

Full-length article

Visual recognition memory is related to basic expression level of NMDA receptor NR1/NR2B subtype in hippocampus and striatum of rats¹Shu-jun XU, Zhong CHEN, Li-jun ZHU, Hai-qing SHEN, Jian-hong LUO²*Department of Neurobiology, Zhejiang University School of Medicine, Hangzhou 310031, China***Key words***N*-methyl-*D*-aspartate receptors; NR1 subunit; NR2B subunit; Western blotting; visual recognition memory

¹ Project supported by Key National Basic Research Program of China (No G2002CB-713808), and Outstanding Cross-Century Faculty Grant of the Ministry of Education of China, the National Natural Science Foundation of China (No 30270436 and 30371638).

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Received 2004-08-11

Accepted 2004-11-15

doi: 10.1111/j.1745-7254.2005.00032.x

Abstract

Aim: To examine the basic expression levels of *N*-methyl-*D*-aspartate (NMDA) receptor NR1 and NR2B subunits in six brain regions of Sprague-Dawley (SD) rats with different visual recognition memory. **Methods:** Rats were tested by a novel-object-recognition model and grouped into the high and the low visual recognition memory groups. The expression levels of NR1 and NR2B subunits in the cortex, hippocampus, striatum, amygdala, diencephalon, and olfactory bulb were measured by semiquantitative immunoblotting. **Results:** The NR1 and NR2B subunit protein levels in the hippocampus of the high visual recognition memory group were 35.9% ($P < 0.01$) and 53.4% ($P < 0.05$) higher respectively than those in the low group. In addition, the NR2B level in the striatum in the high visual recognition memory group was 25.0% ($P < 0.05$) higher than that in the low one. However, no significant difference was found in the levels of the subunits between the two groups in other brain regions. **Conclusion:** The visual recognition memory in rats is related to the basic expression level of NMDA receptor NR1/NR2B subtype in the hippocampus and striatum.

Introduction

N-Methyl-*D*-aspartate (NMDA) receptors, the major ionotropic glutamate receptors in the central nervous system, play key roles in excitatory synaptic transmission and plasticity, and are involved in many physiological and pathological processes^[1,2]. NMDA receptors are mainly composed of NR1 subunit and NR2A, NR2B, NR2C, or NR2D subunit, respectively, and the different subunit combinations form different NMDA receptor subtypes with distinct functional properties^[3,4].

It has been demonstrated that NMDA receptors are involved in long-term synaptic plasticity and certain forms of learning and memory^[5-8]. For example, numerous data indicate that NMDA receptor activity and NMDA receptor-dependent synaptic plasticity in the hippocampus are crucial for spatial recognition memory^[7,8]. However, there have been a few reports about the role of NMDA receptors in visual recognition memory^[9-12], and there are some discrepant or even contradictory results in the visual recognition memory with regard to different doses of NMDA receptors antagonist,

and animal species. Furthermore, most of those findings were mainly obtained from animals with specific brain region injuries, pharmacological manipulated animals or transgenic mice with one of NMDA receptor subunits abnormally expressed. Little is known whether the basic expression levels of NMDA receptors is related to different visual recognition memory in normal adult rats.

Therefore, the objective of our studies was to examine the relationship between the basic expression levels of NR1 and NR2B subunits in six specific regions (cortex, hippocampus, striatum, amygdala, diencephalons, and olfactory bulb) and visual recognition memory in rats.

Materials and methods

Animals All experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animal. The animals used in this study were 6-week old male Sprague-Dawley (SD) rats, weighing 175–190 g (Grade II, Certificate No 22-9601018, Experimental Animal Center, Zhejiang University, China), maintained in

an air-conditioned room with controlled temperature (22–26 °C) and humidity (40%–70%), housed in cages with a 12-h light/dark cycle (lights on from 8:00 AM–20:00 PM) with food and water available. Experiments were carried out each day between 10:00 AM–17:00 PM.

Novel object recognition task Rat was individually habituated to an open-field box (50 cm×50 cm×30 cm high) for 3 d. During training sessions, two novel objects were placed into the open field and the animal was allowed to explore for 5 min. The time spent for exploring each object was recorded. During retention tests, the animal was placed back into the same box after a 24-h delay, in which one of the objects used during training was replaced by a novel object, and allowed to explore freely for 5 min. Behaviour was considered exploratory when an animal was touching or directed towards the object at a distance ≤ 2 cm with its nose; while behaviour was not considered exploratory when an animal was turning around or sitting on the object. To avoid interference effect of the olfactory cue due to scent traces left, after each experiment, both the objects and the arena were scraped by 10% ethanol. The preference index, a ratio of the amount of time spent exploring any one of the two objects (training session) or the novel one (retention session) over the total time spent exploring both objects, was used to measure recognition memory. All 40 male SD rats were ranked by the preference index. From these, eight superior and eight inferior rats were used as high visual recognition memory group and low visual recognition memory group, respectively, in the present study.

