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Neuroscience Letters 420 (2007) 179–183
Neuroscience Letters
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Early music exposure modifies GluR2 protein expression in rat auditory cortex and anterior cingulate cortex

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Received 15 March 2007; received in revised form 29 April 2007; accepted 1 May 2007

Abstract

GluR2, a major subunit in AMPA receptor, plays an important role in brain functional activity. We studied the effect of music exposure during development on the expression level of GluR2 proteins in the auditory cortex (AC) and anterior cingulate cortex (ACC) of SD rats. Rats were divided into three groups, Music1 (exposed to Nostalgy) group, Music2 (exposed to Wishmaster) group, and control (no music exposure) group. For music exposure groups, rats were exposed to music from postnatal day (PND) 14, and the expression levels of GluR2 proteins were determined at PND 28, 42 and 56. For the control group, the expression levels of GluR2 proteins were determined at PND1, 3, 5, 7, 9, 11, 14, 21, 28, 42, and 56. Results showed an age-dependent expression of GluR2 proteins in control rats. In AC, exposure to Music2 dramatically increased the expression of GluR2, while exposure to Music1 had no effect. In ACC, we found remarkable discrepancies in time-dependent expression of GluR2 between music exposed rats and control rats. These results indicate that exposure to music can modify the expression level of GluR2 protein in AC and ACC.

Keywords: Rat; Auditory cortex; Anterior cingulate cortex; Music exposure; GluR2; Western blotting

Glutamate receptors (GluRs) mediate most of the excitatory neurotransmission in the central nervous system (CNS). They also participate in plastic changes in the efficacy of synaptic transmission underlying learning, memory and formation of neural networks during development [12,17]. AMPA receptor, one type of GluRs, consists of four subunits (named GluR1–GluR4). It mediates fast excitatory neurotransmission at a majority of synapses in the CNS. Previous studies have shown that the distribution and density of GluR1–GluR4 exhibited regional expression patterns in adult rat brain. The expression pattern also changes during developmental stages. In the cerebral cortex, the expression patterns of GluR1, GluR3 and GluR4 mRNAs differ among layers, while GluR2 mRNAs are even [15].

GluR2 subunit has a relatively lower Ca^{2+} permeability compared with other subunits. Glutamate-induced Ca^{2+} entry occurs through three kinds of channels: NMDA receptor channel, Ca^{2+}-permeable AMPA receptor channel, and voltage-dependent Ca^{2+} channel [6]. Any changes in the channels will ultimately influence the Ca^{2+} influx. It has been demonstrated that excessive Ca^{2+} entry under pathological conditions leads to neuronal cell death [10]. Because GluR2 regulates Ca^{2+} permeability, we speculate that GluR2 may participate in the regulation of synaptic plasticity.

Studies on rats have shown that music exposure during pregnancy resulted in elevated neurogenesis in hippocampus and enhanced spatial learning ability [9]. Auditory deprivation or auditory experience also affects cortical plasticity [2,13]. Therefore, sound or music does have impacts on brain plasticity. However, it remains unclear whether music exposure can modify the expression of GluR2 subunit. In the present study, we investigate the music effects on the expression level of GluR2 protein in auditory cortex (AC) and anterior cingulalte cortex (ACC) in Sprague-Dawley (SD) rats.

Sprague-Dawley rats were used in this study. The rats were raised in a sound-attenuated room with a background noise level less than 45 dB SPL re 20 μPa. The inside room was maintained at a temperature of $22 \pm 3\,^{\circ}C$ and on a light/dark cycle with 12 h light throughout the experiment.

In experiment 1, 44 rats were used. The GluR2 protein expression in AC and ACC were measured at postnatal days (PNDs) 1, 3, 5, 7, 9, 11, 14, 21, 28, 42, and 56. Each sampling age included four rats.

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doi:10.1016/j.neulet.2007.05.005

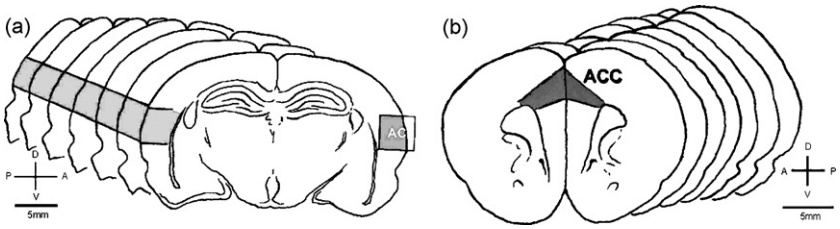

Fig. 1. A series of coronal sections of the rat brain. The extent of separated areas of auditory cortex (AC) region (a) and anterior cingulate cortex (ACC) region (b) are shown in grey.

