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Contribution of the dorsal subiculum to memory for temporal order and novelty detection using objects, odors, or spatial locations in the rat

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ABSTRACT

The contribution of the dorsal subiculum (DS) to memory for temporal order and novelty detection was assessed using a spontaneous exploration paradigm with objects (visual/tactile stimuli), odors, or spatial locations (Hunsaker, Fieldsted, Rosenberg, & Kesner, 2008). Rats with selective excitotoxic lesions of the DS were compared to sham-operated rats (SHAM) in the two exploration tests. In temporal order tests, two previously explored stimuli were presented and normal rats typically show a preference for exploring the stimulus that was first explored compared to the other stimulus. In novelty detection tests, a familiar and a new stimulus were presented and normal rats typically have a preference for exploring new stimuli. In temporal order tests, results indicated that Group SHAM explored significantly more the first than the last stimulus they met when the stimuli were odors or objects. In addition, SHAM rats predictably displayed a significant preference for the new stimulus in the novelty detection tests with objects, odors, and spatial locations. Group DS did not differ from controls on the temporal order and the novelty detection tests with objects or odors. However, on the novelty detection test with spatial locations, Group DS differed from Group SHAM. These results suggest that the DS is necessary for the memory of spatial locations but not of objects and odors.

1. Introduction

A broad literature exists on the role of the hippocampal formation in memory. This brain region appears critical for many types of memory and could as well contribute to the general category called "declarative memory" (for reviews see Morris, 2007; Squire, Stark, & Clark, 2004). The hippocampal formation comprises the dentate gyrus (DG), the CA3, CA2, and CA1 fields, the subiculum, and the entorhinal cortex (EC). Research suggests that each of these components has a specific function (e.g. Hoge & Kesner, 2007; Leutgeb, Leutgeb, Moser, & Moser, 2007; Steffenach, Witter, Moser, & Moser, 2005). Within the hippocampal formation, the subiculum is under-investigated and little is known about its behavioral functions. Damage to this part of the brain can yield spatial memory deficits (Bolhuis, Stewart, & Forrest, 1994; Galani, Coutureau, & Kelche, 1998; Galani, Weiss, Cassel, & Kelche, 1998; Jarrard, 1986; Jarrard, Davidson, & Bowring, 2004; Morris, Schenk, Tweedie, & Jarrard, 1990). However, damage limited to its ventral portion does not produce obvious spatial memory impairment (Riegert et al., 2004). Accordingly, O'Mara (2005, 2006) proposed that the dorsal subiculum has a functional specialization in processing the information about space, movements, and memory whereas the ventral subiculum could be a physiological interface between memory and motivation. This proposition is also supported by more recent data demonstrating that the dorsal subiculum is necessary for the memory of self-motion cues (Potvin, Doré, & Goulet, 2007) and pattern separation of spatial memories (Potvin, Doré, & Goulet, 2009).

The strategic anatomical position of the subiculum suggests a major role for this subdivision within the hippocampal formation circuit. Two main paths link the information processed by the DG and CA3 to the entorhinal cortex, which is the major output structure of the hippocampal formation. One path crosses the CA1 field directly to the EC while the other goes through CA1 and the subiculum before reaching the EC (Finch, Nowlin, & Babb, 1983; Kloosterman, Witter, & Van Haeften, 2003; Kosel, Van Hoesen, & Rosene, 1983; Naber, Witter, & Lopes da Silva, 2001; Steward & Scoville, 1976). Thus, the subiculum represents an additional stage of information processing in the hippocampal formation loop circuit and the specific functions of this indirect route to the EC remains unknown.

Recently, Kesner and colleagues (Hoge & Kesner, 2007; Hunsaker & Kesner, 2008; Hunsaker et al., 2008) studied the behavioral functions of CA1 in a paradigm assessing memory for temporal order and novelty detection. Three types of stimuli were used: visual/tactile objects, odors, and spatial locations. Rats with lesions to the dorsal CA1 were impaired in temporal order of objects and spatial locations, but not of odors. On the other hand, CA1 dorsal lesion left

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novelty detection of objects or spatial locations intact. In the present experiment, we examined the functions of the dorsal subiculum using Kesner et al.'s paradigm. Since the subiculum forms a neuronal path to the EC that is distinct from the one going directly from the CA1 field to the EC, we hypothesized that lesions of the dorsal subiculum would produce different effects than those observed with lesions to CA1.

