening to either light or classical music exhibited a notable attenuation of BDNF declines by immunofluorescence (Fig. 3A and B), PCR (Fig. 3C) and Western blot (Fig. 3D, Fig. S1) assays. Specifically, light music appeared to restore the levels of BDNF in the hippocampus closer to those observed in non-stressed Normal. This suggests that the therapeutic effects of light music and classical music may, in part, be mediated through the modulation of neurotrophic factor and the activation of BDNF signaling.

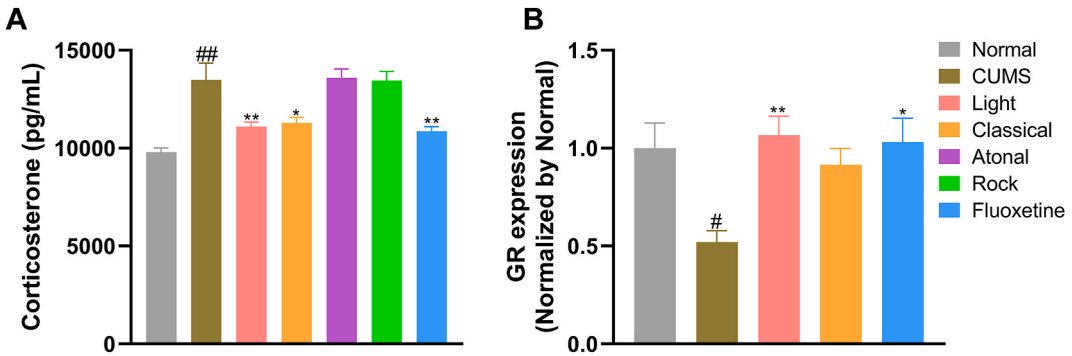

Fig. 2. The effects of different music on serum corticosterone levels and hippocampal glucocorticoid receptor expression in mice exposed to CUMS. (A) Serum corticosterone levels. (B) Hippocampal glucocorticoid receptor mRNA expression. N = 6 or 11 in each group. #p < 0.05 and ##p < 0.01 vs Normal group. *p < 0.05 and **p < 0.01 vs CUMS group.

