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## Original Article

# The effects of music and darkness on radionuclide distribution during mice FDG-PET scan

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## Abstract

**Background:** There is growing interest in the therapeutic potential of music or light in different human disorders. **Aims:** This study aimed to evaluate the effects of music as well as darkness on FDG uptake in 4T1 tumor-bearing BALB/c mice using a PET scan. **Methods:** The music, darkness, and music plus darkness groups were subjected to either song or darkness and their combination, respectively, 30 min before the radiopharmaceutical injection until the end of the experiments. The control group was imaged in silence under ambient conditions. **Results:** Our results revealed that music did not significantly alter the range of tumor SUVmean, but showed a slight increase in brain SUVmean (18.2%) and about 100% increase in brain percentage of injected dose per gram (%ID/g) in *ex vivo* analysis. In contrast, heart SUVmean and heart %ID/g were approximately half those of the silence group. The muscle SUVmean and blood activity measurements showed a decrement upon music exposure. Also, results showed a significant difference in tumor-to-muscle ratio (85% increment) and brain-to-muscle ratio (105% increment) between the silence and music groups. The muscle SUVmean decreased by 50%, and tumor-to-muscle and brain-to-muscle ratios were observed to increase by 44% and 60% in the group exposed to darkness, respectively. **Conclusion:** Our results suggest that music and environmental factors may influence FDG uptake in small-animal PET imaging, and provide important insights into the reliability of FDG-PET imaging for music intervention research and may aid researchers in investigating the effects of music on brain changes and tissue metabolism.

**Key words:** Light, Music intervention, Preclinical imaging, Small-animal PET, Tumor

## Introduction

There is growing interest in the use of music or illumination for therapeutic purposes. It has been shown that music has significant effects on different biological systems (Abbott, 2002; Zatorre, 2005). Particularly, it alters cardiopulmonary (Allen *et al.*, 2001; Sutoo and Akiyama, 2004) and immune (Uchiyama *et al.*, 2012; Gao *et al.*, 2016) functions. Also, it influences the process of neuroplasticity (Kirste *et al.*, 2015) as well as brain function in an array of neuropsychiatric models including anxiety (Escribano *et al.*, 2014), depression (Rauscher *et al.*, 1994; Rauscher *et al.*, 1998) learning and cognitive function (Johnson *et al.*, 1998; Foster and Valentine, 2001), seizure (Turner, 2004), and movement disorders (Pachetti *et al.*, 2000). Likewise, short-term music has also been shown to provide benefits in spatial tasks (Xing *et al.*, 2016). In this regard, classical music,

especially Mozart K.448, is the most common type of music which is used in different experimental studies (Kühlmann *et al.*, 2018).

In the same manner, light deficiency as well as over-exposure to light have been linked to different pathologies. Light undoubtedly influences the human circadian rhythm, and deviations from normal illumination are linked with an array of disorders (Wehr, 1998; Schernhammer and Schulmeister, 2004; Skene and Arendt, 2006). Furthermore, controlled light exposure is found to be useful in endocrine and mood disorders (Dallaspezia *et al.*, 2015; Schwartz and Olds, 2015; Fernandez, 2019; Jermann *et al.*, 2020). In this regard, some of the effects of the circadian hormone, melatonin, are attributed to a change in light exposure (Blasko, 2009; Engwall *et al.*, 2014; Rocha *et al.*, 2015; Shen *et al.*, 2022), beyond its receptor pharmacology.

Such outcomes are thought to be mediated not only

via the relaxing effects of music or darkness but also via the alteration of physiological parameters in different organs (Emmer *et al.*, 2018; Kühlmann *et al.*, 2018). In the meantime, the exact mechanisms remain to be elucidated using new assessment methods and technologies. This is critical when it comes to the potential interference of music and darkness with therapeutic or diagnostic processes.