Preparation of tissue The rats were killed by decapitation and the brains were quickly removed. The brains were dissected on ice into six specific regions (cortex, hippocampus, striatum, amygdala, diencephalon, and olfactory bulb), and homogenized twice in 100 volumes (100×wet weight) of ice-cold 10 mmol/L Tris-HCl (pH 7.4) containing 320 mmol/L sucrose with a Tissueizer (Heidolph DIA×900) at speed 5 for 10 s with a 20 s interval between bursts. The tissue homogenate was centrifuged at 700×*g* for 10 min at 4 °C. The supernatant was collected and centrifuged at 37 000×*g* for 40 min at 4 °C. This high-speed pellet was resuspended in 10 mmol/L Tris-HCl (pH 7.4). Protein concentrations were determined using Folin phenol reagent with bovine serum albumin as a standard.

Semiquantitative Western blot Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transfer of proteins to nitrocellulose membranes were performed according to conventional methods. The denatured membrane proteins were separated on 7.5% polyacrylamide gels and transferred to nitrocellulose membranes in transfer

buffer (Tris 25 mmol/L, glycine 192 mmol/L, 20% methanol and 0.05 % SDS, pH 8.3). The membranes were incubated with a blocking buffer of 5% non-fat dry milk in TBST (Tris-HCl 20 mmol/L, NaCl 140 mmol/L, 0.1% Tween-20, pH 7.4) at room temperature for 1 h. The membranes were then incubated with anti-NR1, and anti-NR2B antibodies (1 g/L) in blocking buffer overnight at 4 °C. After several washes with TBST, the membranes were incubated with horseradish peroxidase-conjugated secondary antibody in blocking buffer for 1 h at room temperature. Following washes for 20 min with four intermediate changes with TBST, proteins were visualized with enhanced chemiluminescence. The resulting film was scanned and analyzed using a laser densitometer and GelScan XL software (Pharmacia LKB). In order to make different gel samples evaluable, every gel was run with three lanes of the same cortex proteins as a standard^[13,14].

Statistical analysis All results were expressed as mean±SD. Differences were analyzed by group comparisons *t*-test with Mann-Whitney Test by SPSS 11.0 software. *P*<0.05 was considered statistically significant.

Results

Novel object recognition task The preference indexes for the high visual recognition memory group and the low visual recognition memory group were summarized in Figure 1. During the training sessions, no significant difference was found in the exploratory preference between the two groups (0.43±0.12 vs 0.49±0.11, *P*>0.05). During the retention sessions, the exploratory preference of the high visual recognition memory group was significantly higher than that of the low one (*P*<0.01).

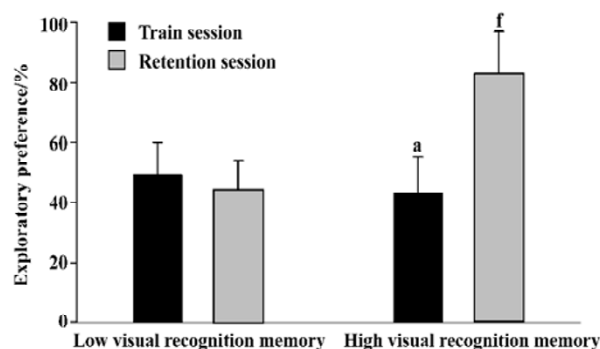


Figure 1. Exploratory preference of rats with different visual recognition memory during the training session and the retention session in the novel-object-recognition task. *n*=8. Mean±SD. ^a*P*>0.05 vs the training session of the group with low visual recognition memory. ^f*P*<0.01 vs the retention session of the group with low visual recognition memory.

Relationship between the basic expression level of NR1 subunit and visual recognition memory in rats The protein level of NR1 subunit in the hippocampus in the high visual recognition memory group was 35.9% higher than that in the low group (1.89 ± 0.18 vs 1.39 ± 0.39 , $P < 0.01$). However, no significant difference was found in the protein levels of NR1 subunit in the cortex, striatum, amygdala, diencephalon and olfactory bulb of rats between the two groups (Figure 2).

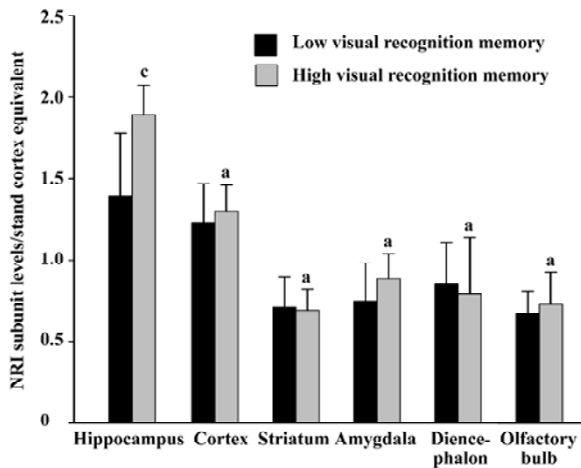


Figure 2. Relative expression levels of NR1 subunits in six brain regions in rats with different visual recognition memory. $n=8$. Mean \pm SD. ^a $P > 0.05$, ^c $P < 0.01$ vs the group with low visual recognition memory.