In experiment 2, 45 SD rats were divided into three groups: Music1 (exposed to Nostalgy) group, Music2 (exposed to Nightwish) group and control group. Each group included 15 rats. The Music1 or Music2 exposure (70 dB SPL) was started at postnatal day 14 (PND14) in a sound attenuated room throughout the dark period for 12 h per day. After music exposure, the rats were put back in the sound-attenuating room in which the control rats were raised. The control rats were not exposed to music throughout the experiment. For each group, the expression of GluR2 protein in AC and ACC was measured at PND28, 42 and 56, with five rats at each sampling age.

SD rats were deeply anaesthetized with injection of sodium pentobarbital (50 mg/kg BW). Immediately after decapitation, the brains were obtained. The right and left AC regions (−3.30 to −6.30 mm anterior to bregma, according to Paxinos and Watson 1998; also according to the blood vessels in brain surface) and ACC regions (3.70 to −1.40 mm anterior to bregma) were separated (Fig. 1), and put in buffer equals to 10 times the volume (137 mmol/l NaCl, 20 mmol/lTris, 1.5 mmol/lNa_3VO_4, 1%NP-40, 10%glycerol, freshly added with 1 mmol/l PMSF, 10 μg/ml aprotinin, 0.2 μg/ml leupeptin). Centrifugation was done at 16,000 rpm for 10 min after homogenization. The supernatants were then collected. All the above procedures were done at 4 °C. Concentration was measured by Bradford methods: the standards contained a range of 0–100 μg protein (BSA) in 100 μl volume, and were added to 5 ml Coomassie Brilliant Blue G-250 (100 mg G-250 in 50 ml 95% ethanol, and 100 ml 85% (w/v) phosphoric acid). After 3 min incubating, the absorbance at 595 nm was measured. The standard curve is: $Y = 555.56X - 0.04$. Samples were boiled at 100 °C in the presence of sample buffer (included 250 mM Tris–HCl, PH6.8, 4% sodium dodecyl sulfate (SDS), 1% β-mercaptoethanol, 1% bromophenol blue, and 20% glycerol) for 5 min, and the final concentration of sample protein was adjusted to 5 μg/μl. Samples were preserved at −80 °C.

Proteins (15 μl of each sample) were separated on a 7.5% SDS-polyacrylamide gel and transferred to a nitrocellulose membrane (PALL). Blocked with 5% non-fat dry milk dissolved in 1% TBST for 2 h, the immobilized proteins were incubated at 4 °C for 12 h with primary antibody (goat-anti-rat GluR2 antibody, 1:200, Santa Cruz; goat actin antibody, 1:2000, Santa Cruz). After being washed with 1% TBST, membranes were incubated with secondary antibody (rat-anti-goat IgG HRP, 1:1000, Jacksonimmuno) for 2 h at room temperature, and then washed again. Proteins were visualized by using enhanced chemiluminescence reagents (Pierce) for 5 min followed by exposure to medical X-ray film (Kodak) for 20 min. Blots were scanned, and analyzed by using Scanband software.

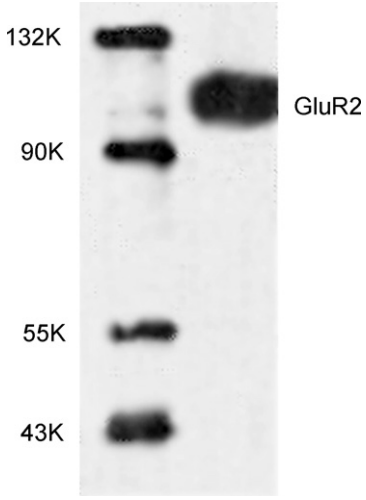

Fig. 2. The specificity and the band location of protein loading. The molecular weight of GluR2 is about 108 kDa.