Positron emission tomography (PET) is a highly sensitive noninvasive functional imaging modality suited for the monitoring of pathophysiological processes in the course of diseases *in vivo* (Krause *et al.*, 2013; Adler *et al.*, 2022). PET with fluorodeoxyglucose (FDG), the most common glucose metabolic probe, is one of the common diagnostic tools for the clinical management of cancer (Agrawal and Rangarajan, 2015), brain disorders such as dementia, Parkinson's disease, Huntington disease, and seizure localization (Noble, 2021), infection and inflammation (Casali *et al.*, 2021; Ten Hove *et al.*, 2021).

In preclinical research, the use of various imaging techniques can provide valuable insights into the metabolic effects of music interventions and other stimuli (Pashazadeh *et al.*, 2015; Tanha *et al.*, 2017; Abbaspour *et al.*, 2018).

FDG-PET imaging allows for the visualization of glucose metabolism, which is a key indicator of tissue function and health. By monitoring changes in FDG uptake in response to music interventions, researchers can gain a better understanding of how music impacts various tissues in the body, including the brain.

However, before using FDG-PET imaging to evaluate the effects of music interventions on laboratory animals, it is important to establish baseline data on FDG distribution in the animal body. This information is critical for accurately interpreting any changes in FDG uptake that may be observed during music interventions.

In this study, we aimed to investigate the effects of music and darkness on radionuclide distribution in tumor-bearing mice during FDG-PET scans. Our study will provide important insights into the effects of music on FDG uptake in the body and the reliability of FDG-PET imaging as an imaging method for music intervention research. The findings of our study will be crucial for researchers interested in investigating the effects of music on brain changes and tissue metabolism in response to music interventions using FDG-PET imaging.

## Materials and Methods

### Ethics statement

All experiments were carried out according to the local guidelines for the Care and Use of Animals with IR.TUMS.MEDICINE.REC.1399.1101 license code.

### Animals

Breast cancer xenografts, 4T1 cells, were implanted subcutaneously in female BALB/c mice, provided by the Cancer Institute of Tehran University of Medical

Sciences, between 7 and 8 weeks of age. On the 11th day of tumor implantation, the mice were transferred to the animal house at our department, and maintained on a 12 h light/12 h dark rhythm with lights on at 7 a.m. in a controlled ambient temperature ( $22 \pm 2^\circ\text{C}$ ) and free access to water and food for three days. Then, they were grouped and subjected to light/music interventions and PET scan. All experiments were carried out according to the local guidelines for the Care and Use of Animals with IR.TUMS.MEDICINE.REC.1399.1101 license code.

Tumor-bearing animals were randomly assigned to four groups (4 mice in each group):

- 1) The silent-light (control) group was kept in silence and ambient light
- 2) The music-light group was subjected to Mozart's piano sonata, K. 448 and ambient light
- 3) The silence-dark group was maintained in silence and a dark room
- 4) Music-dark group were subjected to Mozart's piano sonata, K. 448 in a dark room

Mozart's piano sonata, K.448, approximately 24 min in duration, was played repeatedly by a player, with sound levels of about 70 dB in the cages. Music and/or light interventions began 30 min before radiopharmaceutical injection and continued during anesthesia and imaging for a total of 90 min.

### FDG-PET scan and image analysis

Six h before FDG injection, access to food was ended but the animals had free access to water. The mice were anesthetized with 1.5% isoflurane evaporated in oxygen at a flow rate of 0.5 L/min on a warm table, and ~12 MBq FDG (200  $\mu\text{L}$ ) was injected via the tail vein. The activity of the syringes was measured before and after the injection for the calculation of injected activity. After recovery from anesthesia, the animals were subjected to light/music interventions. Imaging was conducted 60 min post-injection under isoflurane anesthesia for 4 min. PET scans were performed on Xtrim small-animal PET scanner (Parto Negar Persia Co., Iran) using XtrimVision image acquisition software. Reconstruction was performed in XtrimPro software with OSEM with five iterations and four subsets. Attenuation corrections were applied, and the reconstructed voxel size was  $0.78 \times 0.78 \times 1.07 \text{ mm}^3$ .

