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Effects of enriched environment on gene expression and signal pathways in cortex of hippocampal CA1 specific NMDAR1 knockout mice

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Abstract

N-methyl-D-aspartate glutamate receptor 1 (NMDAR1) plays a pivotal role in different forms of memory. Indeed, hippocampal CA1 region specific knockout (KO) of NMDAR1 in mice showed memory impairment. Recently, it has been reported that environmental enrichment enhanced memory and rescued the memory deficits of the NMDAR1-KO mice. It is well known that cortex has synaptic connections with hippocampus and is the storage region of the brain for long-term memory. To understand the molecular mechanisms of the memory impairments in the NMDAR1-KO mice, we have examined gene expression profiles in cortex from the receptor KO mice compared to wild type mice. Furthermore, since memory deficits were rescued after exposure of the NMDAR1-KO mice to enriched environment, we also analyzed the gene expression in the cortex of the KO mice after 3 hours, 2 days and 2 weeks enrichment. We found that the expression levels of 104 genes were altered in the cortex of NMDAR1-KO mice. Environmental enrichment for 3 hours, 2 days and 2 weeks affected the expression of 45, 34 and 56 genes, respectively. Genes involved in multiple signal pathways were regulated in the NMDAR1-KO mice, such as neurotransmission, structure, transcription, protein synthesis and protein processing. It is not surprising that since enriched environment rescued the memory decline in the NMDAR1-KO mice, the expression changes of a number of genes involved in these signal pathways were recovered or even reversed after enrichment. Our results further demonstrated that reelin and Notch signal pathways could be involved in the enrichment effects on memory improvement in the KO mice.

Keywords: Microarray; Memory deficits; Neurotransmission; Signal transduction

1. Introduction

Extensive studies indicated that NMDA receptor activity in the hippocampus was essential for the formation of both spatial [44] and nonspatial memory [52]. Analysis of inducible NMDAR1-KO revealed the crucial role of the NMDA receptor in preserving remote memory [14] as well as the consolidation and storage of conditioned taste aversion memory [13,58]. The hippocampal CA1 region has been shown to be important for converting new memories to long-term memories through the main input and output to the cortex [58]. It has been reported that cortical plasticity was essential for the establishment, consolidation and retrieval of permanent memory [66]. Therefore, NMDAR1 activity in the hippocampus could play an essential role in transferring the new memories from the hippocampus to the cortex for permanent storage. Indeed, NMDAR1-KO at hippocampal CA1 region showed both short-term and long-term memory impairments [52]. However, the molecular mechanisms underlying the effects of NMDAR1 deletion at CA1 region on the long-term memory processes remain unknown.

Enriched environment has been shown to have a significant beneficial impact on the brain. Extensive studies have shown that the enrichment could improve spatial and nonspatial memory as well as long-term recognition memory [7,41,52]. Furthermore, enriched experience reversed learning and memory impairments in different memory-deficient animal models [17,21,48,52]. In addition, the enrichment training reduced amyloid β peptide (Aβ) levels in the brain of amyloid precursor protein (APP) transgenic mice [40]. Recently, it has been reported that the enrichment rescued the nonspatial memory deficits in CA1 NMDAR1-KO mice and promoted an increase of the synapse density [52]. Our previous work demonstrated that enriched

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environment had significant effects on the expression of genes involved in neurotransmission and other signal pathways in the cortex of mice [51]. Moreover, we found that a number of genes that changed their expression levels in the cortex of aged mice were oppositely regulated by enriched environment [36]. To further understand the molecular mechanisms of the effects of enriched experience on memory improvement for NMDAR1-KO mice, we analyzed the gene expression changes in the cortex of the mice after exposure to the enriched environment for 3 hours, 2 days and 2 weeks. Our results have shown that the enrichment may rescue the memory deficits in NMDAR1-KO mice through modulating multiple signal pathways. These findings provide insight into the molecular basis of NMDAR1 regulation of memory and how this regulation was affected by enriched environment.

2. Materials and methods

2.1. Animals and environment enrichment training

The NMDAR1-KO mice and wild-type (WT) littermates were bred with six mice per cage as described [60]. Adult NMDAR1-KO mice were randomly divided into four groups. One group animals were kept in standard cages (naïve group, six mice), and the other three groups trained in an enriched environment for 3 hours daily for 1 day, 2 days and 2 weeks (enriched groups, six mice for each group). The enrichment training was performed in specially designed box (1.5 m × 0.8 m × 0.8 m), in which various toys, running wheels and small houses were changed every other day. Food and water were available in the box.

