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Research report

Global view of the mechanisms of improved learning and memory capability in mice with music-exposure by microarray

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ABSTRACT

Music has been proved beneficial to improve learning and memory in many species including human in previous research work. Although some genes have been identified to contribute to the mechanisms, it is believed that the effect of music is manifold, behind which must concern a complex regulation network. To further understand the mechanisms, we exposed the mice to classical music for one month. The subsequent behavioral experiments showed improvement of spatial learning capability and elevation of fear-motivated memory in the mice with music-exposure as compared to the naïve mice. Meanwhile, we applied the microarray to compare the gene expression profiles of the hippocampus and cortex between the mice with music-exposure and the naïve mice. The results showed approximately 454 genes in cortex (200 genes up-regulated and 254 genes down-regulated) and 437 genes in hippocampus (256 genes up-regulated and 181 genes down-regulated) were significantly affected in music-exposing mice, which mainly involved in ion channel activity and/or synaptic transmission, cytoskeleton, development, transcription, hormone activity. Our work may provide some hints for better understanding the effects of music on learning and memory.

1. Introduction

Music is the universal language of mankind. Pioneering studies have demonstrated that effects of early music training are beneficial to the spatial memory and neurogenesis in the hippocampus of rodents from fetus to adult [13,21]. Meanwhile, it has been certified that music therapy has certain effects on neuropsychiatric disorders, such as dementia [30], depression [8], schizophrenia [37], and so on. The interaction of the gene expression products with the environment must have an influence on the development and function of the nervous system. However, the molecular mechanisms underlying memory improvement induced by music-exposure remain poorly understood.

It is well known that cDNA microarray has been a powerful tool for parallel monitoring the relative expressions of thousands of transcript levels. The application of microarray technology to study the brain function has been used widely in neuroscience [24]. One advantage is to discover the transcriptional network in the regional complexity of the brain [15] and to search new candidate genes for brain functions such as learning and memory progress [22], another superiority is to evaluate molecular events associated with neurological disorders [14,34].

Previous study had reported that the expression levels and/or activity of some genes were regulated after music-exposure, such as BDNF, NGF [1], TrkB [7], NR2B [40], and GluR2 [39]. While it is believed that the effects of music on learning and memory are manifold with very complex mechanisms, so we employed microarray to achieve a global view of the gene expression profile in the mice with music-exposure, and some biological relevant pathways revealed in our study may provide some tips for later fundamental or clinical application of music.

2. Materials and methods

2.1. Animals and music-exposure

Forty male C57BL/6J (B6) mice with 28 days of age were purchased from Shanghai SLAC Laboratory Animal Co. Ltd. These mice were randomly divided into two groups: music group and naïve group. The animals were housed under standard conditions of humidity, room temperature and 12 h light/dark cycles and had free access to water and food. All experiments in this study were approved by the Animal Ethics Committee in ECNU, China.

Music mice ($n = 20$) were exposed daily to Mozart's piano sonata, K. 448, which was usually used in studies on the "Mozart effect" [20,31] (8 h per day, from 22:00 p.m. to next 6:00 a.m.; between 50 and 60 dB) for 30 consecutive days. Control mice ($n = 20$) were placed in a similar room without music (ambient noise; ~50 dB).

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On day 31, 5 mice from each group were decapitated, the brains removed and the forebrain cortexes and hippocampuses were dissected on the ice immediately. Then the tissues were put into liquid nitrogen as soon as possible for further RNA preparation. The rest mice ($n = 15$ for each group) were used for further behavior analysis.

2.2. Open field

A 15-min open field test was performed in an open field apparatus (40.5 cm × 40.5 cm, 30-cm high wall) with TruScan 2.0 system (Coulbourn Instruments) to measure the basic movement capability of the two groups of mice. The data of move velocity (cm/s), distance (cm) and center time (s) were recorded automatically by the apparatus and analyzed with the TruScan software. Center time was defined as the total time spent in the arena-center, a region that is more than 2.5-beam spaces away from the arena walls. Data were presented as mean ± S.E.M., and the difference between the two groups was determined by Student's *t*-test. $P < 0.05$ was considered as statistical significance.

2.3. Morris water maze

The hidden platform water maze task [27] was used to investigate the spatial learning of the two groups mice. The apparatus consisted of a cylindrical tub (200 cm diameter; 40 cm depth) that was filled with water (26 ± 1 °C temperature). The water was rendered opaque by the addition of white, non-toxic titanium dioxide (Jianghu Titanium White Product Co. Ltd., Shanghai). Before the task, both groups were handled for three days, 5 min per day, and then trained with a seven-session training protocol, in which each session contained four trials. During each trial, the escape latency to find the hidden platform was recorded, with the max permitted exploring time of 60 s. The mice were allowed staying on the platform for 15 s after they found the platform.

On the test days, the platform was removed, and the mice were permitted to explore in the pool for 60 s, and spatial specificity was measured by duration of time spent in the target quadrant (% of total time, chance level = 25%). All the behavioral variables were quantified and analyzed by the software (water maze, ACTIMETRICS software). Data were presented as mean ± S.E.M., the difference between trials for day1–7 was determined by two-way ANOVA and the difference between the two groups was determined by Student's *t*-test. $P < 0.05$ was considered as statistical significance, and $P < 0.01$ was considered as highly statistical significance.