Regions of interest (ROIs) were drawn manually around the tissues. Tumor-to-brain ratio (TBR), mean standardized uptake values (SUVmean), and tissue-to-muscle ratios of all analyzed tissues including tumor-to-muscle ratio (TMR), brain-to-muscle ratio (BMR), and heart-to-muscle ratio (HMR) were determined. Tissue-to-muscle ratios were calculated as the ratio of the organ's count rate per pixel (CRPP) divided by the left leg muscle's CRPP for tumor, brain, and heart.

### Biodistribution studies

The animals were euthanized immediately after the PET scan by overexposure to isoflurane (Underwood and Anthony, 2020), and the tumor, brain, heart, liver,

kidneys, and left leg muscle were dissected, weighed, and measured in a dose calibrator (CURIEMENTOR 4, PTW, Freiburg). Subsequently, the %ID/g of each sample was calculated.

### Statistical analysis

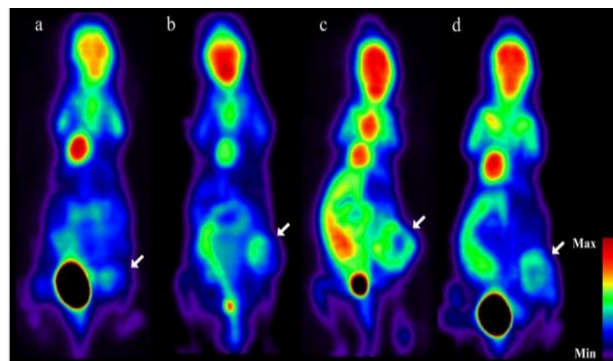
Differences in quantitative parameters between any two groups were analyzed using the Mann-Whitney U-test, with a statistical significance level of  $P < 0.05$ .

## Results

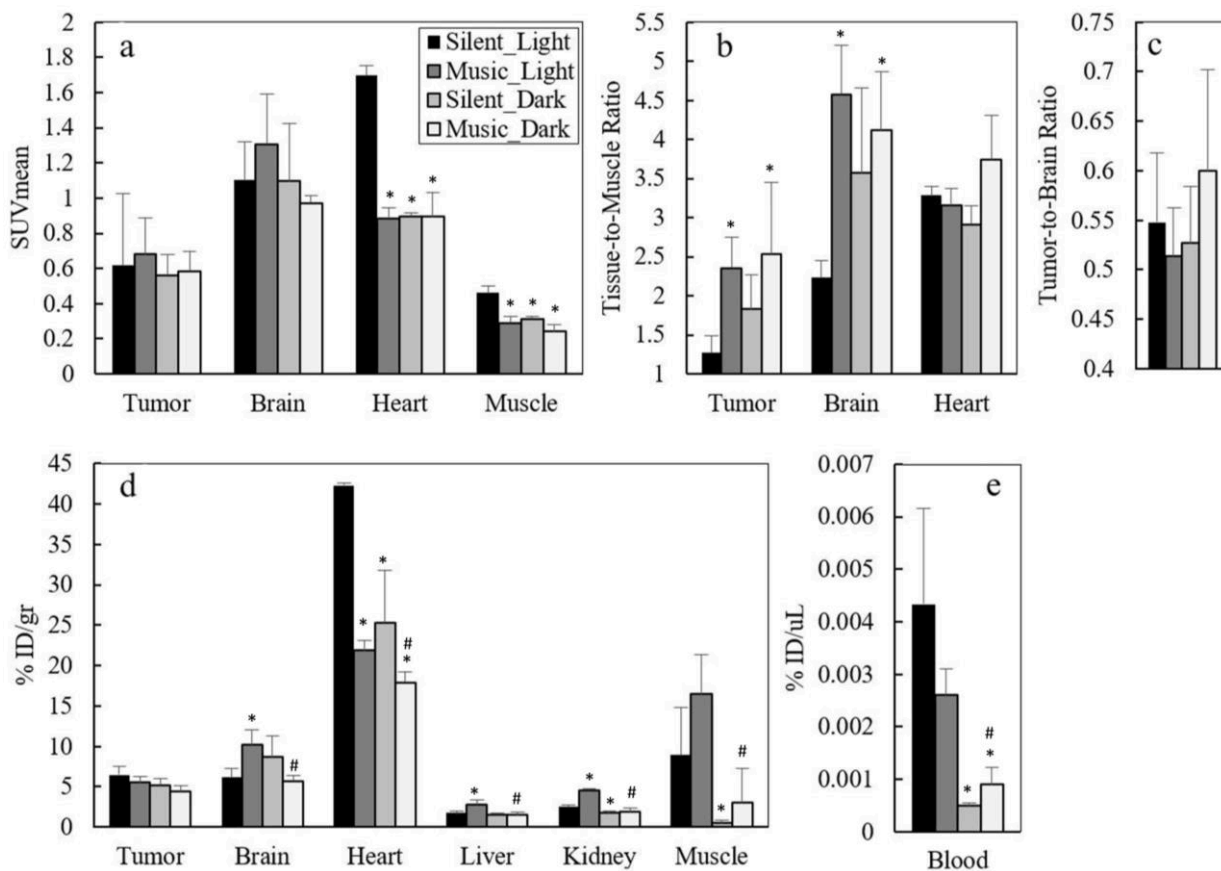
### The effects of music on tumor and organ uptake of FDG

