Basic Science
Research Paper

OPEN

PAIN REPORTS

Dopamine dynamics in chronic pain: music-induced, sex-dependent, behavioral effects in mice

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Abstract

Introduction: Chronic pain is a debilitating disease that is usually comorbid to anxiety and depression. Current treatment approaches mainly rely on analgesics but often neglect emotional aspects. Nonpharmacological interventions, such as listening to music, have been incorporated into clinics to provide a more comprehensive management of chronic pain. However, the underlying mechanisms of music-mediated pain relief are not fully understood.

Objectives: Our aim was to evaluate the effects and mechanisms of music exposure in an animal model of chronic pain.

Methods: We injected mice with the complete Freund adjuvant (CFA) inflammatory agent into the hind paw and housed them for 14 days with background music, or ambient noise, during their active period (Mozart K.205, overnight). The effect of music exposure on nociception, anxiety-like behaviors, and depression-like behaviors was evaluated through different paradigms, including the hot plate, Von Frey, elevated plus maze, splash, and tail suspension tests. In addition, we conducted fiber photometry experiments to investigate whether music influences dopamine dynamics in the nucleus accumbens (NAcc), a crucial region involved in pain processing, anhedonia, and reward.

Results: Our findings indicate that music exposure prevents the decrease in NAcc activity observed in CFA-injected mice, linking with a sex-dependent reduction in allodynia, anxiety-like behaviors, and depression-like behaviors. Accordingly, female mice were more sensitive to music exposure than male mice.

Conclusion: Collectively, our findings provide compelling evidence for the integration of music as a nonpharmacological intervention in chronic pain conditions. Moreover, the observed effect on NAcc suggests its potential as a therapeutic target for addressing chronic pain and its associated symptoms.

Keywords: Music, Chronic pain, Anxiety, Depression, Dopamine, Nucleus accumbens, Fiber photometry

1. Introduction

Chronic pain, with a global prevalence ranging from 10% to 40%, is a critical public health problem.[9,19] It is not only a primary reason for seeking medical care but also exerts a substantial socioeconomic effect on society.[9,19] Beyond its physical effects, chronic pain significantly affects the quality of life and mental well-

Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

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Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.painrpts.com).

PR9 10 (2025) e1205

http://dx.doi.org/10.1097/PR9.0000000000001205

being of patients, often manifesting as comorbid symptoms, such as anxiety, anhedonia, and depression.[9,17,50] Furthermore, emerging evidence suggests a reciprocal exacerbation between physical and psychological symptoms, emphasizing the need for comprehensive multimodal approaches to the management of chronic pain conditions.[50]

In recent years, there has been a paradigm shift in chronic pain management, acknowledging the need for a more holistic perspective to address this condition. Nonpharmacological interventions have emerged as valuable complements to analgesics, helping people better anticipate, perceive, and respond to pain.[56] Listening to music is a nonpharmacological intervention commonly accepted for its noninvasiveness, safety, cost-effectiveness, and user-friendly nature.[6,32,42,51,56] Clinical studies assessing the effect of listening to music on chronic pain consistently report improved pain relief, along with improvements in associated comorbidities, such as anxiety and depression.[21,27,29,32] However, the neurological mechanisms underlying the effects of music in reducing pain and associated comorbidities remain mostly unknown.

As we recently reviewed, the mesolimbic system, which connects the ventral tegmental area (VTA) and the nucleus accumbens (NAcc), has gained attention in the intersection of pain and mood disorders.[14] Listening to music has been shown to induce changes in neural activity, triggering the release of neurotransmitters such as dopamine or endogenous peptides in different brain areas, including the NAcc.[2,8,12,28,35,37,41,52] In addition, listening to music can induce alterations at the peripheral level (eg, changes in heart rate), with the autonomic and descending pain modulatory systems being the main players in these changes.[8,16,32]

The effects of music exposure in animal models, particularly in the context of pain and mood disorders, have gained considerable attention. Numerous studies have investigated how music exposure influences behavioral responses to pain, stress, and emotional states in different animal models.[26,34,39] Of note, animals may not listen to music in the same way humans do. For instance, human perception of music is complex and influenced by cultural, emotional, and cognitive factors.[32,67] Similarly, the complexity of the human brain allows for intricate processing of music, engaging different brain areas associated with emotions, prediction, memory, and reward.[8,32,67] By contrast, animals demonstrate an ability to respond and discriminate rhythmic patterns, tones, and melodies, indicating that music can perceptibly affect different species.[5,24,26] Recent research has shown differences in animal responses when exposed to forward and backward music, evidencing a certain capacity to decode the internal structure of music.[65] Besides, rodents display innate spontaneous beat synchronization and neural tuning in the auditory cortex.[24]

Despite the differences with humans, animal studies could offer valuable insights into the potential benefits of music and its mechanisms of action. In addition, they could help to underscore whether music-mediated effects are dependent on the sex. Here, we used an animal model of chronic pain to contribute to the understanding of music-mediated effects in this condition.

2. Materials and methods

2.1. Animals

Adult male and female CD-1 mice (animal facility of University of Barcelona) weighing 30 to 40 g were used. Animals were housed and tested in compliance with the guidelines provided by the Guide for the Care and Use of Laboratory Animals[69] and following the European Union directives (2010/63/EU). The University of Barcelona Committee on Animal Use and Care approved the protocol. Mice were randomly assigned to each experimental group and housed in standard cages with ad libitum access to food and water and maintained in a 12 h dark/light cycle (starting light period at 8:00 AM), 22°C temperature, and 66% humidity (standard conditions). All animal experiments were done in a period between 9:00 AM and 6:00 PM by a researcher blind to treatments.

2.2. Reagents

Complete Freund adjuvant (CFA) was purchased from Sigma-Aldrich (St. Louis, MO). The antibodies used were as follows: rabbit antityrosine hydroxylase antibody (1:1000, AB152; Merck Life Science SLU, Darmstadt, Germany), chicken anti-GFP (1:500, A10262; Thermo Fisher Scientific Inc, Waltham, MA), donkey antirabbit Alexa Fluor 647 (1:1000, A31573; Thermo Fisher Scientific Inc), and goat anti-chicken Alexa Fluor 488 (Thermo Fisher Scientific Inc).

2.3. Experimental design

2.3.1. Induction of chronic pain and behavioral assessment

Chronic pain was induced by subplantar injection of 0.03 mL of CFA into the left hind paw, under brief isofluorane-induced anesthetic conditions (3%), as previously reported.[11] Mice were randomly divided into 2 groups: vehicle injected and CFA injected. It should be noted that each group was subdivided into 2 groups according to sex (male and female). After 13 days, the effects of CFA injection were evaluated on nociception, whereas the effects on anxiety-like and depression-like associated symptoms were assessed on day 14. A detailed description of the different paradigms used: hot plate test, Von Frey test, elevated plus maze, splash test, and tail suspension test, is provided in the Supplementary Methods (available at http://links.lww.com/PR9/A259).