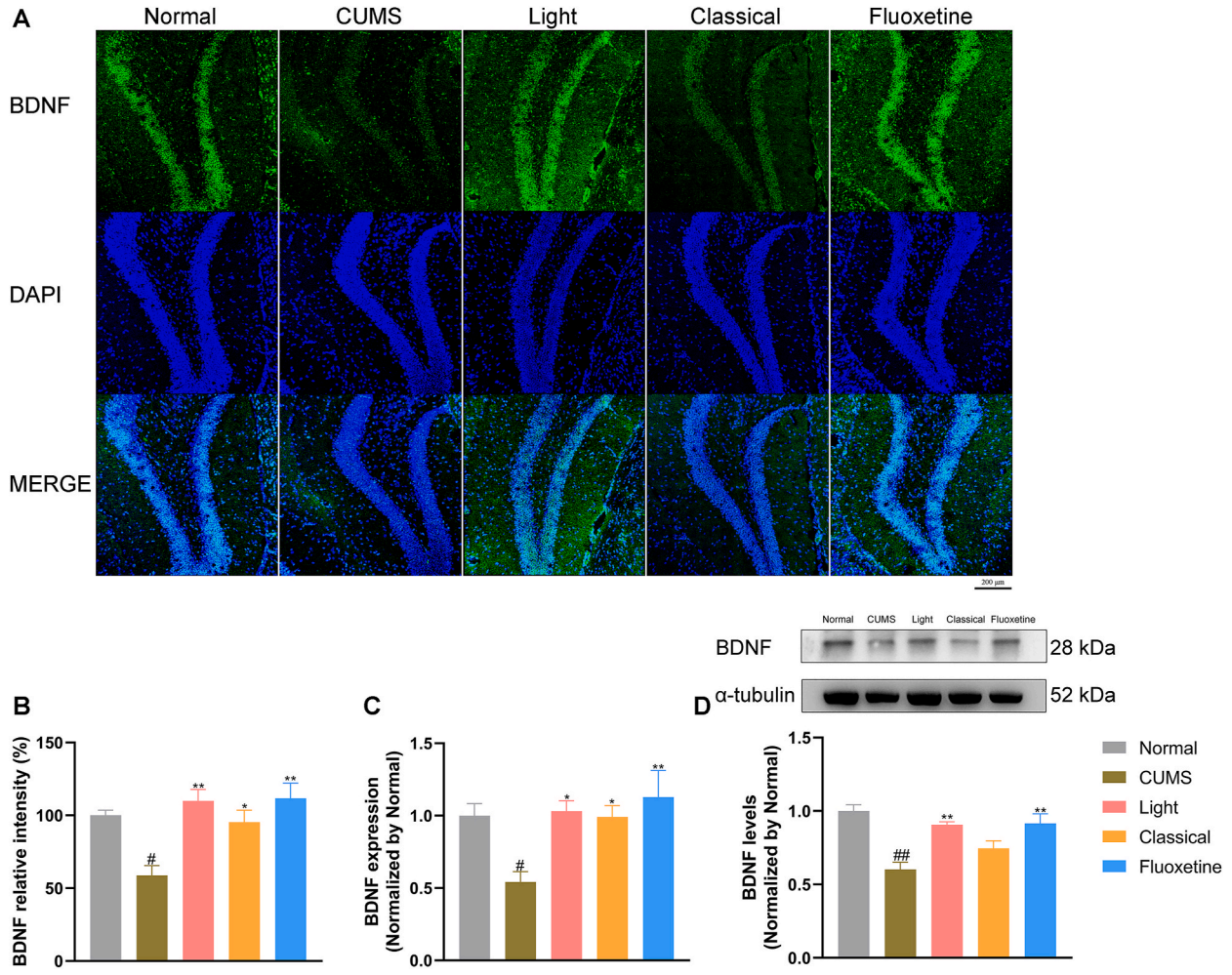

Fig. 3. The effects of light music and classical music on hippocampal BDNF signaling pathway in mice exposed to CUMS. (A) Representative BDNF immunofluorescence image. (B) Histogram of hippocampal BDNF intensity. (C) BDNF mRNA expression. (D) BDNF protein levels. N = 4–6 in each group. #p < 0.05 vs Normal group. *p < 0.05 and **p < 0.01 vs CUMS group.

3.4. Light music and classical music therapy increased synaptic proteins in the hippocampus

In addition to assessing BDNF signaling, our study also evaluated the expression of synaptic proteins in the hippocampus. This investigation was particularly relevant given the known modulation of synaptic proteins by BDNF signaling pathway. As shown in Fig. 4, the results indicated a notable impact of CUMS on synaptic integrity. In detail, CUMS was found to significantly reduce the mRNA expression of the presynaptic protein synaptophysin (Fig. 4A) and the postsynaptic protein PSD95 (Fig. 4B) in the hippocampus, in comparison to the mice in the Normal-vehicle group. This reduction is indicative of synaptic degradation and potential disturbances in neural communication and plasticity, which are critical factors in the pathophysiology of depression. In contrast, our findings revealed that both light music therapy significantly increased both synaptophysin and PSD95 expression at gene levels. Classical music therapy only effectively reversed the reduction in PSD95 mRNA expression. Moreover, the protein levels of synaptophysin and PSD95 were significantly decreased by CUMS (Fig. 4C–E, Fig. S2), while mice exposed to light music therapy showed the attenuations in the loss of both synaptophysin and PSD95. In addition, mice exposed to classical music therapy showed an increase of synaptophysin levels in the hippocampus.