Figures 1a-d shows the representative images of mice in each group. To avoid suppressing the activities of other organs in the FDG-PET images of each mouse, the bladder activity was deliberately removed from the images. This allowed us to more accurately observe and analyze the metabolic activities of the other organs in the mouse. Figures 1a and b demonstrate that music intervention did not cause significant changes in tumor uptake. Additionally, the quantitative results of the image analysis depicted in Figs. 2a-e show that music did not

alter the range of tumor SUVmean. The tumor SUVmean ranged from 0.49 to 0.85 in the silent-light group, and from 0.51 to 0.95 in the music-light group. Similarly, as can be seen in Fig. 2d, *ex vivo* bio-distribution analysis did not reveal a significant difference of tumor %ID/g between the two groups.



**Fig. 1:** Maximum intensity projection (MIP) views of FDG-PET scans of four different female BALB/C mice with 4T1 tumors in the right flanks of animals (white arrows). The animals are selected from silent-light (a), music-light (b), silent-dark (c), and music-dark (d) groups. The images represent acquired data 60 min after FDG injection



**Fig. 2:** Quantitative analysis of the FDG-PET images. Calculated SUVmean (a), tissue-to-muscle ratio (b), tumor-to-brain ratio (TBR) (c), measured %ID/g for tumor, brain, heart, liver, kidney and muscle (d), and blood %ID/uL (e) for scanned animals in silent-light, music-light, silent-dark, and music-dark groups. Mean values and standard deviations are shown for each group. All groups were compared with the silent-light group as the control (\*  $P < 0.05$ ). Additionally, we compared the music-light and music-dark groups (#  $P < 0.05$ )

However, listening to music significantly decreased the activity of the heart and increased activity in the brain. Music slightly increased brain SUVmean (18.2%) compared with the silent group. Accordingly, in music-exposed animals, brain %ID/g in *ex vivo* analysis ( $10.16 \pm 1.83$  vs.  $6.13 \pm 1.11$ ) and muscle %ID/g in *ex vivo* experiments ( $16.51 \pm 4.83$  vs.  $8.9 \pm 5.87$ ) were almost double that of the silent-light group. In contrast, heart SUVmean ( $0.88 \pm 0.06$ ), and heart %ID/g in *ex vivo* experiments ( $21.88 \pm 1.21$ ) in the music-light group were approximately half those of the silent-light group ( $1.7 \pm 0.05$  SUVmean, and  $42.32 \pm 0.27$  %ID/g). Also, blood activity measurements showed a 39.5% decrement in the music-light group. A similar declining pattern in muscle SUVmean ( $0.46 \pm 0.04$  vs.  $0.29 \pm 0.03$ ) was observed upon music exposure.

