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Lesions of the dorsal subiculum and the dorsal hippocampus impaired pattern separation in a task using distinct and overlapping visual stimuli

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ABSTRACT

The contribution of the dorsal subiculum (DS) and of the dorsal hippocampus (DH) to memory for distinct and overlapping visual stimuli was examined. Rats with selective lesions of the DS or the DH were compared to sham-operated rats on a delayed matching-to-place task guided by distal visual cues in a modified radial-arm maze. Overlapping distal visual cues could be perceived from three arm entrances (adjacent arms) and a unique set of distal cues were more likely to be seen from the other two arm entrances (distinct arms). Rats with DS lesions were impaired on trials with baited adjacent arms, but not on trials with baited distinct arms. Rats with DH lesions were impaired on both types of trials. These results suggest that the DS and the DH are necessary for pattern separation and that they may have different contributions to memory.

1. Introduction

In the last decades, the roles of the medial temporal lobe in memory have been intensively investigated. Experiments with humans and animals established that this brain region is essential for declarative memory (for reviews see: Milner, Squire, & Kandel, 1998; Squire, Stark, & Clark, 2004). The medial temporal lobe comprises the hippocampus (dentate gyrus and Ammon's horn), the subiculum, as well as the entorhinal, postrhinal (parahippocampal in primates), and perirhinal cortices. Each component appears to have a specific contribution to declarative memory. For example, in rodents, the perirhinal cortex is central for object recognition (Mumby, Piterkin, Lecluse, & Lehmann, 2007; Winters & Bussey, 2005a, 2005b; Winters, Forwood, Cowell, Saksida, & Bussey, 2004) and the hippocampus is crucial for spatial memory (O'Keefe and Nadel, 1978). Unlike most of the hippocampal formation components, the subiculum did not receive much attention and only a few experiments examined its behavioral functions. The anatomical position of the subiculum within the medial temporal lobe circuit indicates that it might play an important role: it shares reciprocal connections with the entorhinal, postrhinal, and perirhinal cortices and it receives most of its inputs from CA1 (Finch, Nowlin, & Babb, 1983; Kloosterman, Witter, & Van Haeften, 2003; Kosel, Van Hoesen, & Rosene, 1983; Naber, Witter, & Lopes da Silva, 2001; Steward & Scoville, 1976). Moreover, the subiculum is considered as the major output structure of the hippocampal formation (Amaral & Witter, 1995). Damage to the subiculum often produces spatial memory deficits smaller than those observed following damage to the hippocampus (Bolhuis, Stewart, & Forrest, 1994; Galani, Coutureau, & Kelche, 1998a; Galani, Weiss, Cassel, & Kelche, 1998b; Jarrard, 1986; Jarrard, Davidson, & Bowring, 2004; Morris, Schenk, Tweedie, & Jarrard, 1990). The learning impairment caused by damage to both the hippocampus and the subiculum is also generally more severe than the one resulting from lesions of the hippocampus or of the subiculum alone (Allen, Potvin, Doré, & Goulet, 2004; Bunsey & Eichenbaum, 1995; Cho & Jaffard, 1995; Jarrard, 1986; Morris et al., 1990; Potvin, Allen, Thibaudeau, Doré, & Goulet, 2006; Potvin, Doré, & Goulet, 2007). The effects of hippocampal and subicular damage suggest that the subiculum and the hippocampus contribute to related functions in memory. However, the specific contributions of each structure remain unknown.

Many experiments demonstrated that the dorsal, unlike the ventral, portion of the hippocampus is essential for the learning of spatial memory tasks (for reviews see Bannerman et al., 2004; Moser & Moser, 1998). A similar dorsal/ventral dissociation was also proposed for the subiculum (O'Mara, 2005, 2006). Recently,

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we demonstrated that the integrity of the dorsal portions of both the subiculum and the hippocampus was required to perform a radial-arm maze task in the dark and it suggests that the dorsal subiculum is critical for the memory of idiothetic stimuli or self-movements (Potvin et al., 2007). In the dark, performance was disrupted by selective lesions of either the dorsal subiculum (DS) or the dorsal hippocampus (DH) whereas in the light, when proximal visual cues were available, learning was impaired by combined lesions of the DS and the DH but not by lesions of only one of two. Since idiothetic stimuli and proximal visual cues could be used together in the light, it was impossible to determine whether the combined DS–DH lesions affected visual memory and why damage to only one component did not affect performance. The main goal of the present experiment was to examine the specific contributions of the DS and the DH to visual memory.