2.4. One-trial passive avoidance task

The passive avoidance task was performed with Avoidancemonitor Station (Hamilton-Kinder, LLC), as described in the website of Neurogenetics and Behavior Center (http://nbc.jhu.edu/protocols/PassiveAvoidanceProtocol.aspx). In brief, on habituation day, mouse was placed in the light compartment, allowed to explore for 30 s. After 30 s the door was raised and the mouse was allowed to explore freely. When the mouse entered the dark compartment with all four paws, the guillotine door was closed, and the latency to enter was recorded. Once the mouse crossed to the dark chamber the door closed and the mouse was immediately removed and returned to the home cage.

On training day mouse was placed in the light compartment, and allowed to explore for 10 s. After 10 s the guillotine door was lifted. When mouse entered the dark compartment with all four paws, the guillotine door was closed, and the latency to enter was recorded (from the time the door was lifted). 3 s after the door was closed a footshock (0.65 mA, 2 s duration) was delivered. 30 s after the footshock the mouse was removed to its home cage.

On test day (24 h after training), the mouse was returned to the light compartment. After 10 s, the guillotine door was lifted. When the mouse entered the dark compartment with all four paws, the guillotine door was closed, and the latency to enter the dark compartment was recorded (from the time the door was lifted). The mouse was then removed and returned to the home cage.

Data were presented as mean ± S.E.M., and the difference between the two groups was determined by Wilcoxon Signed Rank Tests. $P < 0.01$ was considered as highly statistical significance.

2.5. DNA microarray analysis and quantitative PCR

To eliminate the individual difference among the mice in one group, five forebrain cortex and/or hippocampus tissues from the same group were mixed for RNA isolation, named MC (music forebrain cortex), MH (music hippocampus), NC (naïve forebrain cortex), and NH (naïve hippocampus), respectively. Total RNA was isolated using Trizol (Gibco), and then purified with RNeasy Kit (Qiagen). The final amount of isolated RNA was determined and the quality was assessed in each sample by Agilent 2100 Bioanalyzer.

Microarray experiment was performed following manufacturer's protocols by Shanghai Biochip Co., Ltd. Briefly, cDNA were synthesized from 2 μg RNA samples with the M-MLV kit (Invitrogen) and a primer containing poly (dT) and a T7 RNA polymerase promoter sequence, in vitro transcription using double-stranded cDNA as a template in the presence of aaUTP (Ambion, AM8436) was carried out using Low RNA Input Linear Amplification kit (Agilent, 5184-3523). The Cy3-labled cRNA was purified and hybridized to an Agilent mice 4*44K chip. The Agilent G2565BA Microarray Scanner was used to scan the chip with the scan resolution of 5 μm, PMT 100% and 10%. The data were normalized with Feature Extraction software and gene with expression fold changes ratio of ≥2 were subtracted for further functional classification analysis based on the MAS 2.0 System (Capitalbio., http://bioinfo.capitalbio.com/mas/).

Real-time quantitative PCR reaction was carried out using the DNA Engine Opticon 3 System (MJ Inc.). The PCR reactions were then performed in a total volume of 20 μl with 1 μl of cDNA, 0.2 mM dNTP, 0.4 μM of both upstream and downstream primers, 2 μl of the 10× polymerase reaction buffer, 0.125 μl of ex*Taq* polymerase (Takara), and 0.2 μl of Eva Green™ (Biotium, Inc.). The primers were synthesized by Invitrogen Co., Ltd., and the sequences were listed in Table 1. PCR was performed at 94 °C for 5 min, and then repeated 35 cycles at 94 °C for 30 s, annealing (the annealing temperatures for individual gene were indicated in Table 1) for 30 s, 72 °C for 45 s. GAPDH was used as the control. Each reaction was performed in triplicate. Gene expression level was calculated as $2^{-\Delta\Delta Ct}$ values. Data were presented as mean ± S.E.M. Statistical significance was determined by Student's *t*-test. $P < 0.05$ was considered as statistical significance.

3. Results

3.1. No change of open field activity after music-exposure

The open field task was usually used to measure the general activity level, gross locomotor activity and exploration habit in mice. As showed in Fig. 1, the mice in music group showed no statistical significance as compared to those in naïve group in velocity and distance in a 15 min open field task. The center time, usually reflects the anxiety of the animals, also appeared no difference.

3.2. Elevated spatial learning capability after music-exposure

In the training procedure of Morris water maze task, although both music group and naïve group showed a decrease in escape latency to find the hidden platform, music group exhibited a much faster learning curve than the naïve mice (Fig. 2A) especially on the second day of training as compared to the naïve mice (Student's *t*-test, $P < 0.01$). Although after a seven days training, both group mice were considered to have learned the task, the music mice took only an average 11 s to find the platform, whereas naïve mice took approximately 16 s. On the first probe test, which was performed one day after the training, both two groups showed longer time spent in the target quadrant than in other quadrants, but there was no statistical difference between the two groups (Fig. 2B). It is not surprising since both groups had successfully learned the task. While three days after the first test, both groups were tested again. Compared to the naïve mice, which showed an extinction of memory in the target quadrant, the music mice still spent longer time in the target quadrant, although much lower than that in the first test (Fig. 2C). The results of Morris water maze task showed that the learning capability of the mice after music-exposure was elevated and the memory could last longer when compared to the naïve mice.