The PET scan revealed a significant difference in TMR (85% increment) and BMR (105% increment) between the silent-light and music-light groups. TBR in the silent-light group ( $0.54 \pm 0.07$ ) was almost similar to that of the music-light group ( $0.51 \pm 0.05$ ) in the PET scan.

### The effects of darkness on tumor and organ uptake of FDG

Animals maintained in silence and darkness displayed a marginal decrease in tumor SUVmean ( $0.56 \pm 0.11$  vs.  $0.61 \pm 0.4$ ) compared with the control group. However, *ex vivo* experiments showed no difference between these groups in terms of %ID/g.

SUVmean in the brain did not significantly differ between the silent-dark and silent-light groups, but heart and muscle SUVmean in the silent-light group were twice as more as those in the silent-dark group. There was no significant difference in TBR ( $0.527 \pm 0.058$  vs.  $0.548 \pm 0.07$ ), while TMR ( $1.83 \pm 0.43$  vs.  $1.27 \pm 0.22$ ) and BMR ( $3.57 \pm 1.06$  vs.  $2.23 \pm 0.22$ ) were found to be 44% and 60% higher in animals kept in darkness compared with those kept in ambient light, respectively. Additionally, the blood activity was found to be 88% lower in terms of %ID/g in animals kept in darkness.

### The combined effects of music and darkness on tumor and organ uptake of FDG

The calculated tumor SUVmean showed marginal but not significant variations in silent-light ( $0.61 \pm 0.4$ ), silent-dark ( $0.56 \pm 0.11$ ), music-light ( $0.68 \pm 0.2$ ), and music-dark ( $0.58 \pm 0.1$ ) groups. Similarly, the *ex vivo* bio-distribution showed no significant difference between the tumor uptake of radionuclide in silent-light ( $6.5 \pm 1.09$ ), silent-dark ( $5.24 \pm 0.75$ ) music-light ( $5.55 \pm 0.76$ ), and music-dark ( $4.44 \pm 0.69$ ), groups in terms of %ID/g. The highest TMR was found in the music-dark group (ranging between 1.7 and 4.06) and the lowest value was found in the silent-light group (ranging from 0.72 to 2.21). The results showed that TMR in the music-dark group ( $2.53 \pm 0.91$ ) was significantly higher (38% increase) compared with the silent-dark group ( $1.83 \pm 0.43$ ). Additionally, TMR in the music-dark group was nearly twice as high as that in the silent-light group ( $1.27$

$\pm 0.22$ ). Moreover, TMR in the music-light group ( $2.35 \pm 0.4$ ) was higher (28% increase) than that in the silent-dark group.

A marginal difference was observed between the brain SUVmean of music-light ( $1.3 \pm 0.28$ ) and music-dark ( $0.97 \pm 0.04$ ) groups, which was masked by the high variance of this value in silent-light ( $1.1 \pm 0.22$ ) and silent-dark ( $1.09 \pm 0.32$ ) groups. *Ex vivo* bio-distribution revealed a significant difference between the music-light ( $10.16 \pm 1.83$  %ID/g) and music-dark ( $5.74 \pm 0.67$  %ID/g) groups in the brain, while this factor for silent-light ( $6.13 \pm 1.11$  %ID/g) and silent-dark ( $8.68 \pm 2.59$  %ID/g) groups fall between the two former groups. Calculated BMR in the music-light group ( $4.57 \pm 0.63$ ) was higher than those of the music-dark ( $4.11 \pm 0.75$ ), silent-light ( $2.23 \pm 0.22$ ), and silent-dark ( $3.56 \pm 1.08$ ) groups. Albeit, TBR was almost similar in silent-light ( $0.54 \pm 0.07$ ), silent-dark ( $0.52 \pm 0.05$ ), music-light ( $0.51 \pm 0.05$ ), and music-dark ( $0.6 \pm 0.1$ ) groups.

