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Research report

Mice lacking hippocampal left-right asymmetry show non-spatial learning deficits

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ABSTRACT

Left-right asymmetry is known to exist at several anatomical levels in the brain and recent studies have provided further evidence to show that it also exists at a molecular level in the hippocampal CA3-CA1 circuit. The distribution of N-methyl-D-aspartate (NMDA) receptor NR2B subunits in the apical and basal synapses of CA1 pyramidal neurons is asymmetrical if the input arrives from the left or right CA3 pyramidal neurons. In the present study, we examined the role of hippocampal asymmetry in cognitive function using β2-microglobulin knock-out (β2m KO) mice, which lack hippocampal asymmetry. We tested β2m KO mice in a series of spatial and non-spatial learning tasks and compared the performances of β2m KO and C57BL6/J wild-type (WT) mice. The β2m KO mice appeared normal in both spatial reference memory and spatial working memory tasks but they took more time than WT mice in learning the two non-spatial learning tasks (i.e., a differential reinforcement of lower rates of behavior (DRL) task and a straight runway task). The β2m KO mice also showed less precision in their response timing in the DRL task and showed weaker spontaneous recovery during extinction in the straight runway task. These results indicate that hippocampal asymmetry is important for certain characteristics of non-spatial learning.

1. Introduction

Left-right asymmetry is a fundamental feature of brain organization that has been found in different brain regions and across numerous species [1–6]. For many years, conventional research on laterality focused primarily on gross anatomical and cognitive laterality [7,8]. Recent studies, however, have revealed that laterality also exists at molecular and microscopic levels in the zebrafish habenula and the mouse hippocampus [9–15].

Kawakami et al. [12] have demonstrated that the distribution of N-methyl-D-aspartate (NMDA) receptor (NMDAR) NR2B (ε2) subunits in the wild type (WT) mouse hippocampus is asymmetrical between synapses at the apical and basal dendrites of individual neurons, and between synapses formed by inputs from the left and right CA3 pyramidal neurons (Fig. 1)[15]. Specifically, a larger amount of NR2B subunits are localized at apical synapses that receive projections from the left CA3 (NR2B-dominant synapses), whereas a lower amount of NR2B subunits are distributed at apical synapses that receive inputs from the right CA3 (NR2B-non-dominant synapses) [12,14]. The relationship between NR2B dominance and left-right CA3 inputs is reversed at the basal dendrite, i.e., NR2B-dominant synapses receive inputs from the right CA3 and NR2B-non-dominant synapses receive inputs from the left CA3. Importantly, this asymmetrical allocation of NR2B subunits was found to be similar in left and right CA1 pyramidal neurons [12] but was not observed in CA1 interneurons [15]. Functionally, NR2B-dominant synapses show lower thresholds for activity-dependent long-term potentiation (LTP) than NR2B-non-dominant synapses [12].

To test the significance of hippocampal asymmetry in hippocampal-dependent cognitive function, we used β2-microglobulin knock-out (β2m KO) mice, which lack hippocampal asymmetry (Fig. 1 right-hand panel) [12]. β2-microglobulin is a component of major histocompatibility complex (MHC) Class 1 molecules and is required for the cell surface expression of MHC Class 1 [16]. MHC Class 1 molecules play a role in cellular immunity in the discrimination of self versus non-self proteins. It was believed that MHC was absent in the brain but recent studies have revealed the expression and functions of MHC Class 1 molecules in the brain [see 17 for review]. MHC Class 1 molecules are expressed in the cerebral cortex, hippocampus [18–20], motoneurons [21,22], and the mouse vomeronasal organ [23,24]. They regulate

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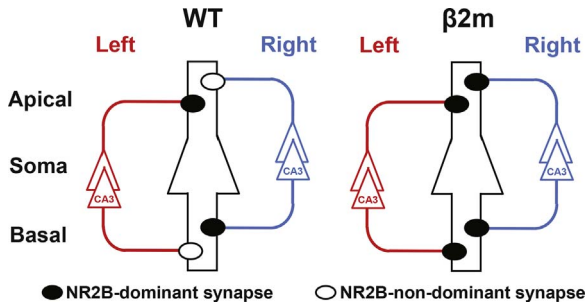

Fig. 1. Schematic drawing of afferent connections to a CA1 pyramidal neuron. Left and right CA3 pyramidal neurons and their axons are shown in red and blue, respectively. A postsynaptic CA1 pyramidal neuron at the center, outlined in black, represents postsynaptic neurons in both left and right hemispheres. Closed and open circles represent NR2B-dominant and NR2B-non-dominant synapses, respectively. Apical: apical dendrites; Basal: basal dendrites. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

activity-dependent refinement of visual nerve connections, synaptic plasticity in hippocampal and cerebellar slices, and spontaneous excitatory postsynaptic currents in dissociated hippocampal cultures [18,19,25–29]. Of particular relevance to the current study is the finding that MHC Class 1 is critical for the generation of hippocampal circuit asymmetry [11]. As shown in Fig. 1, the hippocampus of β2m KO mice lacks NR2B-non-dominant synapses, and thus contains NR2B-dominant synapses only, resulting in a total loss of circuitry asymmetry [11].