2.3.2. Effect of music on chronic pain

Complete Freund's adjuvant–injected mice were divided into 2 groups based on their housing conditions during the 14 days postinjection: (1) ambient noise: ≈45 ± 10 dB and (2) music exposure: Mozart K-205, 20:00 PM to 8:00 AM, ≈55 ± 10 dB. In addition, we included another control group, which was exposed to white noise (≈55 ± 10 dB). Each group was subdivided into 2 groups according to the sex (male and female). It is also important to note that the experimenter responsible for housing the animals did not participate in their evaluation. After 13 and 14 days, the effects of music were evaluated on nociception, anxiety-like symptoms, and depression-like symptoms.

2.3.3. Effect of music on dopamine dynamics in the nucleus accumbens

Dopamine dynamics was evaluated in the NAcc of female mice. First, we performed stereotaxic surgery to inject a dopamine biosensor and implant an optic fiber in the NAcc. After a 4-week period, we performed the subplantar injection of CFA and divided the mice into 2 groups (ambient noise, music exposure; see 2.3.2). Fiber photometry experiments were performed before CFA injection and 7 and 14 days later. Finally, the proper

expression of the biosensor and the placement of the optic fiber were assessed. A detailed description of the protocol to conduct fiber photometry and immunohistochemistry is provided in the Supplementary Methods (available at http://links.lww.com/PR9/A259).

2.4. Data analysis

Data are represented as mean ± SEM with statistical significance set at $P < 0.05$, unless otherwise indicated. The number of animals (n) in each experimental condition is indicated in the corresponding figure legend for each experimental condition. Comparisons between experimental groups were performed by 2-way factor analysis of variance (ANOVA) followed by Tukey or Šídák multiple comparisons post hoc test using GraphPad Prism 9.5.1 (San Diego, CA), as indicated. Outliers were evaluated using the ROUT method assuming a Q value of 0.5% (GraphPad Prism 9.5.1).

3. Results

3.1. Effects of music in nociception, anxiety-like behaviors, and depression-like behaviors associated to chronic pain

The effect of music on chronic pain conditions was evaluated using the CFA-induced inflammatory pain model in mice.[11] First, we explored the effects of CFA injection on nociception and on anxiety-like and depression-like behaviors. The subplantar unilateral injection of CFA induced an inflammatory response readily assessed by measuring the thickness of the paw 1 day after injection, which persisted for at least 14 days. Mice showed hyperalgesia and allodynia 13 days postinflammation induction; they also showed anxiety-like and depression-like behaviors 14 days post-CFA injection (Supplementary Fig. 1, available at http://links.lww.com/PR9/A259). In both nociceptive tests, there were significant differences between both male and female mice following vehicle or CFA injection (Supplementary Fig. 1, available at http://links.lww.com/PR9/A259). These results further validated our previous results demonstrating that CFA induced sustained hyperalgesia and allodynia. Conversely, we examined anxiety-like and depression-like behaviors associated to chronic pain using the elevated plus maze paradigm, the splash test, and the tail suspension test. Our data indicate that CFA-injected mice exhibited heightened anxiety-like behavior, spending less time in the open arms than vehicle-injected mice (Supplementary Fig. 1, available at http://links.lww.com/PR9/A259). Similarly, we observed an anhedonic-like behavior. Grooming time in the splash test was lower in CFA-injected mice compared with vehicle-injected mice (Supplementary Fig. 1, available at http://links.lww.com/PR9/A259). In addition, CFA-injected mice exhibited increased immobility time, which is indicative of despair behavior,[1] compared with vehicle-injected mice (Supplementary Fig. 1, available at http://links.lww.com/PR9/A259). Altogether, our findings provided evidence that CFA injection induced nociceptive, anxiety-like behaviors, and depression-like behaviors. These effects were particularly pronounced in female mice, with anhedonic-like behavior exhibiting the clearest and most significant effect.

Next, we interrogated whether music exposure could preclude CFA-induced effects. To this end, after CFA injection, the animals were subjected to a regimen of music or ambient noise (see Methods, **Fig. 1A**). First, the potential antinociceptive effect of music was explored. Sex-based differences in thermal latencies were analyzed using 2-way ANOVA, which revealed that there was no effect of sex ($F_{(1,44)} = 0.004$, $P = 0.95$) or music exposure ($F_{(1,44)} = 0.043$, $P = 0.84$) and that there was no interaction between both factors ($F_{(1,44)} = 0.554$, $P = 0.46$) (**Fig. 1B**). By contrast, music elevated mechanical nociceptive thresholds. An effect of music exposure ($F_{(1,44)} = 7.152$, $P = 0.01$) but not sex ($F_{(1,44)} = 0.649$, $P = 0.42$) was shown, whereas no interaction between both factors ($F_{(1,44)} = 1.788$, $P = 0.19$) was observed. Tukey post hoc analysis showed significant differences between female mice after exposure to ambient noise or music ($P = 0.0417$) but not between male mice ($P = 0.09$). These findings indicated that music exposure reversed allodynia in female mice (**Fig. 1C**). Of note, we conducted the same experiments in a group of CFA-injected mice following a regimen of white-noise exposure. These animals showed similar nociception than those exposed to ambient noise, supporting the specificity of the effects of music exposure (Supplementary Fig. 2, available at http://links.lww.com/PR9/A259).

Next, we evaluated the effect of music exposure and possible differences between sexes in CFA-induced anxiety-like behavior. We used the machine learning–based package DLC to analyze the video recordings and assess both the time spent in the arms and the distance travelled. In **Figure 1D,** representative plots in which the time spent in the arms by the different groups is represented as a heat map. Quantification of these analyses showed that there was an increase in the percentage of time spent in the open arms of music-exposed mice compared with that of the ambient-noise group. Interestingly, this effect manifested both in male and female mice (**Fig. 1E**). The 2-way ANOVA revealed that there was a significant effect of music exposure ($F_{(1,41)} = 24.760$, $P < 0.0001$) and sex ($F_{(1,41)} = 8.195$, $P = 0.006$), with no interaction between both factors ($F_{(1,41)} = 0.906$, $P = 0.01$). Tukey post hoc analysis showed significant differences in both male and female mice when comparing ambient-noise vs music conditions ($P = 0.008$ and $P = 0.004$, respectively). Thus, data indicated that music exposure was effective in mitigating anxiety-like behavior across sex. We also assessed the total distance travelled during the test. Spontaneous activity may not be an optimal measure for anxiety-like behavior, but an increase in locomotor activity in the maze may also reflect antianxiety effects.[62] Indeed, music exposure augmented locomotor activity in music-treated mice but, in contrast to the percentage of time spent in the open arms, no sex differences were observed (**Fig. 1F**). The 2-way ANOVA revealed an effect of music exposure ($F_{(1,43)} = 4.753$, $P = 0.04$) but not sex ($F_{(1,43)} = 2.078$, $P = 0.16$), without interaction between the 2 factors ($F_{(1,43)} = 0.558$, $P = 0.31$). Tukey post hoc analysis showed no significant differences between the groups. Overall, our findings support that music effectively reduced anxiety-like behavior in CFA-injected mice.

