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Landmark-based but not vestibular-based orientation elicits mossy fiber synaptogenesis in the mouse hippocampus

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Abstract

This study tries to shed light on the paradoxical finding that two inbred strains of mice C57BL/6 (C57) and DBA/2 (DBA), with differences in hippocampal function, perform similarly in the water maze (WM). Mice from both strains were trained on WM protocols permitting or preventing the use of vestibular signals. Hippocampal involvement in performance was then assessed by estimation of post-training mossy fiber (MF) synaptogenesis. We found that C57 and DBA mice performed similarly when both visual and vestibular information were available but only C57 mice exhibited new MF synapses. Disruption of vestibular inputs impaired performance in DBA mice but not in C57 mice which still exhibited a post-training increase of hippocampal MF synaptic terminals. This strain-specific dissociation indicates that DBA mice can navigate successfully by relying on vestibular signals without engaging their hippocampus. In contrast, vestibular signals are irrelevant for C57 mice since their suppression neither disrupts their behavior nor prevents the formation of new hippocampal synapses. These findings suggest some caution is required in considering performance on standard WM protocols as an index of hippocampus-based learning. Estimating the extent of post-training mossy fiber synaptogenesis would be helpful in solving this issue.

Keywords: Inbred mice; Morris water maze; Vestibular signals; Mossy fiber synaptogenesis

1. Introduction

A number of findings have implicated the mossy fiber (MF) pathway in spatial memory. Examples include disruption (Handelmann & Olton, 1981; Steffenach, Sloviter, Moser, & Moser, 2002) and reversible inactivation (Lassalle, Bataille, & Halley, 2000; Meilandt, Barea-Rodriguez, Harvey, & Martinez, 2004) of MF neurotransmission in rodents which affects spatial learning in the water maze (WM). Moreover, computational models of the CA3 region, predict that blocking MF or perforant path projections to CA3 would cause spatial learning impairments (Treves & Rolls, 1992, 1994; Rolls & Kesner, 2006).

In inbred mice, the area of the MF pathway strongly varies between strains (Barber, Vaughn, Wimer, & Wimer, 1974) and this variation is largely heritable (Crusio, Genthner-Grimm, & Schwegler, 1986). One such well-documented hereditary trait is density of intra/infrapyramidal MF projections (IIP-MF) formed by the terminal boutons of granule cells axons on the basal dendrites of pyramidal cells in the CA3 region (Barber et al., 1974; Vaughn, Matthews, Barber, Wimer, & Wimer, 1977). Of interest one, correlations between the extent of the IIP-MF projections and spatial learning have been demonstrated (see Crusio & Schwegler, 2005 for a review) with the larger IIP-MF projections found in those inbred mouse strains which perform well on either the radial maze (Crusio, Schwegler, & Lipp, 1987, 1993; Jamot, Bertholet, & Crusio, 1994; Schwegler, Crusio, & Brust, 1990) or the water maze (Bernasconi-Guastalla, Wolfer, & Lipp, 1994; Schöpke, Wolfer, Lipp, & Leisinger-Trigona, 1991; Schwegler, Crusio, Lipp, & Heimrich, 1988). These data have led us to consider that variation in MF distribution could predict performance in spatial tasks.

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Contradictory findings emerge from comparisons between the C57BL/6 (C57) and DBA/2 (DBA) mouse strains. In a number of studies, extensive evidence has shown that C57 mice outperform DBA mice in a variety of spatial situations (Ammassari-Teule & Caprioli, 1985; Crusio et al., 1987; Middei, Restivo, Sgobio, Passino, & Ammassari-Teule, 2004; Passino, Middei, Restivo, Bertaina-Anglade, & Ammassari-Teule, 2002; Upchurch & Wehner, 1989) with the latter strain relying poorly on extra-maze cues for orienting (Ammassari-Teule, Milhaud, Passino, Restivo, & Lassalle, 1999). The limited spatial capacities of DBA mice seem to be closely tied to their poor hippocampal plasticity (Matsuyama, Namgung, & Routtenberg, 1997; Nguyen, Abel, Kandel, & Bourtchouladze, 2000; Wehner, Sleight, & Upchurch, 1990) and reduced synaptic architecture (Barber et al., 1974; Schwegler & Crusio, 1995). However, despite their observed differences in hippocampal function (Ammassari-Teule et al., 1999) several reports indicate that C57 mice and DBA mice perform similarly when trained in the WM (Owen, Logue, Rasmussen, & Wehner, 1997; Wahlsten, Cooper, & Crabbe, 2005).