Heart and muscle SUVmean in the silent-light group was higher compared with other groups. *Ex vivo* bio-distribution revealed higher radionuclide uptake in the heart of silent-light ( $42.32 \pm 0.27$ ) compared with silent-dark ( $25.35 \pm 6.45$ ), music-light ( $21.88 \pm 1.21$ ), and music-dark ( $17.92 \pm 1.36$ ) groups. The muscle uptake in the music-light group ( $16.51 \pm 4.83$ ) was much higher than in music-dark ( $3.03 \pm 4.28$ ) and silent-dark ( $0.47 \pm 0.36$ ), and silent-light ( $8.91 \pm 5.87$ ) groups.

Finally, TMR in the music-dark group was twice as high as that in the silent-light group. Additionally, the music-dark group showed an 84% higher BMR compared with the silent-light group. The music-light group exhibited higher measured activity in the liver ( $2.84 \pm 0.59$ ) and kidneys ( $4.60 \pm 0.19$ ) compared with other groups in terms of %ID/g of tissue. Blood activity in the silent-light group ( $0.0043 \pm 0.0018$  %ID/g) was higher than in the other groups.

## Discussion

We evaluated the effects of music intervention on FDG uptake in 4T1 tumor-bearing BALB/c mice. To the best of our knowledge, this is the first report that uses a small animal 18F-FDG PET imaging and quantitative analysis to show changes in glucose metabolism in mice undergoing music exposure. Our experiments show that the tumor FDG uptake in xenograft mice remained stable after music intervention. No major alterations were observed in the *ex vivo* measurements. However, the average SUVmean in the tumor was higher in the music group. In contrast, the uptake in the brain tissue had a significant increase in the music exposure group. Exposure to music in ambient light conditions was observed to have a substantial impact on TMR and BMR, with an increase of 85% and 105%, respectively, observed in the music-exposed group compared with the control group. Our findings are consistent with previous research that has demonstrated the potential role of music in enhancing brain metabolism, as reflected by the approximately twofold increase in BMR observed in the

music-exposed group. Furthermore, our results support the notion that listening to music can activate a broad network of brain areas, as previously shown in other studies (Hughes and James, 2001).

For the heart and muscle, the PET analysis showed a significant reduction of SUV<sub>mean</sub> in the music group. The *ex vivo* biodistribution study confirmed these results for the heart but muscle uptake was higher in the music group in terms of %ID/g. These findings are consistent with previous research demonstrating the blood pressure-lowering effect of music and the potential role of music in counteracting the adverse effects of stress. The blood pressure-lowering effect of music may be attributed to the attenuation of autonomic function through stress reduction. In line with this, previous studies have demonstrated that music interventions were associated with reduced corticosterone levels, an important biomarker for stress, in several animal models (Chikahisa *et al.*, 2006; Tasset *et al.*, 2012). Averaged blood activity in the music group was lower than in the silent group. However, we observed high variations in %ID/ $\mu$ L in the silent group. Additionally, the kidney and liver showed a significant increase (%46 and %39, respectively) of %ID/g. This finding was somewhat unexpected, and further experiments are required to understand this effect.