Next, we assessed whether music exposure was also effective at reducing depression-like symptoms. In the splash test, we observed that music exposure increased grooming time, thus reducing anhedonic-like behavior. The statistical analyses showed a main effect of music exposure ($F_{(1,41)} = 6.988$, $P = 0.01$) but not sex ($F_{(1,41)} = 0.115$, $P = 0.74$), with no interaction between both factors ($F_{(1,41)} = 1.032$, $P = 0.32$). Tukey post hoc analysis showed significant differences in female mice under music exposure or ambient noise ($P = 0.049$), but not in male mice ($P = 0.7$) (**Fig. 1G**). Similarly, we could observe an effect of music exposure when measuring despair behavior using the tail suspension test (**Fig. 1H**). The 2-way ANOVA showed no main effects of music exposure ($F_{(1,43)} = 3.427$, $P = 0.07$) or sex ($F_{(1,43)} = 0.132$, $P = 0.72$) but revealed an interaction between both factors ($F_{(1,43)} = 5.500$, $P = 0.02$). Tukey post hoc analysis indicated significant differences only in female mice with music

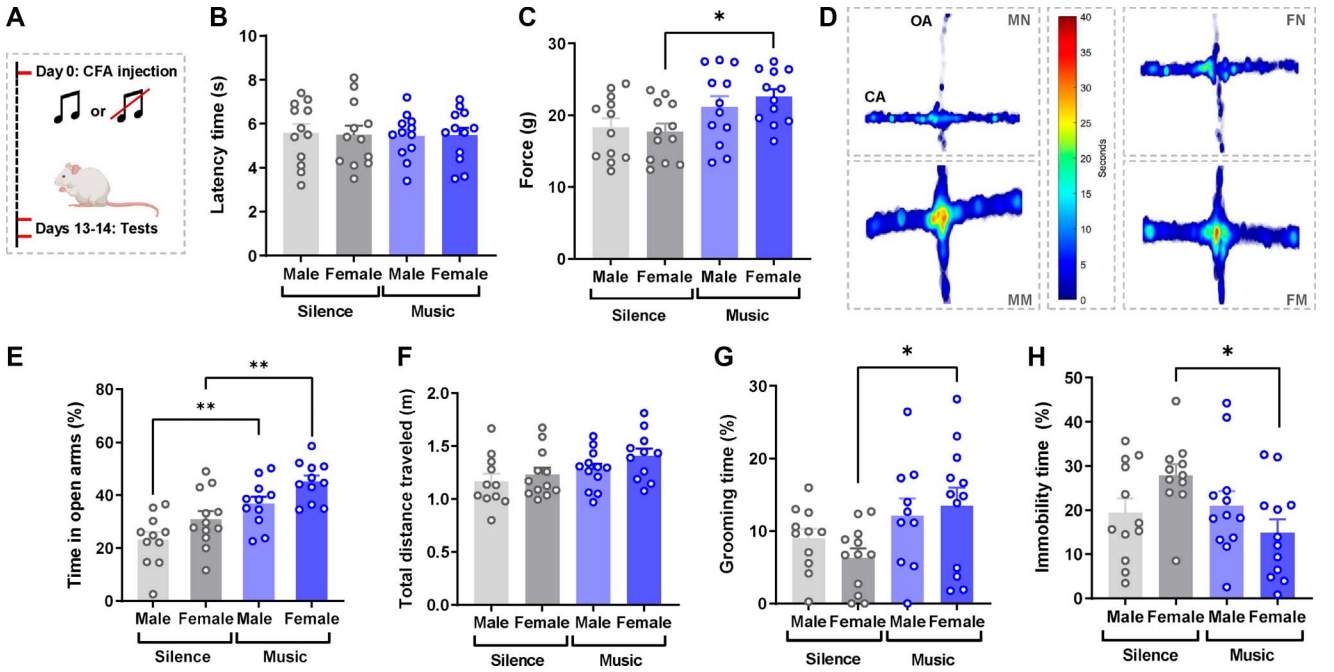

Figure 1. Effects of music on nociception, anxiety-like, and depression-like behaviors linked to chronic pain. (A) Experimental time line: mice were subplantarly injected with complete Freund's adjuvant (CFA) and the potential effects of music exposure (Mozart K-205, O/N) evaluated 13 to 14 days later. (B) Effect of music on CFA-induced thermal hyperalgesia differentiated by sex. Latencies (s) compared between ambient-noise (silence) and music groups, including the sex. Data are presented as mean ± SEM of 12 animals per group. $P > 0.05$; 2-way analysis of variance (ANOVA), Tukey post hoc test. (C) Effect of music exposure on CFA-induced mechanical allodynia differentiated by sex. Force (g) inducing withdrawal compared between ambient-noise (silence) and music groups, including the sex. Data are presented as mean ± SEM of 12 animals per group. $^*P < 0.05$; 2-way ANOVA, Tukey post hoc test. (D) Representative plots depicting the performance of CFA-injected mice: (1) male + ambient noise (MN), (2) female + ambient noise (FN), (3) male + music (MM), and (4) female + music (FM), generated using the DLC software. The tracking of the animal across the maze is colored in relation to time. CA, closed arm; OA, open arm. (E) Quantification of the percentage of time spent in open arms, compared between ambient-noise (silence) and music groups including the sex. Data are presented as mean ± SEM of 11 to 12 animals per group. $^{**}P < 0.01$; 2-way ANOVA, Tukey post hoc test. (F) Distance travelled (m) compared between ambient-noise (silence) and music groups, including sex. Data are presented as mean ± SEM of 11 to 12 animals per group. $P > 0.05$; 2-way ANOVA, Tukey post hoc test. (G) Effect of music on anhedonic behavior differentiated by sex. Grooming time (s) compared between ambient-noise (silence) and music groups including the sex. Data are presented as mean ± SEM of 11 to 12 animals per group. $^*P < 0.05$; 2-way ANOVA, Tukey post hoc test. (H) Effect of music on despair behavior differentiated by sex. Immobility time (s) compared between ambient-noise (silence) and music groups including the sex. Data are presented as mean ± SEM of 11 to 12 animals per group. $^*P < 0.05$; 2-way ANOVA, Tukey post hoc test.

compared with female mice with ambient-noise exposure ($P = 0.0264$). In summary, the effects of music on anhedonia and despair behavior were specifically observed in CFA-injected female mice. Interestingly, we conducted an additional series of experiments to validate the specificity of music-mediated effects in chronic pain conditions. Thus, we assessed whether music exposure did affect anxiety-like and depression-like behaviors in noninflamed mice. We did neither observe an effect of music nor white noise on the behavior of vehicle-injected mice (Supplementary Fig. 3, available at http://links.lww.com/PR9/A259), excluding a nonspecific influence of auditory stimuli on the observed behavioral outcomes.