3.5. Light music and classical music therapy increased hippocampal neurogenesis in CUMS mice

In the final phase of our analysis, we focused on examining neurogenesis (Fig. 5A), particularly in response to the light music and classical music therapies. Our observations revealed that exposure to CUMS led to a marked reduction in the number of DCX positive cells within the dentate gyrus region of the hippocampus (Fig. 5B). However, in a notable contrast, exposure to both light music therapy resulted in a significant increase in DCX positive cells. And the classical music exerted a tendency to increase DCX positive cells

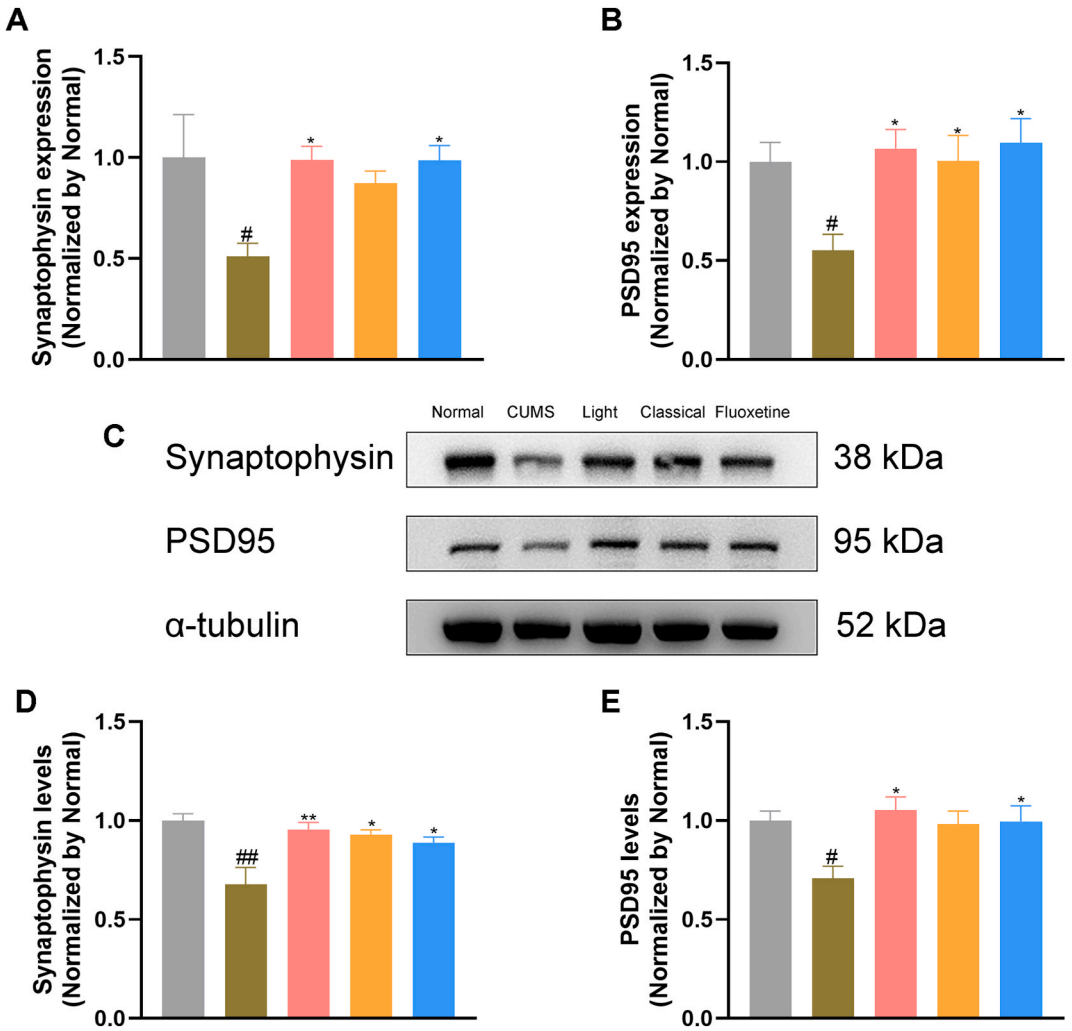

Fig. 4. The effects of light music and classical music on hippocampal presynaptic protein synaptophysin and postsynaptic protein PSD95 expression in mice exposed to CUMS. (A) Synaptophysin mRNA expression. (B) PSD95 mRNA expression. (C) Representative blots. (D) Synaptophysin protein levels. (E) PSD95 protein levels. N = 4 or 6 in each group. #p < 0.05 and ##p < 0.01 vs Normal group. *p < 0.05 and **p < 0.01 vs CUMS group.

in CUMS mice. This finding suggests an enhancement of neurogenesis attributable to the specific genres of music therapy.