2. Methods

2.1. Subjects

The subjects were 21 *Long-Evans* male rats (Charles River, St-Constant, Canada) weighing 300–320 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 am). Access to food and water was unrestricted. All behavioral testing was conducted during the light phase. 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 two groups: bilateral excitotoxic lesions of the dorsal subiculum (DS, $n = 12$) or sham-operated controls (SHAM, $n = 9$). DS lesions were meant to remove approximately one third 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 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). The stereotaxic coordinates relative to bregma of the four injection sites were (1) anteroposterior (AP): −5.1, mediolateral (ML): ±1, dorsoventral (DV): −3.7; (2) AP: −5.8, ML: ±2.8, DV: −3.2; (3) AP: −6.2, ML: ±3, DV: −3.3; (4) AP: −6.4, ML: ±3.8, DV: −3.4. The injection volumes of NMDA varied between 0.05 and 0.07 μl. After each injection, the micropipette was left in place for 1 min to allow diffusion of the solution. 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 subiculum. At the end of surgery, the rats were hydrated (NaCl, 0.9%; 3 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

Two opaque acrylic cages (36.0 × 81.5 × 30.5 cm) were used for tasks with visuotactile objects and odors. The cage for olfactory tasks was dimly lit by distant neon lighting whereas the cage for object tasks was illuminated by a 40-W light bulb located above the centre of the apparatus. Visuotactile stimuli were two copies of ten three-dimensional objects made of various materials (plastic, wood, ceramic, etc.) and presenting different shapes and colors. These objects ranged from 7.5 to 20 cm in height and from 7 to 20 cm in width. Likewise, olfactory stimuli were two copies of ten odors (curry, talc, coffee, paprika, mint, citrus/pepper, cloves, cinnamon, ginger, and garlic). Odors sources were stored in 20 identical salt shakers (9 cm high × 5 cm in diameter) with 11 holes on top (diameter: 0.5 cm). Visuotactile objects and salt shakers could be fixed in the middle of each half of the floor boxes. The spatial tasks were conducted in an open field (100 × 100 × 29.5 cm) with transparent walls that was illuminated with neon lighting located above the apparatus. Many extramaze objects (posters, bucket, etc.) that differed in shapes and colors surrounded the open field and their positions remained constant throughout testing. The stimuli employed for the spatial tasks were three identical translucent rectangular pots (9.5 × 9.5 × 18 cm). A white noise (70–73 dB) served to mask external sounds throughout behavioral testing. During all tasks, video cameras recorded rats' behavior.

2.4. Behavioral procedure

2.4.1. General procedure

Throughout behavioral testing, the experimenters were blind to the nature of treatment. Training began between 26 and 40 days after surgery. Rats were tested in three temporal order tasks and three novelty detection tasks. The procedure was based on Hunsaker et al. (2008). For each type of stimuli (odors, objects, and spatial locations), one task assessed temporal order and the other, novelty detection. In each apparatus, a session of familiarization was administered the day before a task began and consisted of free exploration during 10 min without stimuli. For a given type of stimuli, the temporal order test was conducted 48 h or 72 h before the novelty detection test. Exposure to each of the three types of stimuli (odors, objects, and spatial locations) was counterbalanced across three cohorts of rats. The stimuli used in a particular task were randomly assigned to each rat. Stimuli were only used for one of the two tasks. Rats were individually placed in the apparatuses throughout testing. To control odor traces on each apparatus and stimulus, these were cleaned between each phase with a mixture of water and alcohol (30%). Rats' behaviors were recorded and later analyzed. Locomotor activity was recorded for all tests phases. In olfactory and object tasks, the measure of locomotor activity was the number of times a rat crossed the midline of the cage. In spatial location tasks, the open field was divided into four zones and the locomotor activity was expressed as the number of zone crossings.

2.4.2. Objects and olfactory tasks

The temporal order task consisted of three successive 3-min sample phases, separated by 3 min. A 3-min test phase occurred 10 min after the third sample phase. In each sample phase, the rat was free to explore two copies of an object or odor. Different objects or odors were presented on each of the three sample phases. In the test phases, the rat could explore one stimulus from the first sample phase and one stimulus from the third sample phase, left and right locations of stimuli being randomly assigned. Novelty detection tasks were identical to temporal order tasks, except that during the test phase a new stimulus and a familiar stimulus (from the first sample phase) were presented. Exploration of objects was recorded if the rat's nose was at least 1 cm from the object for at least 0.5 s. Since some objects could be chewed whereas other could not, the time spent biting the objects was excluded from the exploration time to avoid a preference for chewing objects rather than for exploring new or first explored objects. Moreover, since few rats were trying to escape from the cage by climbing on top of objects, the time spent climbing on objects was not considered as exploration. Exploration of odors was