3.3. Increased fear-motivated memory after music-exposure

The passive avoidance performance was an adaptive response to a stressful experience used to assess an ability of learning and memory. On the habituation and training day, the mice showed the instinctive trend of avoidance to the light part, and there were no statistical significance on the escape latency between the music mice and the naïve mice. While after the foot shock on the training day, the mice learned to remain in the light part, avoiding the shock in the dark part. The music mice showed longer escape latency than the naïve mice (Fig. 3), which suggested that besides the spatial learning, the fear-motivated memory was also increased in the mice with music-exposure.

Table 1
Primers of selected genes for real-time PCR reaction (GAPDH gene as the internal control).

Gene name	Accession number	Forward primer	Reverse primer	Annealing temperature
Chrnb3	NM_173212	TGGCTTTGCATGAAGGACCC	TGCGGTCAAGAACCTGAGCC	56.0
Chrnb4	NM_148944	TCCGCCTGGAGCTATCACTG	AGGTCCCATCGGCATTGTTG	56.0
Gabbr1	NM_019439	GATGGCATGGACGCTTATCG	ACAATGCCCAGGCTGGAGAG	56.0
Kcnk5	NM_021542	AGAATCGGGTGCCCAGCTTG	TCGTCTGAGAGGCCGGTTTC	56.0
Arc	NM_018790	GGAGAACTGCCTGAACAGGAG	TTCATGTGGTTCTGGATCTGG	56.0
GH	NM_008117	ATGGAATTGCTTCGCTTCTCG	TTGAGGATCTGCCCAACACG	57.7
STAT5b	NM_011489	GGCACAGACACTGCAGCAGTAC	TCTCGGCCAGCTTCTCACAC	59.6
Clic6	NM_172469	CCGAACACGAGGAGGAATCC	GGCAGTTTCCGATGCTCTCG	58.8
Igfbp2	NM_008342	TGGAGGAGCCCAAGAAGTTGC	GCGCTGTCCGTTCAGAGACATC	60.0
Igfbpl1	NM_018741	CCGGTCATCACCTGGAAGAAG	TGGGACTGAGCCTCTCCAATG	58.3
GAPDH	NM_32599	AGGAGCGAGACCCCACTAACAT	GTGATGGCATGGACTGTGGT	58.0

3.4. Large-scale gene expression change in music-exposure mice

The Agilent microarray results showed that the expression levels of approximately 454 genes in forebrain cortex (200 genes up-regulated and 254 genes down-regulated) and 437 genes in hippocampus (256 genes up-regulated and 181 genes down-regulated) were significantly affected in music mice. Interestingly, among the numerous genes, only few genes were affected both in forebrain cortex and hippocampus after exposing to the music. Further function classification revealed that these affected genes are mainly involved in ion channel activity and/or synaptic transmission, cytoskeleton, development, transcription, hormone activity (Tables 2 and 3). We also performed the real-time PCR analysis to confirm a subset of genes to avoid the possible fake positive on the microarray. The primers for the selected ten genes in the real-time PCR experiments were shown in the Table 1. Our RT-PCR results confirmed the expression patterns of those genes on the microarray data, which also demonstrated that music-exposure influenced the relevant gene expressions (Fig. 4).

3.4.1. Genes involved in neurotransmission

Via functional classification, we found a serial of ion channel and/or synaptic transmission activity related genes was notably affected in the mice with music-exposure.

Nr4a2, which regulates dopamine metabolic process, was up-regulated in the forebrain cortex; and Slc6a3 (solute carrier family 6 (dopamine transporter), member 3) was up-regulated in the hippocampus of music mice. Clic6, up-regulated by 2.6-fold in music hippocampus, is a member of the intracellular chloride channel family [18], which is not only involved in regulation of chloride ion transport, also in regulation of cell proliferation as a part of the complex with D2-like receptor.

Chrnb4, Chrnd and Chrnb3, which belong to nicotinic cholinergic receptors, were differentially affected: Chrnb4 in the forebrain cortex and Chrnd in the hippocampus were up-regulated respectively, whereas Chrnb3 was down-regulated in the hippocampus of music mice.

Trpm8 (transient receptor potential cation channel, subfamily M, member 8), P2rx1 (purinergic receptor P2X, ligand-gated ion channel, 1), Cacna1 s (calcium channel, voltage-dependent, L type, alpha 1S subunit), Trpv4 (transient receptor potential cation channel, subfamily V, member 4), Pkd2l2 (polycystic kidney disease 2-like 2), and Sypl2 (synaptophysin-like 2) are genes involved in cellular calcium ion homeostasis and/or calcium channel activity. Among these genes, Trpm8 and P2rx1 were down-regulated in the forebrain cortex, while the rests were up-regulated in the hippocampus of music mice.

Some genes encoding potassium voltage-gated channel subfamilies were differentially affected in the forebrain cortex of music mice: Kcnk7 and Kcnk5 were up-regulated, whereas Kcne2 and Kcnd1 were down-regulated. Interestingly, these genes had no change in the hippocampus of music mice. Correspondingly, a serial of solute carrier family 6 genes concerning to sodium ion transport, Slc16a8, Slc26a9, Slc13a4 were up-regulated only in the music hippocampus.

Other genes detected on the microarrays involved in the synaptic transmission and/or ion channel activity include Gabbr1 (GABA-B receptor, 1), Gabra6 (GABA-A receptor, subunit alpha 6), Glra1 (glycine receptor, alpha 1 subunit), Cngb3 (cyclic nucleotide gated channel beta 3) and so on (see Tables 1 and 2 for details).