The band intensity for each sample was calculated as total gray value. The values were then normalized to the actin gray value. The statistical significance was determined by *t*-test in SigmaPlot software.

The specificity and the band location of GluR2 protein loading was shown in Fig. 2. Expression of GluR2 proteins in AC (Fig. 3a, Table 1) and ACC (Fig. 3b, Table 1) exhibited age-dependent increasing trend. Evidence showed that excitatory synaptic response mainly depended on NMDA receptors in infant, and the role of NMDARs showed age-related decrease

Table 1
GluR2 protein expression level in auditory cortex and anterior cingulate cortex from PND7 to PND56 (nmol/mg)

Postnatal days	Auditory cortex ($n = 4$)	Anterior cingulate cortex ($n = 4$)
1	79.68 ± 3.11	70.02 ± 11.10
3	92.58 ± 12.64	128.52 ± 13.08
5	127.70 ± 18.40	160.65 ± 10.66
7	154.88 ± 23.33	182.98 ± 6.91
9	201.04 ± 36.78	223.65 ± 7.49
11	251.21 ± 41.53	273.30 ± 10.74
14	272.94 ± 54.19	376.26 ± 58.52
21	428.41 ± 64.32	598.17 ± 107.26
28	485.31 ± 69.84	740.27 ± 140.00
42	603.19 ± 150.29	936.96 ± 122.25
56	889.78 ± 131.70	1503.66 ± 303.05

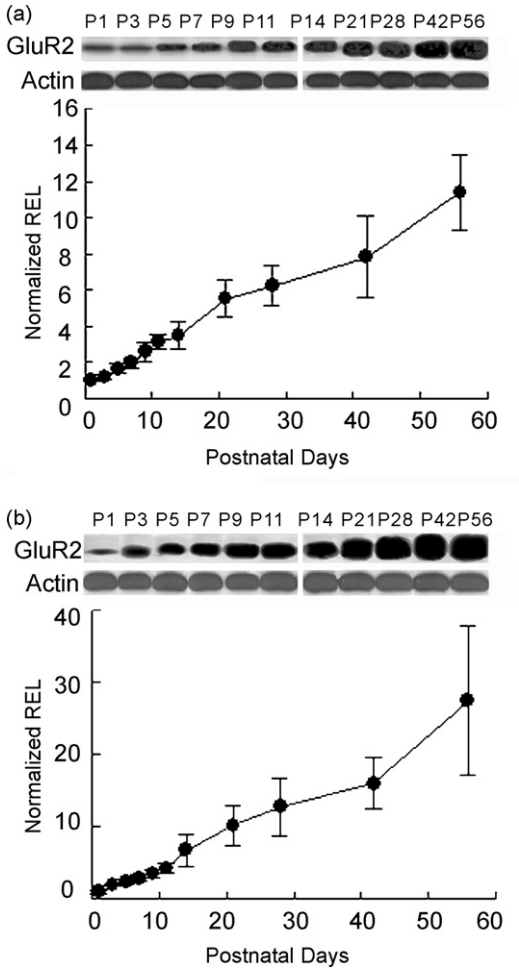

Fig. 3. Age-dependent expression of AMPA receptor subunit GluR2 protein in AC and ACC. (Panel a) AC; (Panel b) ACC. The expression of GluR2 protein determined by Western blotting method at different ages is shown on the top of each panel. Normalized REL: normalized relative expression level of GluR2 protein.

while the effect of AMPA increased [8]. Our results are consistent with the increasing expression of GluR2 proteins in telencephalic membranes and mRNAs in frontal cortex with advanced age [14,4].

We detected an increasing expression of GluR2 protein in AC of rats exposed to Music2 at all age ($P < 0.05$); however, no increasing trend in Music1 group was found (Fig. 4, Table 2). The results indicate that different music may have different impacts on the expression of GluR2 in AC.

Table 2
Comparison of GluR2 protein expression level in auditory cortex among Con., Music1 and Music2 at PND28, PND42 and PND56 (nmol/mg)

Postnatal days	Con. ($n = 5$)	Music1 ($n = 5$)	Music2 ($n = 5$)
28	476.55 ± 26.74	475.02 ± 28.69	643.51 ± 57.23*
42	620.81 ± 53.76	755.42 ± 94.70	1252.34 ± 171.08**
56	860.31 ± 64.31	562.79 ± 95.87†	1499.47 ± 114.55‡

(*) $P < 0.05$ (vs. Con.), (**) $P < 0.05$ (vs. Con.), (†) $P < 0.01$ (vs. Music2), (‡) $P < 0.05$ (vs. Con.).