Theoretical models proposed that pattern separation, the ability to distinguish overlapping representations, is a central feature of declarative memory (Marr, 1971; O'Reilly and Rudy, 2001; Treves & Rolls, 1994). Empirical data (Bakker, Kirwan, Miller, & Stark, 2008; Gilbert, Kesner, & Lee, 2001; Leutgeb, Leutgeb, Moser, & Moser, 2007; Leutgeb, Leutgeb, Treves, Moser, & Moser, 2004; McHugh et al., 2007; Vazdarjanova & Guzowski, 2004) and computational models (McClelland & Goddard, 1996; O'Reilly and Rudy, 2001; Rolls, 1996) suggest that the dentate gyrus (DG) and CA3 field are important for pattern separation. In these models, it is believed that the representations resulting from pattern separation are retrieved through the CA1-entorhinal pathway. However, it is unknown whether the CA1-subiculum-entorhinal pathway plays a role in this process. In this experiment, the possible contribution of the subiculum in pattern separation of visual stimuli was examined in rats. Using a delayed matching-to-place paradigm in a modified radial-arm maze, we tested if selective lesions of the DS or of the DH (DG, CA3, and CA1) disrupted the memory of distinct and/or overlapping stimuli. The experimental setup included three adjacent arms (overlapping arms) in which the same distal visual cues could be perceived from the arm entrances and two other arms (distinct arms) in which a unique set of distal cues was more likely to be seen from each of the two arm entrances. To minimize the contribution of idiothetic cues to performance, the experimenter positioned the rat in different start locations in the sample phase and the test phase of each trial. It was assumed that performance on trials with baited overlapping arms required visual pattern separation abilities whereas performance on trials with baited distinct arms did not.

2. Methods

2.1. Subjects

The subjects were 35 *Long–Evans* male rats (Charles River, St-Constant, Canada) weighing 290–310 g at the time of surgery. They were housed individually in cages and kept on a 12:12-h light–dark cycle (light at 7:00 a.m.). All behavioral testing was conducted during the light phase. Throughout the experiment, the experimenters were blind to the nature of treatment. The research received approval from the Comité de Protection des Animaux de l'Université Laval, which is responsible for the application and enforcement of the rules of the Canadian Council on Animal Care.

2.2. Surgery

Rats were randomly assigned to three groups that either received bilateral excitotoxic lesions of the dorsal hippocampus (DH, $n = 12$), of the dorsal subiculum (DS, $n = 13$), or were sham-operated (SHAM, $n = 10$). The DH lesions were aimed at damaging approximately the 30% of the hippocampus from the septal extremity to preserve as much as possible the dorsal portion of the subiculum. The DS lesions were meant to remove approximately 30% of the subiculum from the septal extremity. General anesthesia was induced and maintained by isoflurane (1.0–2.5%) mixed with oxygen. The ears and scalp were locally anesthetized by subcutaneous injections (sc) of Marcaine (7.5 mg/ml; ears: 0.1 ml, scalp: 0.1 ml). Once the rat was placed into the stereotaxic apparatus (Kopf Instruments, Tujunga, CA), a midline incision was made in the scalp to expose the skull. With bregma and lambda flat, the bone overlying the dorsal hippocampus or the dorsal subiculum was removed. Multiple injections of *N*-methyl-D-aspartate (NMDA; Sigma–Aldrich, Saint Louis, MN, USA) dissolved in phosphate-buffered saline (10 μl/μg) were delivered via a micropipette (35–40 μm) by an automatic nanoliter injector (Nanoject II, Drummond Scientific Company, PA, USA). Table 1 shows the stereotaxic coordinates relative to bregma and volumes of NMDA injected for DH and DS lesions. After each injection, the micropipette was left in place for 1 min to allow diffusion of the solution around each injection site. The same procedure was performed for sham-operated rats except that no injection was made and the pipette was lowered in the neocortex without penetrating the hippocampus or the subiculum. Half of the SHAM rats underwent a sham DS surgery and the other half a sham DH surgery. At the end of surgery, the rats were hydrated (NaCl, 0.9%; 6 ml sc) and given an injection of an analgesic (Anafen, 10 mg/ml; 0.15 ml sc). All rats also received an injection of diazepam (0.5 mg/ml; 0.5 ml) immediately after the surgery to minimize the risk of seizures. During the first three postoperative days, an analgesic was administered through food (Anafen, 10 mg/ml; 0.25 ml). Animal care technicians verified that the food was eaten on each day.

2.3. Apparatus

A wooden 8-arm radial maze elevated 60 cm above the floor was used. The octagonal central platform was 37 cm in diameter and was surrounded by an opaque wall, 40 cm high. Five arms, 60 cm long and 9 cm wide, were used as choice arms whereas black ABS tubes (diameter: 11.2 cm; length: 40 cm) replaced the other three arms and served as start runways (Fig. 1). Three of the five choice arms were separated by a 45° angle (overlapping arms) whereas two were separated by a 90° angle (distinct arms). The overlapping arms were separated from each distinct arm by a minimum angle of 90°.

The floor of each of the five choice arms was covered with a black acrylic insert with a 2 cm-high rim. A recessed food well, 1.5 cm in diameter and 1 cm deep, was located at the end of each choice arm opposite to the platform. An opaque acrylic start box ($15 \times 15 \times 15$ cm) was connected to the distal end of each tube.

Table 1
Stereotaxic coordinates and injection volumes of NMDA.

AP	ML	DV	Volume (μl)
Lesion of the dorsal hippocampus (DH)			
−2.9	±1.1	−3.8	0.08
−3.2	±2.3	−3.7	0.06
−3.9	±1.9	−3.7	0.07
−4.1	±3.3	−3.7	0.08
Lesion of the dorsal subiculum (DS)			
−5.1	±1	−3.7	0.05
−5.8	±2.8	−3.2	0.07
−6.2	±3	−3.3	0.05
−6.4	±3.80	−3.4	0.07

Note: AP, anteroposterior; ML, mediolateral; DV, dorsoventral.
All coordinates are given in millimeters and are relative to bregma.