3.4.2. Genes involved in cytoskeleton and structural molecules

Myo15, a member of unconventional myosins, which plays very important role in hearing and deafness [16,23], was up-regulated in both hippocampus and forebrain cortex of music mice. Another unconventional myosin, myo7b, firstly cloned from inner ear [5], was also up-regulated in hippocampus.

Some actin binding-related genes like Flnb, Cgnl1, Ttn, Zp2, Myh2, Actrt2 were also regulated in the forebrain cortex and/or hippocampus of the music mice. Notably, activity-regulated cytoskeleton-associated protein (Arc/Arg3.1), an immediate early gene, and required for the late-phase of long-term potentiation (LTP) and memory consolidation [29,36] was up-regulated by 2.1-fold in the forebrain cortex of music mice.

Some structural molecular activities related genes, such as Ptpn14, Lmnb2, Ntng2, Cldn18, Cldn2, Ush2a, Epb4.1l4a, Krt2 and Pcdh12, were up- or down-regulated under the music-exposure. Among these genes, Pcdh12, encoding protocadherin 12, proposed to participate in neuron recognition and calcium-dependent cell–cell adhesion [41], was up-regulated in the hippocampus of music mice.

3.4.3. Genes involved in regulation of hormone secretion and activity

Many genes involved in the feedback loop mechanism controlling growth hormone signaling transduction were notably regulated in forebrain cortex and/or hippocampus. GH (growth hormone) was up-regulated in both forebrain cortex and hippocampus of music mice by 6-fold. In forebrain cortex, GHRH (growth hormone release hormone), which controls GH secretion, was down-regulated by 2.4-fold. It is not surprising since growth hormone secretion can exert a short feedback inhibitory effect on GHRH secretion.

Another important gene involved in the feedback loop of GH mechanism is IGF-1, whose activity is greatly affected by its binding proteins. Several IGFBPs were detected to have changed differentially in the hippocampus and/or forebrain cortex of the music mice. IGFBP2, the major IGFBP in the cerebral spinal fluid was up-regulated by 2.1-fold in the hippocampus of music mice. IGFBPl1 (insulin-like growth factor binding protein-like 1) was down-regulated in both forebrain cortex and hippocampus of music mice.

Table 2
Gene expression change in the forebrain cortex of the mice with music exposure compared to naive mice.

Pathway name	Gene symbol	Accession number	Fold change
Synaptic transmission/ion channel activity	Chrnb4	NM_148944	6.5
	Gabbr1	NM_019439	2.1
	Gabra6	NM_008068	−7
	Glra1	NM_020492	10.1
	Kcnk7	NM_001004138	7.7
	Kcnk5	NM_021542	12.9
	Kcnd1	NM_008423	−2.3
	Kcne2	NM_134110	−2.2
	Trpm8	NM_134252	−16.4
	P2rx1	NM_008771	−2.2
Cytoskeleton	Flnb	NM_134080	2.5
	Myo15	NM_010862	2.7
	Arc	NM_018790	2.1
Structural molecule activity	Ptpn14	NM_008976	3.9
	Lmnb2	NM_010722	2.2
	Ntng2	NM_133500	3.8
	Cldn18	NM_019815	11.1
	Cldn2	NM_016675	−13.0
	Ush2a	NM_021408	−3.9
	Epb4.1l4a	NM_013512	−2.1
	Krt2	NM_010668	−5.6
Signal transduction	Npas4	NM_153553	2.3
	Pkp1	NM_019645	2.2
	Lrdd	NM_022654	2.1
	Tnfsf14	NM_019418	7
	Stat5b	NM_011489	5.7
	Fos	NM_010234	2.6
	Serpine1	NM_008871	3.6
Hormone activity	Adipoq	NM_009605	4.3
	Prl	NM_011164	4.6
	Gh	NM_008117	6
	Ppy	NM_008918	44.3
	Trhr	NM_013696	−1.9
	Trh	NM_009426	−3.7
	Ucn3	NM_031250	−14
	Ghrh	NM_010285	−2.4
Development and growth	Hoxb13	NM_008267	2.5
	Wnt5b	NM_009525	6.4
	Hoxc10	NM_010462	2
	Dach1	NM_007826	−2.1
	Gpc3	NM_016697	−2.1
	Isl1	NM_021459	−2.8
	Hoxb8	NM_010461	−6.9
	Igfbpl1	NM_018741	−2.3
	Wrn	NM_011721	−2.3
Regulation of transcription	Bhlhb3	NM_024469	−2.7
	Pax4	NM_011038	−2.4
	Egr2	NM_010118	2.7
	Nr4a2	NM_013613	2.6
	Hdac9	AK173017	−2.3
	Sfpi1	NM_011355	−2.4
	Titf1	NM_009385	−4.3
Cell cycle	Cdc6	NM_011799	4.7
	Espl1	NM_001014976	2.4
	Meig1	NM_008579	−2.2
	Spag5	NM_017407	−3.4
	Cdc25b	NM_023117	−2.4
Metabolism	Gsta2	NM_008182	3.6
	Acsm2	NM_146197	4.4
	Aldh3a1	NM_007436	6.2
	Hsd17b3	NM_008291	6.9
	Tbx5	NM_011537	−2.6
	Bhlhb4	NM_080641	−2.3

Besides GH, another member of the cytokine receptor superfamily, Prl (Prolactin) was also up-regulated in both forebrain cortex and hippocampus. Signaling transduction of GH and Prl receptors involve in stat-JAK pathway [3,35], so accordingly, we detected that STAT4 and STAT5b were up-regulated by 2.2-fold in the hippocampus and 5.7-fold in the forebrain cortex of music mice, respectively.