3.2. Effect of music in dopamine release in the nucleus accumbens

Fiber photometry is a cutting-edge tool that allows real-time measurements of neurotransmitter release.[46,47,55] The ability to quantify neurotransmitter release in a subsecond scale offers unprecedented opportunities to understand the neural mechanisms underlying different physiological and pathological processes.[46,47,55] We hypothesized that the effects of music in chronic pain conditions might be mediated, at least in part, by alterations in dopamine dynamics in the NAcc.[14] Accordingly, we investigated potential alterations in dopamine release over time following CFA injection and music exposure. Based on the previous behavioral results, we performed our experiments only in female mice. First, animals underwent stereotaxic surgery to inject the intensity-based fluorescent dopamine biosensor Dlight1.3b[46] and implanting a fiber optic, and, after a 4-week period, we recorded extracellular dopamine transients in free moving animals (**Fig. 2A**). After CFA injection, we repeated photometry experiments at days 7 and 14 days of chronic inflammation with or without music exposure (**Fig. 2A**). **Figure 2B** illustrates the schematic representation of virus injection and insertion of a fiber optic into the NAcc to record spontaneous dopamine transients. Following the last fiber photometry recordings, mice were killed, and the brains were processed for immunohistochemistry to ensure biosensor expression in the NAcc and the correct optic fiber implantation (**Fig. 2C**).

Photometry recordings were conducted in a home-cage environment, where animals had free exploration for 20 minutes. Animals previously underwent habituation for 3 days to habituate to the experimental conditions, including the tethering to the fiber-optic system. Representative traces from a nonmusic-exposed animal at time points 0 and 14 are presented (**Fig. 2D**), alongside traces from an animal exposed to music at the same intervals (**Fig. 2E**). In addition, a representative alignment of dopamine transients average for each condition at different time points is illustrated (**Fig. 2F**). Quantitative analysis across animals showed a progressive decrease in the number of dopamine peaks following CFA injection, with a significant reduction observed at time point 14