4. Discussion

Our study, for the first time, evaluated the effects of four different music on the depressive symptoms in animals, which has yielded significant insights into the potential of music therapy as a treatment for depression-like behaviors in mice. Specifically, we observed that exposure to light music and classical music over a four-week period led to a reduction in symptoms commonly associated with depression. This was evidenced by a marked increase in sucrose preference, indicating a decrease in anhedonia, and a reduction in immobility time during the forced swimming test, suggesting a decrease in despair-like behavior. These behavioral changes are critical indicators in the study of depression, as they closely mimic symptoms observed in human depressive disorders. Conversely, our findings highlighted a stark contrast in the effects of atonal composition and rock music on the same depression-like behaviors in mice. Unlike light music and classical music, atonal composition and rock did not demonstrate a significant therapeutic effect. These genres neither improved sucrose preference nor reduced immobility time, suggesting their limited efficacy in alleviating the symptoms of depression. The differential impact of these music genres underscores the complexity of music therapy as a treatment modality. While light music and classical music showed promise in alleviating depressive symptoms, the lack of effect from atonal composition and rock music suggests that not all types of music are equally beneficial. This could be attributed to various factors, such as the rhythmic structure, melodic content, or the emotional resonance of different genres, which may play crucial roles in their therapeutic potential [13–15].

Corticosterone, a primary glucocorticoid in rodents, plays a pivotal role in the body's response to stress [16]. It's analogous to