Table 3
Comparison of GluR2 protein expression level in anterior cingulate cortex among Con., Music1 and Music2 at PND28, PND42 and PND56 (nmol/mg)

Postnatal days	Con. ($n = 5$)	Music1 ($n = 5$)	Music2 ($n = 5$)
28	719.55 ± 79.71	639.16 ± 116.24	615.15 ± 86.99
42	926.11 ± 115.86	2557.91 ± 696.54*	2598.94 ± 692.77**
56	1490.00 ± 90.63	2215.10 ± 197.45†	2809.37 ± 191.83‡

(*) $P < 0.05$ (vs. Con.), (**) $P < 0.05$ (vs. Con.), (†) $P < 0.05$ (vs. Music2), (‡) $P < 0.01$ (vs. Con.).

In the ACC, significant differences were found in the expression level of GluR2 between the music exposed groups and the control group at PND42 ($P < 0.05$), and between Music2 group and control group at PND56 ($P < 0.01$). However, there is no statistically significant difference in the expression level of GluR2 between the Music1 group and the control group at PND56, and among all groups at PND28 (Fig. 5, Table 3). These results suggest that ACC requires time accumulation for any changes.

Fast excitatory synaptic transmission in the CNS is predominantly mediated by AMPARs. During development, the ratio of AMPAR/NMDAR increases, which leads to a more important role of AMPARs [7]. Consistent with previous studies, the present results also demonstrated an age-dependent increased expression of GluR2 protein in AC and ACC. In neocortical principal neurons, AMPARs showed relatively low gating and low Ca^{2+} permeability. In GABAergic interneurons, AMPARs showed faster gating and high Ca^{2+} permeability. Functional dif-

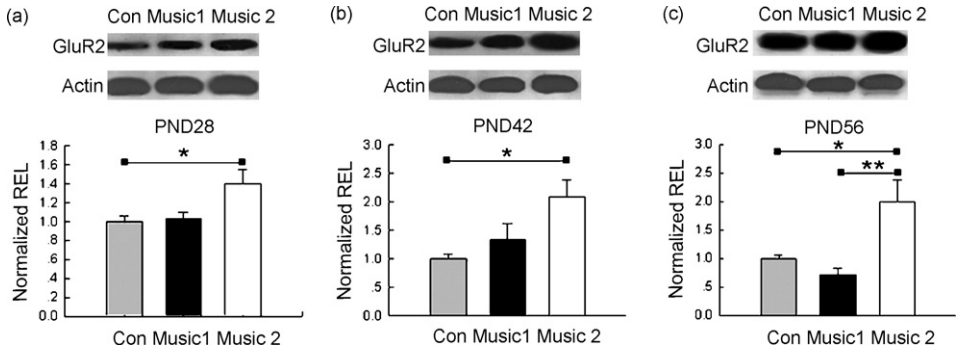

Fig. 4. Normalized relative expression of GluR2 protein in AC for three groups of rats at PND28, 42 and 56. Con: control group. Significant differences were detected between Control and Music2 at PND28 (a), PND42 (b), and PND56 (c) ((*) $P < 0.05$). No difference was found between Control group and Music1 group. A significant difference was detected between Music1 and Music2 at PND56 ((**) $P < 0.01$). Normalized REL: normalized relative expression level of GluR2 protein.