In the present study, we investigated the role of hippocampal asymmetry in the hippocampal-dependent cognitive functions, which include both spatial and non-spatial learning. We administered a battery of behavioral tasks that are believed to depend on hippocampal integrity. The tasks included a spatial reference memory task in a "cheeseboard" open arena, a spatial working memory task in an eight-arm radial maze, a test of response timing and response inhibition using a differential reinforcement of lower rates of behavior (DRL) schedule in operant chambers, and the acquisition and extinction of a simple appetitive approach response in a straight runway task. We also examined other non-specific measures of behavior, including locomotor activity, habituation to a new environment, and motivation for a food reward using a progressive-ratio schedule of reinforcement for instrumental conditioning. The performance of β2m KO mice in each of these tasks was compared to that of age- and sex-matched WT mice.

The β2m KO mice showed a slower initial acquisition of non-spatial tasks but showed normal acquisition and probe performance in the spatial reference memory and spatial working memory tasks. These results suggest that hippocampal asymmetry is important for some aspects of non-spatial learning.

2. Experimental procedure

2.1. General methods

2.1.1. Subjects

Male β2-microglobulin knock-out mice (referred to as "β2m KO mice") were originally purchased from The Jackson Laboratory (Bar Harbor, ME, USA), bred in-house at the animal facility in Kyusyu University (Fukuoka, Japan), and transported to the experimental facility in Keio University for the current study at six weeks of age. C57BL6/J mice served as control subjects and were purchased from the Nippon Bio-supp. Center (Tokyo, Japan). Mice were kept in a temperature-controlled holding room (21–24 °C) under a reversed light-dark cycle (lights off from 08:00 to 20:00). Mice were housed in groups of three to six of the same strain in standard mouse cages (Three to four mice per cage: 17.5 × 24.5 × 12.5 cm [w × d × h]; five to six mice per cage: 22 × 35 × 13.5 cm [w × d × h]). Food and water were available ad libitum except during periods of food or water restriction (see the relevant Procedure sections). All experiments were conducted in experimental rooms that were approved by Keio University Department of Science and Engineering Genetic Modification Experiment Committee (No. 26-16), and in accordance with the Guidelines of the Japanese Society of Animal Psychology. All experimental procedures were approved by the Keio University animal experimental center (No. 08007-6).

2.2. Experiment 1: spatial reference memory in the cheeseboard task (a "dry" version of the Morris water maze task)

2.2.1. Subjects

For the cheeseboard task, 17 β2m KO mice (C57BL6 genetic background, 10–14 weeks old) and 14 C57BL6/J mice (20–27 weeks old) were used. All animals were experimentally naïve. Prior to the start of the experiment, all mice were subjected to a food deprivation schedule and maintained at 90% of their normal body-weight.

2.2.2. Apparatus

A circular arena (90 cm in diameter) surrounded by a circular wall (45 cm high) was used (Fig. 2A). The floor and wall of the arena were made of white acrylic material. The arena stood on a wooden desk (50 cm above the room floor) that was installed in the center of the experimental room (170 × 170 × 280 cm [w × d × h]). There were various extra-maze stimuli in the room, including several posters on the walls, objects suspended from the ceiling, a small desk, and a door. During the experimental sessions, the experimenter always stood in a fixed position by the desk, except when the animals were placed in, and removed from, the arena. On the surface of the arena floor there were 61 equally spaced holes (1 cm diameter, 0.5 cm depth, 10 cm intervals between holes). Each of the holes could contain one oat grain (approximately 25 mg, Kawahara Bird-Animal Trading Co. Ltd, Tokyo, Japan.) as the reinforcer. A speaker mounted near the ceiling of the room broadcast constant white noise (65 dB) during the experiment to mask extraneous noise. A CCD camera was fixed on the ceiling directly above the center of the arena to monitor and record the movement of the animals inside the arena. The image was transmitted from the camera to a computer running on Windows XP, which recorded and analyzed the image with a tracking software (Any-maze, Stoelting Co., Illinois, U.S.A.).

2.2.3. Procedure

The cheeseboard task was conducted over five consecutive days, including the first two days of habituation sessions. Each habituation session consisted of three trials. Before the start of each habituation trial, an oat grain was buried in each of the 61 holes. At the start of each trial, the mouse was introduced into the arena from one of the six potential starting positions and then allowed to explore the entire arena. Once the mouse found the food and started to eat it, the experimenter waited for 15 s before removing the mouse from the arena. If the mouse did not find the food in 120 s, the experimenter guided the animal to one of the randomly chosen holes and encouraged it to eat. After each trial, the experimenter wiped the arena clean with 70% ethanol and re-baited the hole before the next animal was run. Four or five mice were brought into the experimental room together and run in a sequence, so that the inter-trial interval (ITI) for each mouse was about 15 min. Each mouse experienced all six starting positions (one per trial) across the six trials of the habituation phase (two sessions).

Following the habituation phase, the acquisition training was administered for three sessions from Days 3 through 5 in a similar manner to the habituation sessions, except for the followings; first, only one hole at the center of the NE quadrant was baited in each trial; second, each acquisition session consisted of five trials and the mice were released from a different starting position in each trial.

Fifteen min after the completion of the third acquisition session