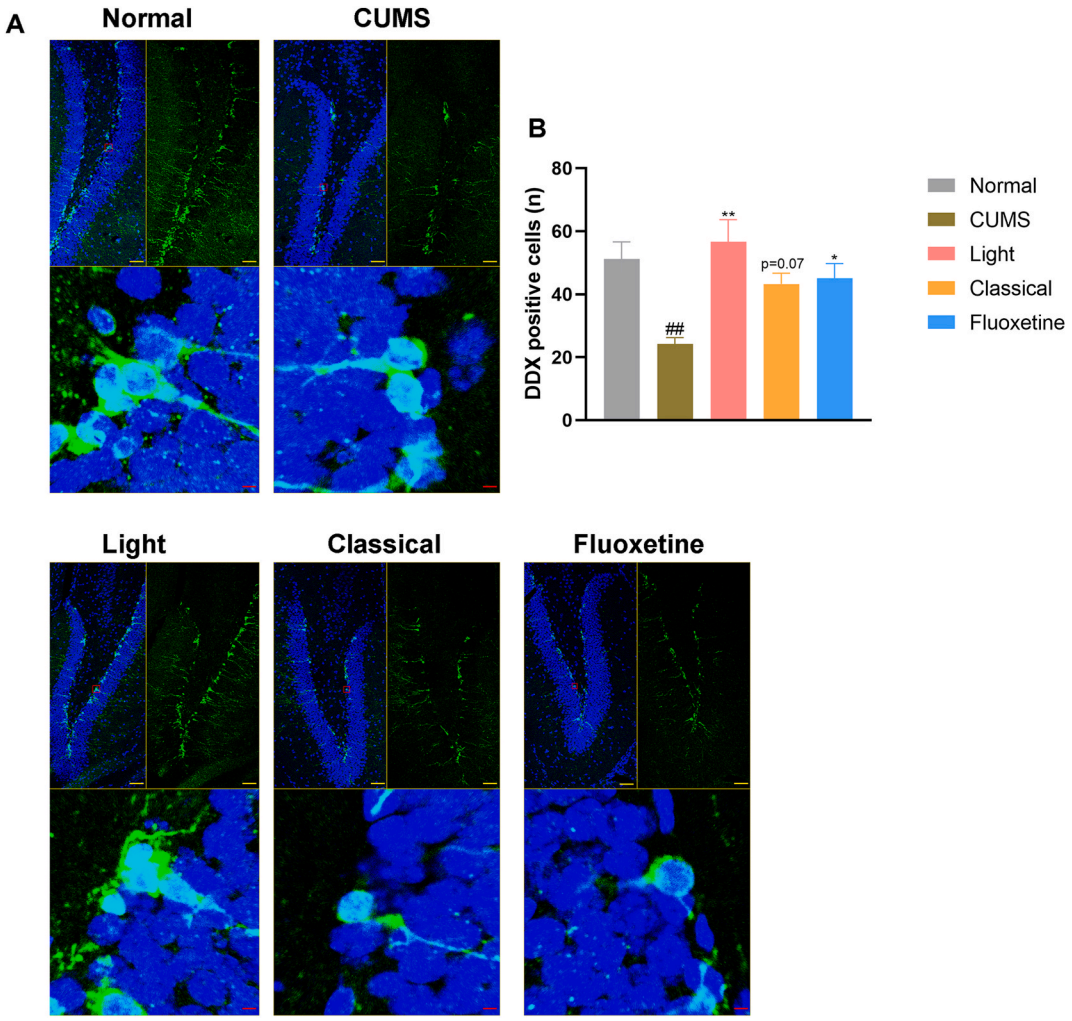

Fig. 5. The effects of light music and classical music on hippocampal neurogenesis in mice exposed to CUMS. (A) Representative DCX positive cells immunofluorescence image. (B) Histogram of hippocampal DCX positive cells. N = 5 in each group. ##p < 0.01 vs Normal group. *p < 0.05 and **p < 0.01 vs CUMS group. Yellow bar: 50 μm; Red bar: 3 μm.

cortisol in humans. Typically, in response to stress, the hypothalamic-pituitary-adrenal axis is activated, leading to increased secretion of corticosterone [17]. This hormone helps mobilize energy reserves and modulate stress responses but, when elevated chronically, can contribute to the pathophysiology of depression. Chronic elevation of corticosterone is linked to neuronal damage, particularly in the hippocampus – a region critical for mood regulation and cognitive function [18]. Elevated corticosterone levels have been associated with several core symptoms of depression, including anhedonia, fatigue, and cognitive dysfunction [19]. In our present study, the reduction of corticosterone levels in mice subjected to light music and classical music suggests an inhibition of the HPA axis hyperactivity typically observed in stress. This finding indicates that these music genres might be capable of modulating the body's physiological stress response, thus contributing to their antidepressant-like effects. The role of glucocorticoid receptor in depression and stress resilience is equally significant. Glucocorticoid receptor, when activated by glucocorticoids like corticosterone, regulate gene transcription and modulate a wide range of physiological processes, including immune response, metabolism, and brain function [20,21]. In the context of depression, the density and sensitivity of glucocorticoid receptors in the brain, particularly in the hippocampus, play a critical role in regulating the HPA axis and mediating the effects of stress. An increase in glucocorticoid receptor levels, as observed in our study following light music and classical music exposure, suggests enhanced feedback regulation on the HPA axis [22,23]. This could lead to a more controlled and balanced stress response, reducing the likelihood of developing stress-related pathologies like depression. Generally, music can evoke powerful emotional responses and induce relaxation. In addition, the rhythm of music can synchronize with physiological processes, such as heart rate and breathing. This emotional engagement and relaxation could lead to a reduction in perceived stress, thereby diminishing HPA axis activation and subsequent corticosterone release [24].