4. Discussion

Although there have been controversial points of the beneficial effect of music on learning and memory, more and more evidence has proved the improvement effect of music in spatial learning and working memory in mouse [7,40], rat [21,28,31] or human [17,20].

Table 3
Gene expression change in the Hippocampus of the mice with music exposure compared to naive mice.

Pathway name	Gene symbol	Accession number	Fold change
Synaptic transmission/ion channel activity	Cngb3	NM_013927	2.7
	Cacna1s	NM_001081023	2.5
	Trpv4	NM_022017	2.1
	Pkd2l2	NM_016927	4.2
	Sypl2	NM_008596	3
	Chrnd	NM_021600	2.3
	Chrnb3	NM_173212	−4.1
	Slc6a3	NM_010020	−2.1
	Clic6	NM_172469	2.6
	Slc16a8	NM_020516	2.1
	Slc26a9	NM_177243	2.2
	Slc13a4	NM_172892	2
Cytoskeleton	Cgnl1	NM_026599	2.3
	Ttn	NM_011652	3.5
	Myh2	NM_001039545	2.2
	Myo15	NM_010862	3
	Pcdh12	NM_017378	2.2
	Cttn	NM_007803	3.2
	Elmo1	NM_080288	−2.2
	Myo7b	NM_032394	−3
	Actrt2	NM_028513	−2.8
Signal transduction	Gnb3	NM_013530	2.7
	Rgs18	NM_022881	10
	Fasl	NM_010177	3
	Lrdd	NM_022654	2.1
	Aph1a	NM_146104	−2.2
Hormone activity	Prl	NM_011164	3.6
	Gh	NM_008117	6
	Trh	NM_009426	2.2
	Pthr2	NM_139270	2.5
	Ptgdr	NM_008962	2.1
	Adrb3	AF193027	7.3
	Ptger3	NM_011196	2.4
Development and growth	Tlx1	NM_021901	6
	Pitx1	NM_011097	3
	Evx1	NM_007966	2.7
	Tlx3	NM_021901	6
	Hoxb13	NM_008267	7.3
	Hoxb5	NM_008268	3.5
	Hoxd9	NM_013555	−8.4
	Igfbp2	NM_008342	2.1
	Bmp5	NM_007555	2.3
Transcription	Six1	NM_009189	2.3
	Pax4	NM_011038	8.3
	Pax5	NM_008782	2.0
Cell junction/adhesion	Amotl1	NM_001081395	2.0
	Cdh1	NM_009864	2.2
	Gjb4	NM_008127	2.1
	Glycam1	NM_008134	2.5
	Ncam2	NM_010954	2.4
Integral to plasma membrane	Itgb6	NM_021359	2.4
	Cd1d2	NM_007640	4.8
	Adora3	NM_009631	4.0
Response to abiotic stimulus	Gpr44	NM_009962	2.0
	Ccr9	NM_009913	2.1
	Il8rb	NM_009909	2.7
	Gnas	NM_010309	−11.4
	Cxcl5	NM_009141	−2.3
Sensory perception	Rpe65	NM_029987	2.2
	Pde6g	NM_012065	2.1
	Gucy2e	NM_008192	2.2
	Tas2r130	NM_199156	6.0
	Rp1hl1	NM_146246	
Metabolism	Soat2	NM_146064	5.4
	Cyp2f2	NM_007817	2.0
	Cyp1a2	NM_009993	2.2
	Ifng	NM_008337	−2.6
Lipid biosynthesis	Ptgis	NM_008968	2.3