Relationship between basic expression level of NR2B subunit and visual recognition memory in rats As shown in Figure 3, the protein level of NR2B subunit in the hippocampus in the high visual recognition memory group was 53.3% higher than that in the low group (2.04 ± 0.22 vs 1.33 ± 0.57 , $P < 0.05$). In addition, the NR2B level in the high one was 25.0% higher than that in the low group in the striatum ($P < 0.05$). While, no significant difference was found in the protein levels of NR2B subunit in other brain regions between the two groups ($P > 0.05$).

Discussion

In the present study, we observed that both NR1 and NR2B subunits in the hippocampus in the high visual recognition memory group were remarkably higher than those in the low group. So the NMDA receptor NR1/NR2B subtype is higher in the high visual recognition memory group, although it is unclear whether there is any difference in the basic expression levels of the other subtypes of NMDA

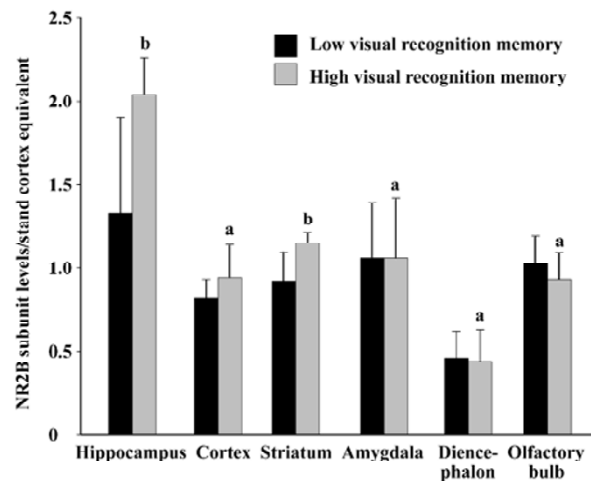


Figure 3. Relative expression levels of NR2B subunits in six brain regions in rats with different visual recognition memory. $n=8$. Mean \pm SD. ^a $P > 0.05$, ^b $P < 0.05$ vs the group with low visual recognition memory.

receptors. The result is consistent with the previous studies, which found that the transgenic mice overexpressing NR2B subunit in the forebrains exhibited an enhancement of novel-object-recognition memory^[10], and the CA1-specific NR1 subunit knockout mice exhibited impairment in object recognition^[15]. Therefore, our data provided more evidence *in vivo* to support the concept that NMDA receptors in the hippocampus are involved in visual recognition memory.

Previous studies showed that lesions to the perirhinal, postrhinal cortex and associated structures induced deficits in the ability to recognize single objects^[16,17], and that NMDA receptor blockade in the perirhinal cortex impaired object discrimination in rats^[18]. It seems that NMDA receptors in the cortex are related to visual recognition memory. However, in our study, no change in the basic expression levels of NR1 and NR2B subunits in the cortex was found between the groups with different visual recognition memory. Quinlan *et al* also reported that the NR2B subunit level in the visual cortex remained unchanged by visual experience^[19]. The explanation for these converse results might be that the cortical coding of object recognition decays quickly and is not sufficient for maintaining information about objects across longer retention intervals (eg 24 h)^[20]. It is also possible that the NMDA receptor NR2B subunit in some sub-regions of the cortex is related to visual memory. So our results at least suggest that the basic expression levels of NR1 and NR2B subunits in the cortex correlated less with visual recognition memory when a 24 h retention interval was imposed.

The striatum is a brain region involved in procedural

memory and diverse forms of implicit memory^[21,22]. It is well known that glutamatergic input from the cortex to the striatum is critical for striatal learning^[23] and NR2B subunit in the striatum is correlated with water maze performance in the inferior group of the aged rats^[24]. Our results here provided more evidences that the basic expression level of NR2B subunit in the striatum was also correlated with visual recognition memory.

The novel-object-recognition task was used in this study, which is based on the spontaneous preference that animals display for novel objects. This task is considered to need only “familiarity judgment” as to whether the objects presented in the choice phase are novel or familiar to the animals^[25]. The advantage of this method is that performance does not depend on the retention of a rule, and it is not influenced by changes in response to reward, which can provide a relatively pure measure of “working memory”^[26].

In conclusion, the main finding of the present study is that the basic expression level of the NMDA receptor NR1/NR2B subtype in the hippocampus and striatum is related to visual recognition memory.

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