Spatial navigation in rodents can be achieved using a number of different mechanisms, such as landmark-based orientation, which implies that the goal position is inferred from spatial relationships between environmental cues and therefore requires the hippocampus (O'Keefe & Nadel, 1978), or path integration which allows reaching an invisible goal but by constant monitoring of self-motion via vestibular signals (Stackman & Herbert, 2002). Our hypothesis is that depressed hippocampal function could decrease the efficiency of landmark-based orientation in DBA mice making them reliant on an alternative mechanism depending less on the hippocampus but warranting an accurate WM performance. To examine this hypothesis, we took note of findings showing that rats extensively trained in a water maze show long-term formation of new mossy fiber synapses (Ramirez-Amaya, Escobar, Chao, & Bermudez-Rattoni, 1999, 2001). Accordingly, we compared the performance of C57 mice and DBA mice on two WM training protocols and controlled the strain-specific involvement of the hippocampus by estimating post-training mossy fiber synaptogenesis in each strain. In experiment 1, mice were subjected to standard water maze testing in a cued-environment allowing implementation of any orientation system. In experiment 2, mice were rotated between trials to disturb vestibular signals and leave landmark-based orientation as the main available modality for navigating.

2. Materials and methods

2.1. Animals

Thirty-six C57BL/6J and 36 DBA/2J adult male mice were obtained from Charles River (Calco, Italy). Mice were housed in groups of three with food and water available *ad libitum* in a room with a 12-h light/12-h dark cycle (lights on at 7 a.m.). In experiment 1, mice from each strain were sub-divided into three groups: trained (TR, $N = 6$), swimming control (SC, $N = 6$), and control cage (CC, $N = 6$) groups. In experiment 2, the same three conditions were considered but mice were subjected to a 1-min rotation protocol (R) (see Section 2.3) before each training (TR-R, $N = 6$) or swimming (SC-R, $N = 6$) episode. The control cage groups were also subjected to the same rotation protocol (CC-R, $N = 6$). Experiments were carried out in compliance with the guidelines laid down by the National Institute of Health (NIH) in the USA regarding the care and use of animals and with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and according to institutional ethical guidelines.

2.2. Apparatus

The WM was a white circular tank (104 cm in diameter) filled with opaque water at a temperature of 22–23 °C. A platform made of grey plastic material (12 cm in diameter) was submerged 0.5 cm below the water surface and 13 cm from the edge of the tank. The tank was placed in a sound-proof room and surrounded by four curtains at a distance of 50 cm with each curtain bearing a distinct cue card (20 × 20 cm). The tank used for SC groups was smaller (60 cm in diameter) and SC sessions were performed without visual cues. The latency to find the platform was recorded by means of a computer-based video tracking system Ethovision® (Noldus, The Netherlands).

2.3. Experimental procedure

Mice were first subjected to one habituation session during which no visual cue was attached on the curtains and each mouse was trained to climb onto the platform signalled by a flag. Mice were left swimming until they rested on the platform for at least 1 min. All mice reached this criterion within 5 min.

Training started on the following day and was carried out for 10 consecutive days (days 1–10) to ensure an extensive training experience. Mice performed four trials per day. On each trial, they were released from one of four fixed points according to a sequence varying randomly from trial to trial. Mice were allowed to swim until they climbed onto the platform; those that did not locate the platform within 60 s were driven on it by the experimenter. Mice were left 20 s on the platform then put in a warmed cage. Inter-trial intervals (ITIs) lasted 3 min. The dependent variables recorded were: (i) latency for reaching the platform and (ii) distance traveled. Mice from the SC groups were allowed to swim for the same time as the slower mice in the trained groups. During the ITIs, the trained and the swimming control mice were left either undisturbed in the warmed cage (experiment 1) or rotated (experiment 2) according to a previously described procedure (Commins, Gemmell, Anderson, Gigg, & O'Mara, 1999; Francia, Santucci, Chiarotti, & Alleva, 2004). Briefly, they were placed in an opaque warmed box (10 × 10 × 8 cm) fixed on a tray lying on a centrifuge basement. Rotation was performed at 56 rpm in a constant direction. During experiment 1 mice from the CC groups remained in their cage, whereas for experiment 2 mice from the CC groups were rotated for one minute. This rotation procedure was repeated three times (3 min inter-trial interval) every day for ten consecutive days (see Commins et al., 1999 for details). For the probe trial (day 11) the platform was removed from the water maze and trained mice were left to swim for 60 s. Control mice (SC) swam in their tank for the same duration. Number of crossings over the platform area (that is the 12-cm diameter circular area corresponding to the space where the platform was previously positioned) was recorded in the trained groups.

2.4. Timm's staining

Six days after the end of behavioral testing, the mice were anesthetized with a solution of chloride hydrate (400 mg/kg) and perfused transcardially with a Sulphide solution (Na_2S; $NaH_2PO_4 \cdot 2H_2O$) for 2 min, 3% glutaraldehyde solution for 3 min and again with a Sulphide solution for 2 min. At the end of the perfusion, brains were rapidly removed and post-fixed for 24 h in a 20% sucrose solution diluted in 3% glutaraldehyde solution. The brains were then snap-frozen in isopentane at −80 °C, cut by