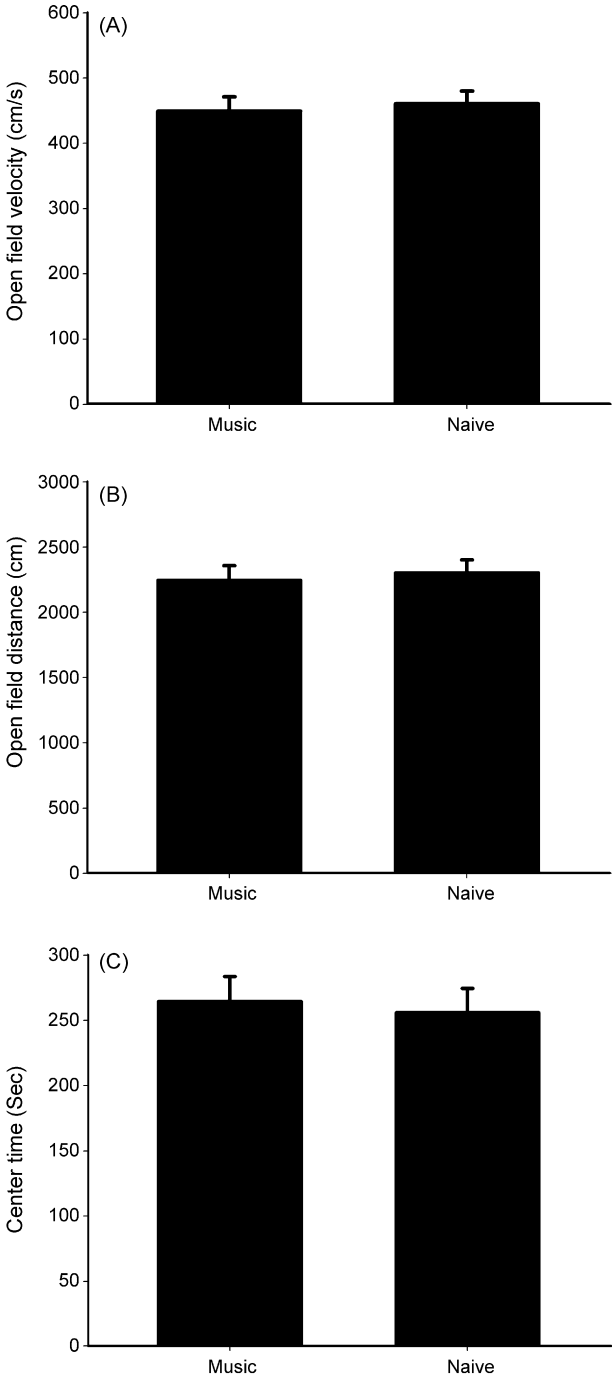

Fig. 1. No open field activity change after music-exposure as measured by a 15-min open field task. (A) Open field velocity. (B) Open filed distance. (C) Center time. All the parameters showed no statistical significance. (By Student's *t*-test, $P < 0.05$ was considered as statistical significance).

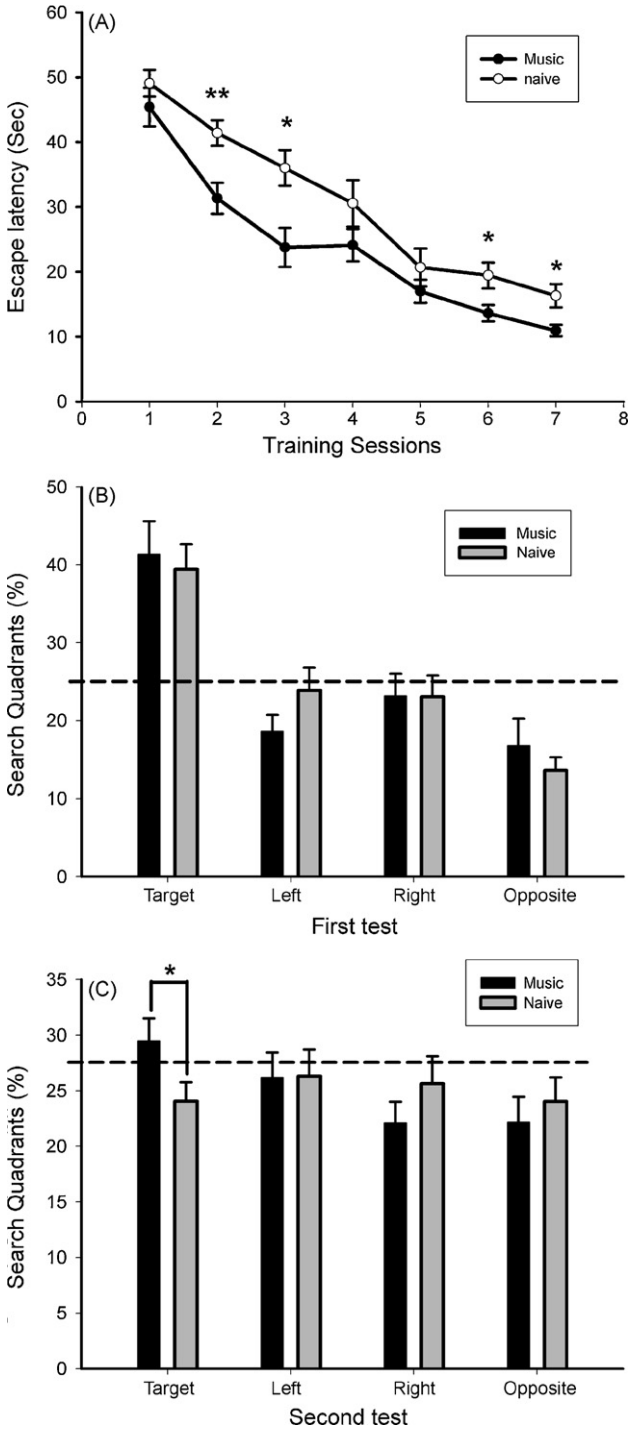

Fig. 2. Enhanced performance of the mice exposed to music in spatial reference memory as measured by Morris water maze. Both naïve and music group mice were subjected to a seven-session training paradigm. (A) Escape latency in training procedures. Although both types of mice showed a decrease in escape latency to find the hidden platform, mice exposed to music exhibited a faster learning cure than the naïve mice (two-way ANOVA for day 1–7 revealed a difference between trials: $F_{(13,140)} = 19.089$, $P < 0.0001$). In addition, a Student's *t*-test showed significant difference in escape latency at the second ($^{**}P < 0.01$), third, sixth and seventh sessions ($^{*}P < 0.05$) between the two group of mice. (B) Place preference in the first probe test (one day after training). The time spent to search in the target quadrant between the two group mice showed no difference, although much higher than other quadrants. (C) Place preference in the second probe test (four days after training). The time spent to search in the target quadrant in music group mice was significantly higher than that in naïve mice (Student's *t*-test, $^{*}P = 0.05$), although decreased when compared to that on 1 day after training.