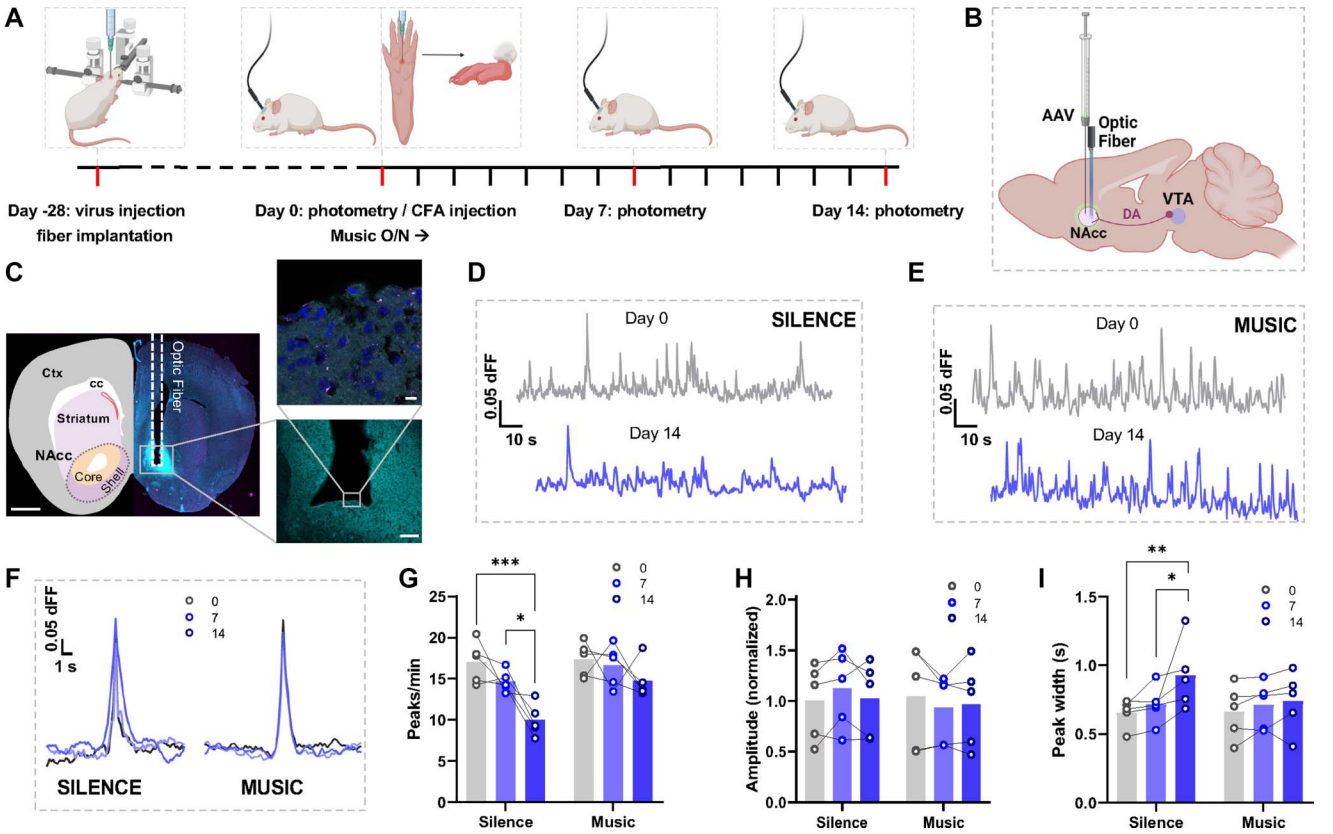

Figure 2. Assessment of dopamine dynamics in the nucleus accumbens. (A) Experimental time line: mice were injected with a dopamine sensor virus followed by the insertion of a fiber optic into the nucleus accumbens (NAcc), and 28 days later submitted to fiber photometry recordings. Next, mice were subplantarly injected with complete Freund's adjuvant (CFA), and the potential effects of music treatment (Mozart K-205, O/N) in dopamine dynamics evaluated after 7 and 14 days. (B) Schematic illustration of surgical intervention for Dlight1.3b AAV injection and optic fiber implantation in the NAcc. (C) Whole brain slice showing the insertion of the optic fiber in the NAcc and immunofluorescence detection of the DLight1.3b sensor with an antibody targeted to a biosensor's GFP moiety. Tyrosine hydroxylase (TH) and cell nuclei (with DAPI) were also immunolabelled. Scale bar = 1000 μm. In-set (down): magnified image (10×) of AAV expression in the vicinity of the optic fiber. Scale bar = 100 μm. In-set (up): magnified image (63×) showing cell nuclei (blue), AAV (green), and TH (magenta) expression. Scale bar = 10 μm. A drawing of brain regions in the cerebral section overlaps on the left hemisphere. cc, corpus callosum; ctx, cortex. (D) Representative traces showing spontaneous dopamine fluorescent transients at different time points (0 and 14 days) for the ambient-noise (silence) group. (E) Representative traces at different time points (0 and 14 days) for the music group. (F) Representative plot of overall peak alignment of the complete recordings at the different time points (0, 7, and 14 days) for both ambient-noise (silence) and music groups. (G) Effects of music treatment on spontaneous dopamine transients in CFA-injected mice. Number of peaks/min compared between ambient-noise (silence) and music groups (5 animals per group) at the different time points (0, 7, and 14 days). $^{*}P < 0.05$, $^{***}P < 0.001$; 2-way analysis of variance (ANOVA), Šídák post hoc test. (H) Peak amplitude, normalized to the mean of each condition at day 0, compared between ambient-noise (silence) and music groups (5 animals per group) at the different time points (0, 7, and 14 days). $P > 0.05$; 2-way ANOVA, Šídák post hoc test. (I) Peak width compared between ambient-noise (silence) and music groups (5 animals per group) at the different time points (0, 7, and 14 days). $^{*}P < 0.05$, $^{**}< 0.01$; 2-way ANOVA, Šídák post hoc test.

(**Fig. 2G**). Importantly, music exposure exhibited a preventive effect on this decline. The 2-way ANOVA revealed that there was a main effect of time ($F_{(2.16)} = 12.180$, $P = 0.0006$) and music exposure ($F_{(1,8)} = 10.440$, $P = 0.012$), without interaction between both factors ($F_{(2,16)} = 2.488$, $P = 0.12$). Šídák post hoc analysis showed significant differences between time points 0 and 7 and time point 14 only in the ambient-noise group ($P = 0.0008$ and $P = 0.0265$, respectively). These results revealed a progressive reduction of spontaneous dopamine transients in the NAcc under chronic pain conditions. Music exposure prevented this decline, suggesting that it may exert a potential regulatory role in maintaining dopamine signaling dynamics in the context of chronic pain.

Peak analysis was conducted to discern potential alterations in dopamine transients induced by chronic pain and music exposure. Our results revealed no discernible effects of CFA-injection on peak amplitude, which remained consistent across all time points. Furthermore, music exposure did not exert a modulatory influence on peak amplitude (**Fig. 2H**). The 2-way ANOVA revealed that there was not an effect of time ($F_{(2,16)} = 0.211$, $P = 0.81$) neither of music exposure ($F_{(1,8)} = 0.798$, $P = 0.07$) and that there was not an interaction between both factors ($F_{(2,16)} = 2.462$, $P = 0.117$). Šídák post hoc analysis further substantiated that there were not differences between the time points ($P > 0.05$). Conversely, CFA injection led to an increase in peak width, an effect that music exposure prevented. The 2-way within-subject ANOVA revealed that there was an effect of time ($F_{(2,16)} = 6.941$, $P = 0.007$) but not music exposure ($F_{(1,8)} = 0.342$, $P = 0.58$), without interaction between both factors ($F_{(2,16)} = 2.624$, $P = 0.10$). However, Šídák post hoc analysis disclosed significant differences between time points 0 and 7 and time point 14 exclusively in the non–music-exposed group ($P = 0.0058$ and $P = 0.0402$, respectively). The widening of peaks suggests potential alterations in dopamine synaptic clearance, which music exposure prevented, thereby maintaining the dopamine dynamics in the NAcc of CFA-injected mice.

4. Discussion

Our findings uncovered compelling sex-dependent effects of music exposure under chronic pain conditions. Music exposure

did not modify thermal hyperalgesia but attenuated mechanical allodynia induced by CFA injection, an effect that was exclusively observed in female mice 13 days post-CFA injection. This outcome aligns with an expanding body of literature supporting the efficacy of music across diverse rodent models.[26,34,39] Interestingly, music exposure did not affect the behavior of noninflamed mice, thus supporting the specificity of music-mediated effects in chronic pain conditions. Notably, recent research proposed that the effectiveness of music exposure in rodents would depend on the sound pressure level (that should be ≈50 dB) rather than consonancy.[68] Our music exposure regimen was set at ≈ 55 ± 10 dB, whereas control animals were housed in ambient-noise conditions (≈45 ± 10 dB). We discarded the possibility of a sound-dependent effect by assessing the effects of white noise at the same sound pressure level (≈55 ± 10 dB). In addition, it is noteworthy that the observed behavioral effects align with prior studies investigating similar music regimens and sound levels.[30] Importantly, some of these studies demonstrated that employing nonconsonant or unpleasant music at comparable sound pressure levels led to the loss of music-mediated effects.[26,41,61]

We only observed music-dependent antinociceptive effects in female mice. These sex-specific effects are in accordance with numerous reports highlighting variations in pain perception and analgesic responses between male and female individuals.[1,43] Hence, considering sex as a critical factor in chronic pain research appears imperative. Although our study did not investigate into the potential mechanisms explaining the distinct antinociceptive effects of music exposure, existing research has extensively explored changes in hormonal modulation.[10,25,33,40] These changes would make female subjects more susceptible to interventions that could modulate neuroendocrine responses, such as music.[8] In addition, it has been proposed that estrogens may influence the excitability of nociceptive neurons, alter neurotransmitter's release, or interact with the endogenous opioid system.[13,23,66] For instance, estrogens can influence the expression and function of opioid receptors,[7] potentially enhancing the sensitivity of female subjects to interventions modulating the opioid system, such as music.[36,67]

We explored the effect of music exposure on anxiety- and depression-like behavior associated with CFA-induced pain.[20,31] In both male and female mice, music exposure exerted anxiolytic effects, aligning with previous studies highlighting the effect of music on anxiety.[4,16] These studies investigated into the alterations induced by music on various systems, including the immune and autonomic systems, both recognized for their sensitivity to music interventions.[48] Conversely, we observed an effect of music exposure on depression-like symptoms, specifically in female mice. Our findings are in accordance with previous studies demonstrating the antidepressant effects of music.[16,45] In these investigations, music exposure was shown to restore homeostasis in the hypothalamus–pituitary–adrenal axis, prevent oxidative stress, and counteract neurotrophic factor deficits.[16,26] However, these studies did not specifically explore the influence of sex. The mechanisms through which music may influence depression-like behavior, particularly in female mice, are insufficiently understood. Nevertheless, our results highlight the importance of accounting for sex-specific factors to use music for managing anxiety and depression associated with chronic pain.

