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Research report

Brain networks activated to form object recognition memory

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ABSTRACT

Object recognition memory allows discrimination of familiar and novel objects. Previous studies have shown the importance of several brain regions for object recognition memories; however, the mechanisms underlying the consolidation of object recognition (OR) memory at the anatomic level remain unknown. Here, we analyzed the brain network for the generation of OR memory in mice by measuring the expression of the immediate-early gene c-fos. We found that c-fos expression was induced in the hippocampus (CA1 and CA3 regions), insular cortex (IC), perirhinal cortex (PRh), and medial prefrontal cortex (mPFC) when OR memory was generated, suggesting that gene expression in these brain regions contributes to the formation of OR memory. Consistently, inhibition of protein synthesis in the mPFC blocked the formation of long-term OR memory. Importantly, network analyses suggested that the hippocampus, IC, PRh and mPFC show increased connectivity with other brain regions when OR memory is formed. Thus, we suggest that a brain network composed of the hippocampus, IC, PRh, and mPFC is required for the generation of OR memory by connecting with other brain regions.

1. Introduction

Recognition memory confers the ability to learn and memorize the novelty of entities (Rossato et al., 2007). Importantly, mice and humans generate object memories that allow discrimination of familiar and novel objects (Ennaceur and Delacour, 1988; Antunes and Biala, 2012).

Memory consolidation is the process for the stabilization of a labile short-term memory (STM) and formation of a long-term memory (LTM) (Squire and Alvarez, 1995; Dudai, 1996). A crucial molecular mechanism of memory consolidation is the requirement for the activation of gene expression (Flexner et al., 1965; Davis and Squire, 1984; Silva et al., 1998). Importantly, the activation of gene expression necessary for consolidation is mediated by the transcription factor cAMP-responsive element-binding protein (CREB), which is a central regulator of neural activity-dependent transcription (Bourtchuladze et al., 1994; Kida et al., 2002).

CREB activates the transcription of immediate-early genes (IEGs) such as c-fos in an activity- and learning-dependent manner (Sheng et al., 1990; Kaczmarek and Robertson, 2002). Importantly, abundant studies have shown that brain regions showing learning-induced IEG expression play essential roles in gene expression-dependent memory processes including consolidation, reconsolidation, and extinction (Morrow et al., 1999; Santini et al., 2004; Mamiya et al., 2009). Therefore, IEG expression has been widely accepted as a marker to identify brain regions that are activated in response to learning or memory retrieval (Guzowski et al., 2001; Frankland et al., 2004; Mamiya et al., 2009).

Previous pharmacological and lesion studies have shown that multiple brain regions contribute to the learning and memory formation of object recognition. Gene expression is required in the perirhinal cortex (PRh) and insular cortex (IC) for the formation of object recognition (OR) memory. Conversely, several studies have suggested the importance of the hippocampus in the formation of OR memory, while other studies demonstrated that the hippocampus is required for only object location memory (Hammond et al., 2004; Rossato et al., 2007; Cohen et al., 2013; but see, Mumby et al., 2002a; Roozendaal et al., 2010). However, the distinct roles and connectivity of multiple brain regions for the formation of OR memory still remain unclear.

In novel object recognition memory tasks, mice are allowed to learn object novelty through exploration of the object (Ennaceur and Delacour, 1988; Antunes and Biala, 2012). The difference in exploration times between familiar and novel objects reflects the familiarity of a familiar object. In this study, to understand the mechanisms for the formation of OR memory at the anatomical level, we examined the activation of brain regions when OR memory is generated by analyzing the expression of an IEG and tried to identify the brain networks required to generate this memory. We also examined a role of gene expression in the mPFC in the formation of OR memory.

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2. Materials and methods

2.1. Animals

All experiments were conducted according to the Guide for the Care and Use of Laboratory Animals (US National Institutes of Health, Bethesda, MD). All animal experiments were approved by the Animal Care and Use Committee of Tokyo University of Agriculture (authorization number: 250008). All surgical procedures were performed under Nembutal anesthesia, and every effort was made to minimize suffering. Male C57BL/6N mice were obtained from Charles River (Yokohama, Japan). The mice were housed in cages of 5 or 6, maintained on a 12 h light/dark cycle, and allowed *ad libitum* access to food and water. The mice were at least 8 weeks of age at the start of the experiments, and all behavioral procedures were conducted during the light phase of the cycle. All experiments were conducted blind to the treatment condition of the mice.

2.2. Novel object recognition task

A novel object recognition task was performed as described previously (Antunes and Biala, 2012). For the habituation phase, individual adult male mice were placed in a chamber (40 × 40 × 40 cm) and allowed to explore the context freely for 20 min for 3 days. After a period of 24 h, for the training phase, the mouse was placed in the same chamber containing 2 different objects for 15 min and allowed to explore the objects. After a period of 24 h, for the testing phase, one of the objects was exchanged with a new one although the locations of two objects were not changed. The duration of exploration behavior exhibited by the adult mouse was determined with a hand-held stopwatch and exploration preference was calculated using the formula: (time exploring the familiar object)/(time exploring the novel object + time exploring the familiar object) or (time exploring the novel object)/(time exploring the novel object + time exploring the familiar object). A discrimination index was calculated using the following formula: (time exploring the novel object − time exploring the familiar object)/(time exploring the novel object + time exploring the familiar object).