For example, the exposure to the noise or music during pregnancy caused the opposite effects on neurogenesis in the hippocampus, and spatial learning ability in pups [21]. In Nakamura et al.'s work, they proved that auditory stimulation with music, but not with White noise or Etude, significantly decreased renal sympathetic nerve activity and blood pressure in rats [28]. In some pilot projects, music had been certified to exert an enhancing effect on autobiographical memory and an improvement in the social and emotional areas of Alzheimer's disease patients [4,19]. In our spatial learning test, the mice of music group had a faster learning capability and prolonged memory on the target object than the naïve mice. These

improvement effects of music on spatial learning were consistent with previous research results [17,21]. We also proved that music could increase fear-motivated memory, which was shown by the one-trial passive avoidance task. One point must be indicated that since the music-exposure cause multi-effects, not only on learning and memory, but also the stress response, immune system and so on; so it is not surprising that an integrated gene expression profiles would change accordingly. And in this article, we only focused on the genes those possibly related with learning and memory.

Although previous study has identified some individual genes that were affected after music-exposure, a global view of the gene expression profile under music-exposure was essential and worthy for seeking the complex mechanisms of music effect. Our microarray results demonstrated that genes involved in multiple biological processes, such as ion channels, synaptic transmission, cytoskeleton, transcription, signaling transduction, etc., were affected by the music-exposure condition. It is very interesting that there were only few genes (less than 20 genes in total) that were affected both in hippocampus and cortex after music exposing, including Myo15, Gh, Prl, Hoxb13, three genes related with defense response and so on. We speculate that, first, even in normal conditions, the gene expression profiles differ among the parts of the brain, since different function and neural circuitry exist; second, some genes expression change fold didn't meet the thresholds for analyzing the chips results (more than 2-folds), so the number of genes which were affected both in hippocampus and forebrain cortex was possibly decreased in our results. Whether the few common changes in both hippocampus and forebrain reveal some common pathways in which the music-exposure affects the learning and memory and/or other capabilities in the brain of mice should be further studied.

We detected multiple ion channel activity and synaptic transmission-related gene expression changes, which suggest that music-exposure may influence the basic activity of neuron and thus exert profound effects on nervous system. These alterations in gene transcription level concerned important neurotransmitters function, such as dopamine metabolism and activity, nicotinic cholinergic and GABA receptors activity. Dopamine, acetylcholine and GABA all play important role in learning and memory performance [9,12,32]. The increase of Nr4a2, Slc6a3 and Clic6 expression may enhance dopamine synthesis and activity of its receptor. While it is more complex for acetylcholine and GABA function since there were so many receptor subtypes differentially regulated in the mice with music-exposure, which play different roles in certain parts of nervous system. Notably, the interaction among the three neurotransmitters had been proved to be essential in the hippocampus and cortex, e.g. the dopamine D2 receptor (D2R) is involved in the regulation of acetylcholine (ACh) release in the hippocampus [12]; alpha4beta2-type nAChRs are critical for ACh-stimulated GABA release in the cortex, hippocampus, striatum and thalamus [26].

Interestingly, one of the most important excitatory neurotransmitter, glutamate-related genes failed to meet the criteria on our

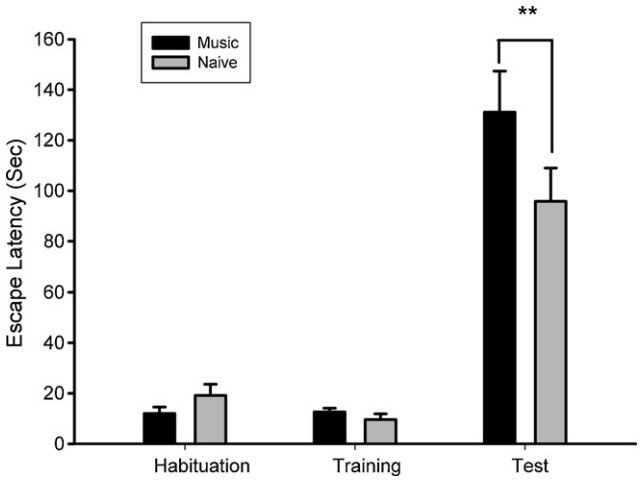

Fig. 3. Enhanced performance of the music mice in fear-motivated memory as measured by one-trial passive avoidance task. The escape latency (s) showed the time when it took the mice to enter the dark part from the light part. On the habituation and training day, the mice of music group showed similar performance with the naïve group, while on the test day, the mice of music group showed longer escape latency (Wilcoxon Signed Rank Tests, $^{**}P < 0.01$).