Possible mechanisms explaining the benefits of listening to music on chronic pain and associated symptoms may involve the modulation of various neurotransmitter systems, including the serotoninergic and dopaminergic pathways.[35,37,41,45,49] Recently, we highlighted the convergence of mechanisms in chronic pain and depression, pointing the VTA as a potential therapeutical hub.[14] Chronic pain conditions are often associated with alterations in the mesolimbic pathway,[28,49,60] which is also involved in the regulation of both mood and reward. Alterations in this pathway have been identified in the context of depression conditions.[22,44,57] We took advantage of the Dlight1.3b biosensor, which provided high temporal resolution (sub-seconds scale) to monitor the spatiotemporal dynamics of dopamine signals in the NAcc.[46] It is worth mentioning that the Dlight series of biosensors exhibit faster off-kinetics compared with other indicators like $GRAB_{D2}$, hence allowing to detect changes in phasic dopamine release, whereas $GRAB_{D2}$ would be more adequate to measure tonic dopamine.[47,59]

We conducted recordings of spontaneous dopamine transients in freely moving animals under basal conditions and following both CFA injection and music exposure. Our data demonstrate that music exposure effectively prevented the reduction in the frequency of spontaneous dopamine transients induced by chronic pain. This observation suggests that chronic pain may induce neuronal changes, potentially affecting the firing patterns or the excitability of dopaminergic neurons.[18,54] Among the potential causes for this phenomenon is the impairment of synaptic dopamine transmission, where alterations in dopamine reuptake or vesicular release could influence the occurrence of spontaneous transients.[54,55] Moreover, we observed that the width of the peaks was wider, indicating potential impairment of dopamine reuptake and/or degradation, likely linked to changes in the kinetics of synapse clearance.[54,55] Under normal conditions, dopamine diffusion is limited by various factors, particularly the dopamine transporter. Impairment of these mechanisms of synapse clearance could enhance the diffusional spread of dopamine from its point of release.[15,58] Interestingly, the peak amplitude remained unchanged, contrasting with findings in other studies,[18,63,64] although the lack of effect on peak amplitude might be because of the biosensor sensitivity and expression. Potential disparities in these results could also originate from differences in the models used (acute vs chronic pain) or the sex (male vs female) of the animals.[18,63,64] Therefore, the changes induced by CFA injection seemed to influence the occurrence rather than the intensity of spontaneous dopamine transients. The sustained decrease in dopamine levels may lead to a reduction in the overall activity within the NAcc.[53,54] Collectively, it appears evident that chronic pain induces alterations in the VTA dopaminergic neurons projecting to the NAcc, and music exposure prevents such alterations. These findings agree with previous studies indicating that dopamine release increases in the mesolimbic system following music exposure.[2,3,12,38,52] Of note, we conducted these experiments exclusively with female mice based on the initial behavioral observations. However, replicating these experiments with male mice could further clarify the specificity of the observed effects.

In conclusion, our findings indicate that music exposure prevented the neurobiological adaptations observed in chronic pain, linking with antinociceptive effects and a reduction in anxiety-like and depression-like behaviors. Importantly, the effects of exposure to music were more pronounced in female mice, suggesting that gender disparities must be taken into account when implementing pain treatments. Our findings not only provide support for the integration of listening to music as a nonpharmacological intervention for a more comprehensive management of chronic pain but also contribute to elucidating the underlying mechanisms of music-induced effects in chronic pain conditions.

Disclosures

The authors have no conflicts of interest to declare.

Acknowledgments

The authors thank Esther Castaño and Benjamín Torrejón from the Scientific and Technical Services (SCT) group at the Bellvitge Campus of the University of Barcelona for their technical assistance. The authors also thank Koosje De Ru, Master student from University Utrecht for her help on the cerebral section drawing.

Author's contribution: Substantial contributions to the conception or design of the work (A.R.-F., J.B., V.F.-D.); or the acquisition (M.F.-G., A.F., E.A., P.O.-L., V.F.-D.), analysis (M.F.-G., P.O.-L., J.B., V.F.-D.), or interpretation of data for the work (all authors). Drafting the work or revising it critically for important intellectual content (all authors). Final approval of the version to be published (all authors). Agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved (all authors).

This work was supported by Fundación Española del Dolor (BF2-19-09); Neuroscience Program, IDIBELL-Bellvitge Institute for Biomedical Research (21VAR007); Plan Nacional Sobre Drogas, Ministerio de Sanidad (2021I068); Ministerio de Ciencia e Innovación, Agencia Estatal de Investigación (RYC-2019-027371-I, PRE2022-103701 and CEX2021-001159-M).

Data availability statement: The data that support the findings of this study are available on request from the corresponding author.

Supplemental digital content

Supplemental digital content associated with this article can be found online at http://links.lww.com/PR9/A259.

Article history:
Received 25 June 2024
Received in revised form 20 August 2024
Accepted 24 August 2024
Available online 9 December 2024