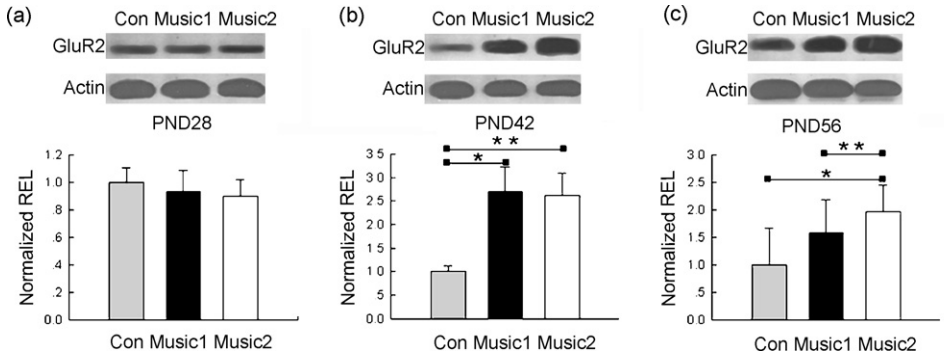

Fig. 5. Normalized relative expression of GluR2 protein in ACC for three groups of rats determined at PND28, 42, and 56. At PND28, no significant difference was found among three groups (a). At PND 42, significant difference was found between control group and Music 1 group, and between control group and Music 2 group (b). At PND 56, there were significant differences between Control and Music2 group ((*) $P < 0.01$), and between Music1 group and Music2 group ((**) $P < 0.05$) (c). Normalized REL: normalized relative expression level.

ference between these two classes of neurons was correlated with the components of AMPARs. In principal neurons, GluR1 and GluR2 subunits expressed abundantly; however, in interneurons, the relative abundance of GluR2 was lower than that in principal neurons. Channels consisted of Q/R site-edited GluR2 subunit always showed low Ca^{2+} permeability [3].

In the present study, the GluR2 level is determined from whole tissue block, whether the increasing parts occur in synaptosme or not requires further research. Here we hypothesize that the increasing expression might be related to the Ca^{2+} influx regulation, changes in AMPA receptor composition, newly formed synapses, and expression changes of other receptors. First of all, Ca^{2+} probably plays a major role in the control of cell migration, differentiation, neuritogenisis and apoptosis [12]. Enduring excitatory impulse probably causes excessive influx of Ca^{2+}, which may influence developmental processes. Occurrence of increasing GluR2 protein might join in the regulation of Ca^{2+} influx. GluR2 is likely to play a role of safeguard. Second, glutamate released from the same presynaptic neuron will exert different effects on postsynaptic target neurons, depending on the functional and molecular characteristics of the postsynaptic AMPARs. Therefore, the increased expression of GluR2 correlating changes in other subunits of AMPAR may be meaningful, however, we did not detect here. A previous study has shown that, in hippocampus and frontal cortex, the (GluR1 + GluR3)/GluR2 ratio declines with increasing age, and the authors predicted a decrease in glutamate-operated Ca^{2+} permeability mediated through AMPA receptors as a function of age [4]. Also, an increase in GluR2 levels might imply the formation of new synapses, which strongly affect the direction and extent of synaptic plasticity, and further strengthen the function of auditory cortex, as well as other regions concerned. Evidence indicates that AMPARs mediate transmission underling experience-dependent dendritic arbor growth by stabilizing branches, and this supports a competition-based model for dendrite growth [5]. Furthermore, increasing expression of GluR2 might lead to Ca^{2+} influx through NMDA receptor channels and voltage-dependent Ca^{2+} channels, which probably relates to changes in expression of other channel proteins like NMDAR, etc. Changes in ratio of different types of channels will directly impact the postsynaptic events.

Auditory experience plays an important role in the development of the primary auditory cortex (A1). Many experiments have shown that sound or music does have influence on the auditory plasticity on rodents. Environmental acoustic exposure may strongly affect the emergent and enduring functional organization of A1 [18,11]. Early auditory deprivation decreased the expression levels of NR2B mRNA and protein in AC during critical period of rat auditory development, while acoustic experience increases expression level of NR2B protein [2]. In our study, music exposure caused an increased expression of GluR2 protein which provides one of the molecular mechanisms of music impact. Interestingly, we found difference in the expression of GluR2 protein between Music1 group and Music2 group. This implies that different types of music can lead to different degrees of influences. The underlying mechanism for the differences is still under investigation.

ACC has been considered as a crucial region in regulation of emotional behavior and modulation of negative mood [16,1]. In the present study, we do not know whether the music affected the mood of these rats. We suggest that changes in GluR2 protein expression in rats exposed to music may be related to the function of ACC.

Acknowledgements

We thank Hui Sun for reading an earlier version of this manuscript. This work is supported by National Science Foundation of China (NSFC No. 30170313, 9020812, 30570595), Shanghai Pujiang Program (No. 06PJ14036), Shanghai Rising Star program (No. 05QMX1420), and Shanghai Shuguang program (05SG28).

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