2.2. High-density oligonucleotide microarray analysis

The animals were decapitated after anesthesia with tribromoethanol and the frontal cortices were dissected and immediately frozen in dry ice. The tissues were pooled and stored at −80 °C before use. RNA was extracted from the pooled samples and stored as previously described [45]. Mu11KsubA and Mu11KsubB arrays (Affymetrix, Santa Clara, CA, USA) containing 13,069 probe sets corresponding to more than 11,000 genes and expressed sequence tags were used. Probe labeling and microarray hybridization were performed according to the manufacturer's protocol. The high-density oligonucleotide microarray analysis was conducted as previously described [46,51]. Data were analyzed using the Affymetrix GeneChip Expression Analysis Software. To ensure the reliability of the data, we conducted hybridization experiments in duplicate microarrays from each RNA sample. In agreement with our previous experience [45,46,51] and quantitative RT-PCR confirmation, we found that the duplicate experiments provided consistent and reproducible results. MAPPFINDER/GENMAPP Software has been used to map the microarray data for functional annotation and classification.

2.3. Real-time PCR

Real-time RT-PCR was performed to validate some selected genes using ABI 7700 Sequence Detection System. Total RNA was isolated from cortex and reverse-transcribed using the SuperScript First-Strand Synthesis System (Invitrogen, Carlsbad, CA, USA). Glyseraldehyde-3-phosphate dehydrogenase (GAPDH) was used as control. The sequences of primers and probes of the tested genes and GAPDH are listed in Table 1. The PCR reaction was performed as described [45]. TaqMan probes for the genes were obtained from Perkin-Elmer. All the probes were labeled with the fluorescent dyes 6-carboxyfluorescein (FAM) and 6-carboxy-tetramethyl rhodamine (TAMRA). TaqMan probe concentrations were maintained at 100 nM, while PCR primer concentrations were systematically varied in all combinations. The fold change of the targets genes was calculated using the $2^{-\Delta\Delta Ct}$ methods.

Table 1
Primers and probes used in the real-time RT-PCR experiments

Accession number	Gene name	Forward primer	Reverse primer	Taqman probe
NM_001001303	GAPDH	CTTCACCACCATGGAGAAGGC	GGCATGGACTGTGGTCATGAG	CCTGGCCAAGGTCATCCATGACAACTTT
X57497	GluR1	CGCCTCAACGCCATCCT	GGTACCCGATGCCGTTCTTT	CCAGATTGTGAAGCTAG
AA537307	Jip3	GGTCTCCCCAGAGTCAGTCCTA	AGCACACCATCCTCCATTAACC	TGCAGGCTTAGCCTC
AA596555	Sf3b1	GGTACCCCTACTCCAGGTCACA	CGCCACGCCTGAAGCT	ATGAGCATGACACCTGAA
W82651	Rab14	GCTTGCTTCATCAATTTACAGAAAAA	TGTACCAAATTCAACACCAATTGTG	AATTTATGGCTGATTTTCCT
AA059763	tubb2	AGAGAACACAGATGAAACCTATTCCA	AGGGTGCGGAAACAGATGTC	TGACAATGAGGCTCTG
L36314	Gdi3	CCGCGTGCGCTCCTT	CCAGCACGATCACGTCGTAT	CCGCCATGAATGAG
M13227	Penk1	TGCAGCTACCGCCTGGTT	CAGTGTGCACGCCAGGAA	CCCAGGCGACATCA
D17584	Tac1	CCGGAGCCCTTTGAGCAT	CTGCTGAGGCTTGGGTCTTC	TGCAGAGAATCGCC
AA117100	Stathmin	GCCGATGTAGGACCGTATAGGT	TCTCCCCTTGAGCCCCTAA	ATCCAGACCGTGAGATGT
AA271910	Map1lc3a	CTTGCCTGTCTGCCCATCTT	TCCTGGCAGGAGCAAAGC	CCCCTCATCCACCTGA
X70764	Mark2	GACCATTGGCAAGGGTAACTTT	TCTTTCCCCGTCAGGATGTG	CCAAGGTGAAGTTGGC
X61940	Dusp1	CCACAGGACACCGCACAAG	GCCCGTTCTGCGGTTCT	TCGACCGACTTTTT
AA051486	eIF-5	TGGGTACCGAGGCATGCT	AGGTGGGTTTTTGAGAATGAATG	ACACACATCATAAACTC
AA107455	Eef2	AGATCCGTGCCATCATGGA	TGGGCGATGACTGACATGTT	AAGAAAGCCAACATCC
D32167	Zic1	ACCTTTGCAAGATGTGCGATAA	GACCTTCATGTGTTTACGCAGAGA	TCCTACACGCACCCC
AA407629	Sbno1	CCTGTGAGGCCACCAACTTTT	GCGGAAAGGTGAAGAAATCACT	TGCTTTTTTGGCTTCTC