References

[1] Bartley EJ, Fillingim RB. Sex differences in pain: a brief review of clinical and experimental findings. Br J Anaesth 2013;111:52–8.
[2] Blood AJ, Zatorre RJ. Intensely pleasurable responses to music correlate with activity in brain regions implicated in reward and emotion. Proc Natl Acad Sci USA 2001;98:11818–23.
[3] Blum K, Simpatico T, Febo M, Rodriquez C, Dushaj K, Li M, Braverman ER, Demetrovics Z, Oscar-Berman M, Badgaiyan RD. Hypothesizing music intervention enhances brain functional connectivity involving dopaminergic recruitment: common neuro-correlates to abusable drugs. Mol Neurobiol 2017;54:3753–8.
[4] Bradt J, Dileo C, Potvin N. Music for stress and anxiety reduction in coronary heart disease patients. Cochrane Database Syst Rev 2013;2013:CD006577.
[5] Celma-Miralles A, Toro JM. Discrimination of temporal regularity in rats (Rattus norvegicus) and humans (Homo sapiens). J Comp Psychol 2020;134:3–10.
[6] Cepeda MS, Carr DB, Lau J, Alvarez H. Music for pain relief. In: Cepeda MS, editor. Cochrane Database of Systematic Reviews. Chichester: John Wiley & Sons, Ltd, 2006.
[7] Chai PR, Carreiro S, Ranney ML, Karanam K, Ahtisaari M, Edwards R, Schreiber KL, Ben-Ghaly L, Erickson TB, Boyer EW. Music as an adjunct to opioid-based analgesia. J Med Toxicol 2017;13:249–54.
[8] Chanda ML, Levitin DJ. The neurochemistry of music. Trends Cogn Sci 2013;17:179–93.
[9] Cohen SP, Vase L, Hooten WM. Chronic pain: an update on burden, best practices, and new advances. Lancet 2021;397:2082–97.
[10] Craft RM. Modulation of pain by estrogens. PAIN 2007;132:S3–12.
[11] Fernández-Dueñas V, Pol O, García-Nogales P, Hernández L, Planas E, Puig MM. Tolerance to the antinociceptive and antiexudative effects of morphine in a murine model of peripheral inflammation. J Pharmacol Exp Ther 2007;322:360–8.
[12] Ferreri L, Mas-Herrero E, Zatorre RJ, Ripollés P, Gomez-Andres A, Alicart H, Olivé G, Marco-Pallarés J, Antonijoan RM, Valle M, Riba J, Rodriguez-Fornells A. Dopamine modulates the reward experiences elicited by music. Proc Natl Acad Sci USA 2019;116:3793–8.
[13] Fitzgerald E, Arcego DM, Shen MJ, O'Toole N, Wen X, Nagy C, Mostafavi S, Craig K, Silveira PP, Rayan NA, Diorio J, Meaney MJ, Zhang T-Y. Sex and cell-specific gene expression in corticolimbic brain regions associated with psychiatric disorders revealed by bulk and single-nuclei RNA sequencing. EBioMedicine 2023;95:104749.
[14] Flores-García M, Rizzo A, Garçon-Poca MZ, Fernández-Dueñas V, Bonaventura J. Converging circuits between pain and depression: the ventral tegmental area as a therapeutic hub. Front Pharmacol 2023;14:1278023.
[15] Ford CP. The role of D2-autoreceptors in regulating dopamine neuron activity and transmission. Neuroscience 2014;282:13–22.
[16] Fu Q, Qiu R, Chen L, Chen Y, Qi W, Cheng Y. Music prevents stress-induced depression and anxiety-like behavior in mice. Transl Psychiatry 2023;13:317.
[17] Garland EL, Trøstheim M, Eikemo M, Ernst G, Leknes S. Anhedonia in chronic pain and prescription opioid misuse. Psychol Med 2020;50:1977–88.
[18] Gee TA, Weintraub NC, Lu D, Phelps CE, Navratilova E, Heien ML, Porreca F. A pain-induced tonic hypodopaminergic state augments phasic dopamine release in the nucleus accumbens. PAIN 2020;161:2376–84.
[19] Goldberg DS, McGee SJ. Pain as a global public health priority. BMC Public Health 2011;11:770.
[20] Guan SY, Zhang K, Wang XS, Yang L, Feng B, Tian DD, Gao MR, Liu SB, Liu A, Zhao MG. Anxiolytic effects of polydatin through the blockade of neuroinflammation in a chronic pain mouse model. Mol Pain 2020;16:174480691990071.
[21] Gutgsell KJ, Schluchter M, Margevicius S, DeGolia PA, McLaughlin B, Harris M, Mecklenburg J, Wiencek C. Music therapy reduces pain in palliative care patients: a randomized controlled trial. J Pain Symptom Manage 2013;45:822–31.
[22] Heshmati M, Russo SJ. Anhedonia and the brain reward circuitry in depression. Curr Behav Neurosci Rep 2015;2:146–53.
[23] Huhn AS, Berry MS, Dunn KE. Systematic review of sex-based differences in opioid-based effects. Int Rev Psychiatry 2018;30:107–16.
[24] Ito Y, Shiramatsu TI, Ishida N, Oshima K, Magami K, Takahashi H. Spontaneous beat synchronization in rats: neural dynamics and motor entrainment. Sci Adv 2022;8:eabo7019.
[25] Kowalczyk WJ, Evans SM, Bisaga AM, Sullivan MA, Comer SD. Sex differences and hormonal influences on response to cold pressor pain in humans. J Pain 2006;7:151–60.
[26] Kühlmann AYR, de Rooij A, Hunink MGM, De Zeeuw CI, Jeekel J. Music affects rodents: a systematic review of experimental research. Front Behav Neurosci 2018;12:301.
[27] Kühlmann AYR, de Rooij A, Kroese LF, van Dijk M, Hunink MGM, Jeekel J. Meta-analysis evaluating music interventions for anxiety and pain in surgery. Br J Surg 2018;105:773–83.
[28] Kuner R, Kuner T. Cellular circuits in the brain and their modulation in acute and chronic pain. Physiol Rev 2021;101:213–58.
[29] Lee JH. The effects of music on pain: a meta-analysis. J Music Ther 2016;53:430–77.
[30] Li WJ, Yu H, Yang JM, Gao J, Jiang H, Feng M, Zhao YX, Chen ZY. Anxiolytic effect of music exposure on BDNFMet/Met transgenic mice. Brain Res 2010;1347:71–9.
[31] Liang HY, Chen ZJ, Xiao H, Lin YH, Hu YY, Chang L, Wu HY, Wang P, Lu W, Zhu DY, Luo CX. nNOS-expressing neurons in the vmPFC transform pPVT-derived chronic pain signals into anxiety behaviors. Nat Commun 2020;11:2501.
[32] Lunde SJ, Vuust P, Garza-Villarreal EA, Vase L. Music-induced analgesia: how does music relieve pain? PAIN 2019;160:989–93.
[33] Manolagas SC, Kousteni S. Perspective: nonreproductive sites of action of reproductive hormones. Endocrinology 2001;142:2200–4.
[34] Mao X, Cai D, Lou W. Music alleviates pain perception in depression mouse models by promoting the release of glutamate in the hippocampus of mice to act on GRIK5. Nucleosides Nucleotides Nucleic Acids 2022;41:463–73.
[35] Martikainen IK, Nuechterlein EB, Pecina M, Love TM, Cummiford CM, Green CR, Stohler CS, Zubieta J-K. Chronic back pain is associated with alterations in dopamine neurotransmission in the ventral striatum. J Neurosci 2015;35:9957–65.