BDNF, a key neurotrophin in the central nervous system, plays a crucial role in neuronal survival, differentiation, and synaptic plasticity, and its activity through the TrkB receptor is essential for maintaining neuronal health and function [25]. Enhanced BDNF

signaling, as indicated by the findings, suggests that music therapy can positively influence this critical pathway, akin to the effects observed with other neurotrophic interventions like exercise, environmental enrichment, and certain pharmacotherapies [26,27]. This enhancement could be attributed to the multi-sensory stimulation provided by music, which engages various brain regions and potentially upregulates BDNF expression, thereby promoting synaptic health and neuronal growth [28]. The increase in synaptic proteins, such as synaptophysin and PSD95, further supports this notion, as these proteins are integral to synaptic structure and function, and their expression is often modulated by BDNF. Synaptic health is critical for effective neural communication and plasticity, processes that are often impaired in depression. Therefore, the restoration or enhancement of synaptic protein levels through music therapy could signify a reversal of these impairments, contributing to improved mood and cognitive function. Similarly, the observed promotion of neurogenesis, particularly in the hippocampus, a region heavily implicated in mood regulation and cognitive processes, underscores the potential of music therapy as a neurogenic agent. The generation of new neurons in the adult brain, a process once thought impossible, is now recognized as an important aspect of brain plasticity and is believed to be beneficial in the treatment of depression [29]. Enhanced neurogenesis, possibly driven by increased BDNF signaling, could lead to improved hippocampal function and resilience against stress-induced neuronal damage [30]. The parallels between the effects of music therapy and other neurotrophic interventions are striking. For instance, physical exercise, known for its ability to boost BDNF levels, similarly promotes synaptic protein expression and neurogenesis, leading to improved mood and cognitive function in depressive disorders. Environmental enrichment, which provides sensory and cognitive stimulation, has also been shown to enhance neurogenesis and synaptic plasticity, potentially through mechanisms similar to those activated by music therapy [31]. These parallels suggest that like exercise and environmental enrichment, music therapy may exert its therapeutic effects through a holistic engagement of the brain's neurotrophic, synaptic, and neurogenic processes. This comprehensive stimulation likely contributes to the overall improvement in brain health and function, which is crucial for combating depression. In conclusion, the enhanced BDNF signaling, synaptic protein expression, and neurogenesis observed in response to light music and classical music therapy highlight the significant potential of music as a neurotrophic intervention.

It should be noted that there are also several limitations in our present study. Firstly, the method of music exposure in our study, through auditory means in a controlled laboratory setting, might not completely mimic how humans typically experience music in a naturalistic environment, which could affect the generalizability of our results. Secondly, although we selected four distinct music genres to cover a broad range of auditory stimuli, this selection is not exhaustive. Other genres or specific pieces of music may elicit different responses, and our study's findings might not be applicable to all types of music.

5. Conclusion

In conclusion, our study underscores the potential of light and classical music therapies in mitigating depression-like behaviors in mice subjected to CUMS, with these therapies modulating the hypothalamus-pituitary-adrenal axis and enhancing BDNF-mediated synaptic plasticity and neurogenesis. The lack of a therapeutic effect observed with atonal and rock music emphasizes the critical role of music genre in determining the efficacy of music therapy. These findings not only corroborate the significance of music's specific characteristics in eliciting therapeutic outcomes but also open avenues for integrating music therapy into existing mental health treatment paradigms. Expanding upon these results, future research could explore the customization of music therapy to align with individual patient preferences and specific depressive symptoms, enhancing therapeutic efficacy and patient engagement. The potential for personalized music therapy could be systematically investigated, considering factors such as musical genre, tempo, and rhythm in relation to patient-specific emotional, cognitive, and neurophysiological profiles. The integration of music therapy into clinical practice requires a multidisciplinary approach, combining insights from psychology, neurobiology, and musicology, to develop evidence-based, patient-centered therapeutic strategies. Further longitudinal studies are needed to understand the long-term effects of music therapy on depression and its potential to be used alongside traditional pharmacological and psychotherapeutic treatments, thereby enriching the therapeutic arsenal available for combating depression.

Ethics statement

The experimental protocol received approval from the Animal Ethics Committee of the Jiangxi University of Chinese Medicine (JZLLSC20230462, approval on 03/24/2023).

Data availability

Data is not available to access in a data repository; however, it will be made available by the corresponding author upon request.

CRediT authorship contribution statement

Hong-Yu Cheng: Writing – original draft, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Hao-Xue Xie:** Investigation, Formal analysis, Data curation. **Qian-Lan Tang:** Methodology. **Li-Tao Yi:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Ji-Xiao Zhu:** Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e34196.

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