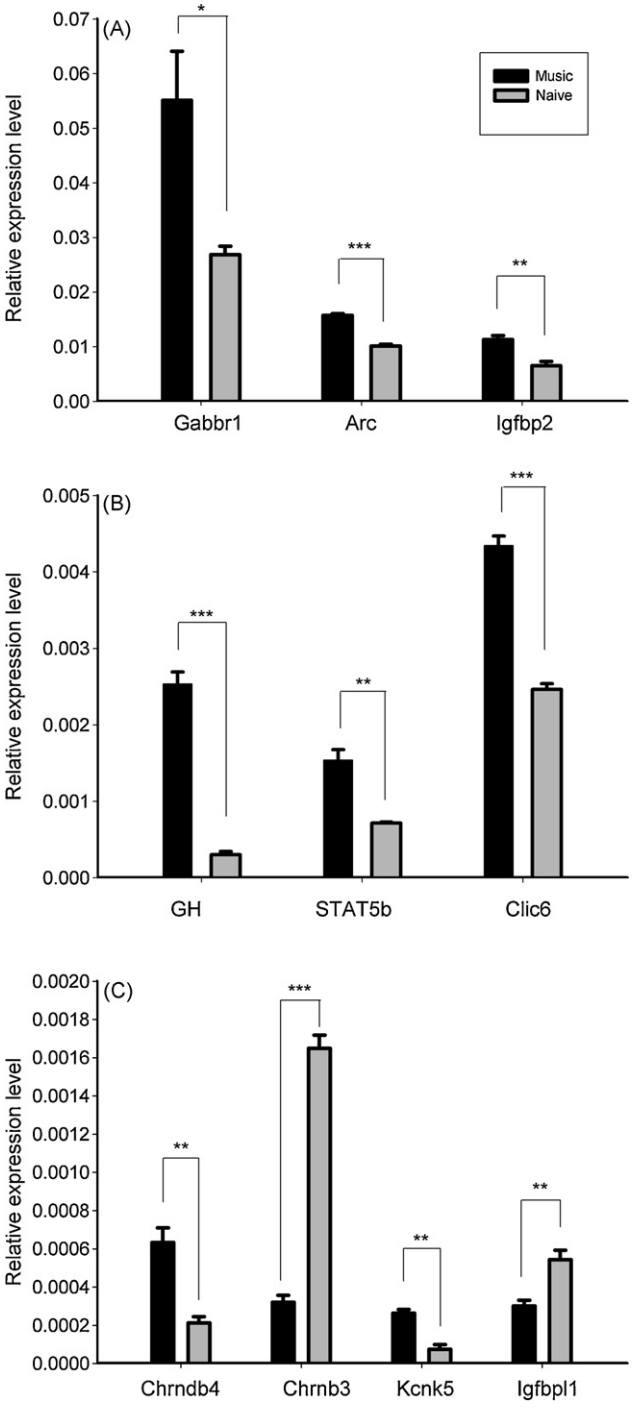

Fig. 4. Validation of gene expression profile on the microarray by real-time PCR. Relative expression levels were indicated as the ratio of the target gene expression level to GAPDH expression level in the same sample. Data were shown as mean ± S.E.M. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $n = 3$.

microarrays. It seemed not corresponding with pervious results that protein levels of NR2B [40] or GluR2 [39] was increased after music-exposure, which may due to different experimental system and different ways and/or threshold for the assay.

Cellular calcium ion homeostasis is essential not only for neuronal development, synaptic transmission and plasticity, but also for the regulation of various metabolic pathways. We detected more than twenty differential expressed genes that were involved in calcium channel or signaling transduction. It must be emphasized that the balance and interaction of various ion transport and neurotransmitters in the brain are very important and essential for normal physiological process and function, so any individual ion channel activity change could not be responsible for illustration of the mechanism of music effect.

A growing body of evidence indicated that growth hormone contribute to the function of center nervous system (CNS). GH is up-regulated during memory consolidation [10] and enhances excitatory synaptic transmission in area CA1 of rat hippocampus [25]. GH replacement had been proved to induce memory and mood improvements [2]. GH also has been verified to attenuate beta-amyloid (1-42)-induced memory impairment in mice [33]. Regulation of the neuro-hormonal axis may partially ameliorate such cognitive declines in healthy normal older adults and potentially in individuals with impaired cognitive function [38]. In the hippocampus and/or forebrain cortex of mice with exposure to music, we had detected a significant GH expression increase and a series of GH regulation related gene expression changes, e.g. GHRH and STAT5b, which might serve as one of the important pathways contributed to the mechanism of music effect on learning and memory.

IGF-1 exerts a diversity of actions and is an indispensable peptide hormone for proper development in the CNS. The activity modification of IGF-1 plays a crucial role in GH feedback loop mechanism, as IGF-1 can be induced by GH, and also in turn exerts a negative feedback influence on GH release. Whereas IGF actions are highly dependent on high-affinity regulatory IGF binding proteins (IGFBPs), therefore a balance of IGF/IGFBP action at different development stages is very important [6,11]. In fact, many in vivo and in vitro studies have indicated both inhibitory as well as stimulatory roles of IGFBP-2 on IGF induced growth, development and survival. During the exposure to music, IGFBP2 was up-regulated in the hippocampus. On the other hand, Igf2bp3 in the hippocampus, Igbp1b in the forebrain cortex, and IGFBP11 both in the hippocampus and forebrain cortex were down-regulated. All these changes revealed an uncertain effect of IGF signal transduction on the CNS of mice with exposure to music. Considering of the GH/IGF-1 feedback loop, a very complex neuron–endocrine system interaction in the CNS should be highly concerned during music-exposure.

In summary, music-exposure exerted a profound effect on the mice brain gene expression profiles, which leaded a behavioral performance alteration on learning and memory. The application of microarray technology revealed multiple signaling pathways that might contribute to the complex process. Besides the synaptic transmission-related genes involved pathways, the neuron–endocrine system interaction may also worth further studying. Furthermore, considering that fear-motivated memory is related with amygdale as well as hippocampus and prefrontal cortex [42], the music effects on more regions of the brain need to be further concerned.

Conflict of interest

None.

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