[36] Mas-Herrero E, Ferreri L, Cardona G, Zatorre RJ, Pla-Juncà F, Antonijoan RM, Riba J, Valle M, Rodriguez-Fornells A. The role of opioid transmission in music-induced pleasure. Ann N Y Acad Sci 2023;1520:105–14.
[37] Mavridis IN. Music and the nucleus accumbens. Surg Radiol Anat 2015; 37:121–5.
[38] Menon V, Levitin DJ. The rewards of music listening: response and physiological connectivity of the mesolimbic system. Neuroimage 2005; 28:175–84.
[39] Metcalf CS, Huntsman M, Garcia G, Kochanski AK, Chikinda M, Watanabe E, Underwood T, Vanegas F, Smith MD, White HS, Bulaj G. Music-enhanced analgesia and antiseizure activities in animal models of pain and epilepsy: toward preclinical studies supporting development of digital therapeutics and their combinations with pharmaceutical drugs. Front Neurol 2019;10:277.
[40] Mogil JS. Sex differences in pain and pain inhibition: multiple explanations of a controversial phenomenon. Nat Rev Neurosci 2012;13:859–66.
[41] Moraes MM, Rabelo PCR, Pinto VA, Pires W, Wanner SP, Szawka RE, Soares DD. Auditory stimulation by exposure to melodic music increases dopamine and serotonin activities in rat forebrain areas linked to reward and motor control. Neurosci Lett 2018;673:73–8.
[42] Moss H, Fitzpatrick K, O'Shea P, Loewy J, Hussey C, Harmon D, Guetin S, Gallagher L, Corcoran J, Clements-Cortes A, Bradt J. An agenda for excellence: the role of music therapy for people living with chronic pain. Music Med 2023;15:925.
[43] Nater UM, Abbruzzese E, Krebs M, Ehlert U. Sex differences in emotional and psychophysiological responses to musical stimuli. Int J Psychophysiol 2006;62:300–8.
[44] Nestler EJ, Carlezon WA. The mesolimbic dopamine reward circuit in depression. Biol Psychiatry 2006;59:1151–9.
[45] Papadakakis A, Sidiropoulou K, Panagis G. Music exposure attenuates anxiety- and depression-like behaviors and increases hippocampal spine density in male rats. Behav Brain Res 2019;372:112023.
[46] Patriarchi T, Cho JR, Merten K, Howe MW, Marley A, Xiong W-H, Folk RW, Broussard GJ, Liang R, Jang MJ, Zhong H, Dombeck D, von Zastrow M, Nimmerjahn A, Gradinaru V, Williams JT, Tian L. Ultrafast neuronal imaging of dopamine dynamics with designed genetically encoded sensors. Science 2018;360:eaat4422.
[47] Patriarchi T, Mohebi A, Sun J, Marley A, Liang R, Dong C, Puhger K, Mizuno GO, Davis CM, Wiltgen B, von Zastrow M, Berke JD, Tian L. An expanded palette of dopamine sensors for multiplex imaging in vivo. Nat Methods 2020;17:1147–55.
[48] Rebecchini L. Music, mental health, and immunity. Brain Behav Immun Health 2021;18:100374.
[49] Ren W, Centeno MV, Wei X, Wickersham I, Martina M, Apkarian AV, Surmeier DJ. Adaptive alterations in the mesoaccumbal network after peripheral nerve injury. PAIN 2021;162:895–906.
[50] Roughan WH, Campos AI, Garcia-Marín LM, Cuéllar-Partida G, Lupton MK, Hickie IB, Medland SE, Wray NR, Byrne EM, Ngo TT, Martin NG, Rentería ME. Comorbid chronic pain and depression: shared risk factors and differential antidepressant effectiveness. Front Psychiatry 2021;12:643609.
[51] Roy M, Peretz I, Rainville P. Emotional valence contributes to music-induced analgesia. PAIN 2008;134:140–7.
[52] Salimpoor VN, Benovoy M, Larcher K, Dagher A, Zatorre RJ. Anatomically distinct dopamine release during anticipation and experience of peak emotion to music. Nat Neurosci 2011;14:257–62.
[53] Salinas AG, Lee JO, Augustin SM, Zhang S, Patriarchi T, Tian L, Morales M, Mateo Y, Lovinger DM. Distinct sub-second dopamine signaling in dorsolateral striatum measured by a genetically-encoded fluorescent sensor. Nat Commun 2023;14:5915.
[54] Schwartz N, Temkin P, Jurado S, Lim BK, Heifets BD, Polepalli JS, Malenka RC. Chronic pain. Decreased motivation during chronic pain requires long-term depression in the nucleus accumbens. Science 2014; 345:535–42.
[55] Scimemi A, Beato M. Determining the neurotransmitter concentration profile at active synapses. Mol Neurobiol 2009;40:289–306.
[56] Shi Y, Wu W. Multimodal non-invasive non-pharmacological therapies for chronic pain: mechanisms and progress. BMC Med 2023;21:372.
[57] Shirayama Y, Chaki S. Neurochemistry of the nucleus accumbens and its relevance to depression and antidepressant action in rodents. Curr Neuropharmacol 2006;4:277–91.
[58] Silm K, Yang J, Marcott PF, Asensio CS, Eriksen J, Guthrie DA, Newman AH, Ford CP, Edwards RH. Synaptic vesicle recycling pathway determines neurotransmitter content and release properties. Neuron 2019;102:786–800.e5.
[59] Sun F, Zhou J, Dai B, Qian T, Zeng J, Li X, Zhuo Y, Zhang Y, Wang Y, Qian C, Tan K, Feng J, Dong H, Lin D, Cui G, Li Y. Next-generation GRAB sensors for monitoring dopaminergic activity in vivo. Nat Methods 2020; 17:1156–66.
[60] Tanasescu R, Cottam WJ, Condon L, Tench CR, Auer DP. Functional reorganisation in chronic pain and neural correlates of pain sensitisation: a coordinate based meta-analysis of 266 cutaneous pain fMRI studies. Neurosci Biobehav Rev 2016;68:120–33.
[61] Tavakoli F, Hoseini SE, Mokhtari M, Vahdati A, Razmi N, Vessal M. Role of music in morphine rewarding effects in mice using conditioned place preference method. Neuro Endocrinol Lett 2012;33:709–12.
[62] Walf AA, Frye CA. The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. Nat Protoc 2007;2:322–8.
[63] Wood PB, Schweinhardt P, Jaeger E, Dagher A, Hakyemez H, Rabiner EA, Bushnell MC, Chizh BA. Fibromyalgia patients show an abnormal dopamine response to pain. Eur J Neurosci 2007;25: 3576–82.
[64] Xie JY, Qu C, Patwardhan A, Ossipov MH, Navratilova E, Becerra L, Borsook D, Porreca F. Activation of mesocorticolimbic reward circuits for assessment of relief of ongoing pain: a potential biomarker of efficacy. PAIN 2014;155:1659–66.
[65] Xing Y, Xia Y, Kendrick K, Liu X, Wang M, Wu D, Yang H, Jing W, Guo D, Yao D. Mozart, Mozart rhythm and retrograde Mozart effects: evidences from behaviours and neurobiology bases. Sci Rep 2016;6:18744.
[66] Xu L, Nan J, Lan Y. The nucleus accumbens: a common target in the comorbidity of depression and addiction. Front Neural Circuits 2020;14: 37.
[67] Zatorre RJ, Salimpoor VN. From perception to pleasure: music and its neural substrates. Proc Natl Acad Sci USA 2013;110:10430–7.
[68] Zhou W, Ye C, Wang H, Mao Y, Zhang W, Liu A, Yang C-L, Li T, Hayashi L, Zhao W, Chen L, Liu Y, Tao W, Zhang Z. Sound induces analgesia through corticothalamic circuits. Science 2022;377:198–204.
[69] Clark J. Derrell, Gebhart Gerald F., Gonder Janet C., Keeling Michale E., Kohn Dennis F.. Special Report: The 1996 Guide for the Care and Use of Laboratory Animals.. ILAR J. 1997;38(1):41–48. doi:10.1093/ilar.38.1.41. 11528046.