Furthermore, our results showed that exposure to darkness had a significant impact on TMR and BMR in silent groups. Specifically, TMR was observed to increase by 44% in the group exposed to darkness, while BMR showed a substantial increase of 60% compared with the control group. Refinetti *et al.* (2006) observed that rodents are more active in darkness, as their nocturnal lifestyle is associated with increased activity levels during this period. In the darkness, rodents engage in various daily activities such as environmental recognition, foraging, feeding, and alertness, which correspond to increases in brain activity in specific brain regions, including the nucleus accumbens, hippocampus, and midbrain (Ohara and Hayton, 1996; Mele *et al.*, 2004; Ito and Feldheim, 2018; Sosa *et al.*, 2018). Our findings are consistent with these observations, as we demonstrated that exposure to darkness had a significant impact on brain metabolism as measured by BMR, which showed a substantial increase in the silent-dark group compared with the silent-light group. This increase in BMR is likely due to the higher brain activity induced by darkness, which in turn leads to increased brain glucose metabolism, as measured by [18F] FDG brain uptake. In addition, the analysis revealed a significant decrease in blood activity, with a reduction of 80% observed in terms of %ID/ $\mu$ L.

Also, we compared the result of music intervention on FDG uptake in animals that were kept in darkness during all steps of PET imaging with animals in a dark and silent room. The results showed that in animals in a dark room, music decreased FDG uptake in brain and heart tissues, while muscle and blood activity concentration increased by music intervention. In contrast, no significant changes were seen in the tumor,

liver, and kidneys of the animals. In the darkness, TMR was found to increase by 38% in the music-exposed group, while BMR showed only a 15% increase compared with the control group.

Our results showed that FDG uptake in tissues of animals exposed to music in a dark room was different from the results of music intervention under ambient light. The average SUV<sub>mean</sub> value in the brain of animals exposed to music in ambient light was higher (with a high SD value) than that of animals exposed to music in darkness which was confirmed by *ex vivo* measurements. This suggests that the effects of music exposure on brain metabolism may be modulated by environmental factors such as light. *Ex vivo* measurements showed a significant reduction of FDG uptake in the heart in darkness, but no significant differences were observed in the heart's SUV<sub>mean</sub> between music-light and music-dark groups. We also observed significant FDG uptake reduction in muscle, liver, kidney, and blood caused by music intervention in darkness in comparison with music-exposed animals in ambient light. These changes in tissue activities show the effect of darkness during the music intervention that has significant effects on FDG uptake in the animal body.

Our study has several limitations. Firstly, the short duration of music exposure may have limited the ability to investigate the long-term effects of music on metabolism. Additionally, the study only used one type of music genre, which may not reflect the effects of other music genres on metabolism. Secondly, the sample size was relatively small, although the number of animals in each group was adequate to detect significant changes in FDG uptake.

In light of recent advancements, including high temporal resolution fPET studies (Hahn *et al.*, 2024), future research could further refine our understanding of the dynamic changes in brain glucose metabolism through improved quantitative methods (Jafarian-Dehkordi *et al.*, 2023; Pashazadeh *et al.*, 2023). Although this study did not employ such methods, incorporating them in future investigations may enhance the precision and depth of metabolic assessments, offering an expanded view of how environmental stimuli, like music, influence brain function.

The results of this study showed that exposure to music or darkness for 90 min before radionuclide injection until the end of the imaging procedure significantly increased the relative FDG uptake parameters in the tumor and brain while decreasing concentrated activity in heart tissue and blood. Our results suggest that the effects of music intervention may vary depending on the ambient light conditions due to the influence of darkness on FDG uptake in tissues. Moreover, the combination of music and darkness resulted in a significant increase in the relative uptake of FDG in the tumor and brain tissues of the animals. These findings provide new insights into the potential effects of music and environmental factors on FDG uptake parameters in small-animal PET imaging and provide important insights into the reliability of FDG-PET

imaging as an imaging method for music intervention research. This may aid researchers investigating the effects of music on brain changes and tissue metabolism.

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## Conflict of interests

The authors have no relevant financial or non-financial interests to disclose.

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