For the first experiment (Fig. 1), we examined the training condition that allowed the mice to form OR memory. The mice were habituated for 3 days or not, and underwent the training and testing periods.

For the second experiment (c-fos immunohistochemistry), the mice were divided into 4 groups as described in the Results (No Habit/Object, No Object, No Habit, and Novel Object Recognition [NOR] groups).

For the third experiment (Fig. 4), the mice were trained as described above and received a microinfusion of the protein synthesis inhibitor anisomycin (ANI, 62.5 μg; Sigma) or artificial cerebrospinal fluid (ACSF) into the mPFC immediately after the training. The mice were tested at 2 or 24 h after the training. ANI was dissolved in vehicle (VEH) solution (ACSF) and adjusted to pH 7.0–7.4 with NaOH.

2.3. Immunohistochemistry

Immunohistochemistry was performed as described previously (Mamiya et al., 2009; Inaba et al., 2015). Ninety min after the training, mice were anesthetized and then perfused with 4% paraformaldehyde. Brains were removed, fixed overnight, transferred to 30% sucrose, and stored at 4° C. Two days later, brains were frozen using liquid nitrogen and then coronal sections (30 μm) were cut in a cryostat. We used a polyclonal rabbit primary antibody for c-fos (1:5000; Cat#PC38, RRID: AB_2106755; Millipore) and biotinylated goat anti-rabbit IgG (SAB-PO kit; Nichirei Biosciences, Tokyo, Japan). Structures were defined anatomically according to the atlas of Franklin and Paxinos (2012). Quantification of c-fos-positive cells in sections (100 × 100 μm) of the olfactory bulb (OB; bregma between +4.28 and +3.92 mm), medial prefrontal cortex (mPFC; bregma between +2.10 and +1.98 mm), anterior cingulate cortex (ACC; bregma between +0.8 and +1.0), IC (bregma between +0.8 and +1.0), amygdala (bregma between −1.22 and −1.34 mm), dorsal hippocampus (bregma between −1.46 and −1.82 mm), temporal cortex (TC; bregma between −3.88 and −4.00), PRh (bregma between −3.88 and −4.00), and entorhinal cortex (EC; bregma between −3.88 and −4.00) was performed using a computerized image analysis system (WinROOF version 5.6 software; Mitani Corporation, Fukui, Japan). Immunoreactive cells were counted bilaterally with a fixed sample window across at least 3 sections by an experimenter blind to the treatment condition. The number of c-fos-positive cells in each group was expressed as the ratio of the averaged values in the No Habit/Object group.

2.4. Surgery for drug microinfusion

Surgeries were performed as described previously (Mamiya et al., 2009; Inaba et al., 2015). Under Nembutal anesthesia and using standard stereotaxic procedures, stainless steel guide cannulae (22 gauge) were implanted through a hole drilled in the skull at anteroposterior +2.7 mm and mediolateral ± 0 mm from bregma at 25° relative to the craniocaudal axis. The mice were allowed to recover for at least 1 week after surgery. Infusions into the mPFC (0.5 μL) were made at a rate of 0.25 μL/min through injection cannula (28 gauge) extending 1.77 mm below the surface of the brain. This dose of locally infused ANI inhibits > 90% of protein synthesis for at least 4 h (Rosenblum et al., 1993). Only mice with a cannula tip within the boundaries of the mPFC was included in the data analysis (Fig. 4C, F).

2.5. Network construction and graph theoretical analysis

Correlation matrices were generated using Pearson's r values from the inter-regional c-fos expression data (Fig. 5A). For comparisons of average correlations between groups, Pearson's r values were calculated. All inter-regional correlations between the hippocampal, IC, PRh, or mPFC were used to assess condition-dependent changes in functional connectivity.

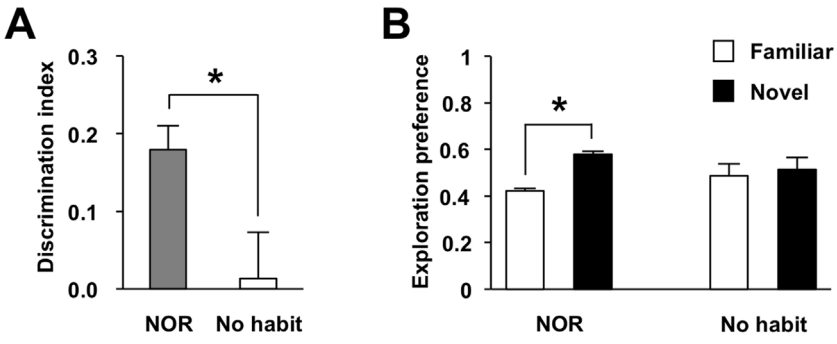

Fig. 1. Habituation to the task chamber is required to generate OR memory. (A, B) The mice were habituated (NOR) or not habituated (No Habit) to the task chamber. (A) Discrimination index. *P < 0.05, compared with the No Habit group. (B) Comparisons of exploration preference at test. NOR group, n = 10; No Habit group, n = 10. *P < 0.05, compared with the exploration preference for the novel object (paired *t* test).