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Spared place and object-place learning but limited spatial working memory capacity in rats with selective lesions of the dentate gyrus

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Abstract

We studied the cognitive performance of rats with colchicine-induced lesions of the hippocampal dentate gyrus (DG) on a range of spatial, non-spatial and mixed spatial/procedural tasks. Rats were assigned to three experimental groups receiving large colchicine lesions (7 μg per hippocampus), small colchicine lesions (1.75 μg per hippocampus) or sham lesions. Stereological estimates of cell density indicated that the colchicine treatments induced dose-dependent damage to the DG, while sparing in large part other hippocampal subfields. Remarkably, the behavioural results showed that the colchicine lesions did not affect the performance of rats in an object discrimination task, in an object-place associative task in which a familiar object was displaced from a given position nor in a spontaneous spatial discrimination task performed in the T-maze. However, rats in both lesion groups were severely impaired in a reinforced non-matching-to-position working memory task conducted in the T-maze. Importantly, performance in the working memory task correlated strongly with cell density in the DG but not with cell density in the CA1 and CA3 areas. Only rats with large-lesions showed a transient deficit in a reinforced rule-based conditional discrimination task. These data demonstrated that rats with selective lesions of the DG readily acquire and retain neural representations relative to objects and places but are specifically impaired in their ability to update rapidly and flexibly spatial information that is essential to guide goal-directed actions.

Keywords: Spatial memory; Working memory; Object recognition; Place recognition; Dentate gyrus; Hippocampus; Colchicine

1. Introduction

It is widely accepted that the hippocampal formation plays a key role in the formation and retrieval of spatial memories. Data from human amnesiacs sustaining insults to the hippocampus documented deficits in various forms of spatial, explicit and associative long-term memory [4,41]. Also, numerous studies involving lesions of the dorsal hippocampus in rats and monkeys demonstrated severe impairments in performance in a wide range of spatial tasks [13,17,39]. There remains, however, considerable controversy with regard to the specific nature of the memories held in the hippocampus. The cognitive map theory of O'Keefe and Nadel [31] postulated a bimodal system for resolving spatial tasks: a place, allocentric strategy involving cognitive mapping of the environment and a taxon, egocentric strategy based on proprioceptive signals and the relative movement of the body axis with respect to fixed contextual elements. In parallel, a critical distinction was made between working memory and reference memory [32], the former referring to trial-specific spatial information and the latter to spatial information which is used to solve successive trials. With regard to the working/reference memory dichotomy, the hippocampus was mainly implicated in working, and to a lesser extent in reference, memory [18,33]. Recent accounts suggested that the hippocampus binds and stores configural associations between elemental stimuli, both spatial and logical [42]. By contrast, path-integration theories [27,45] viewed the hippocampus as a preconfigured network that generates internal representations in two-dimensional space based on self-motion.

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In addition to such contrasting perspectives of the hippocampal network as a whole, an important aspect that has been debated in recent years is the degree to which a functional division of labour exists within the hippocampal formation. Can the different subfields of the hippocampus be regarded as a functional unit or is there evidence for a modularly organized network of functionally segregated subfields? Behavioural studies involving discrete lesions of specific hippocampal sectors, including selective lesions of the dentate gyrus (DG), CA3 or CA1 [11,19–21] have contributed to dissociate some of the functions of each of these areas. Furthermore, current computational models [2,40] are consistent with the notion of functional heterogeneity within the hippocampus.

Here we focused on the hippocampal DG, the first relay station to the hippocampal formation. The DG can be damaged selectively following intragyral injections of colchicine, a neurotoxin which binds to tubulin and preferentially targets the granule cells of the DG and their mossy fiber projections to CA3 [12,16]. Such lesion procedure has been used previously in rats to dissociate some of the functions of the DG with variable, often controversial, results [15,43,46]. Previous evidence indicated that the magnitude of the lesion to the DG, and the degree of cognitive impairment induced as a result, is dependent on the dose of colchicine applied locally [15]. Therefore, we used two concentrations of colchicine to vary systematically the extent of the lesions and we correlated quantitative estimates of the lesions with values of mnemonic function. We compared the performance of rats with colchicine-induced lesions of the DG with that of control rats in a range of tasks which differed in working memory load (single trial versus multiple trials), response directness (spontaneous spatial responses versus instrumental spatial responses) and spatial requirements (spatial versus procedural learning).

2. Materials and methods

2.1. Subjects and surgical lesions

Male Long-Evans rats ($N = 30$) weighing 150–175 g were obtained from Harlan Ibérica S.A. (Spain) and were initially housed in pairs in a 12-h light:12-h dark cycle (lights on at 9:00 a.m.) with water and rodent chow available ad libitum. Rats were allowed to acclimatise to the vivarium before the experiments began, and were handled for 3 days prior to any experimental manipulation. All in vivo experiments were carried out in compliance with current European directives (86/609/ECC). Rats were 250–275 g at the time of surgery. Rats were divided into three lesion groups ($n = 8$ each), receiving injections of either colchicine (7 or 1.75 mg/ml solutions) or sterile 0.9% saline (sham group). Dosage was based on previous studies using colchicine for inducing lesions of the DG [15,45,46]. The animals were anaesthetised with Avertin (a mixture of 2,2,2-tribromoethanol and tertiary amyl alcohol), injected at a dose of 1 ml/100 g, and mounted on a stereotaxic apparatus (Stoelting, Illinois) on a flat skull position. The skull was exposed and an inverted V-shape hole was drilled at the level of the dorsal hippocampus, bilaterally. A stainless steel needle (31G) was mounted on the stereotaxic arm and connected with polyethylene tubing to Hamilton microsyringes driven by a precision pump (Harvard Apparatus). Rats received a total of 10 injections (0.2 μl each) of the corresponding neurotoxic solutions, five into each hemisphere. The needle was carefully lowered into the brain at the following stereotaxic coordinates: AP −2.3, −3.1, −3.8, −4.5, −5.3, ML ±0.7, ±1.10, ±1.80, ±2.4, ±3.2, DV 3.6, −3.7, −3.7, −3.1, −3.2 (from brain surface) [36]. The needle remained in place for 3 min to reduce backflow. The wound was cleansed and two small injections of 2% lidocaine HCl were made under the skin on each side. Sutures were applied as needed. The rats were allowed to recover from the surgery and were housed individually thereafter. The animals were coded and their experimental condition was not revealed to the experimenters until behavioural tests and neurohistological analyses were completed. Rats were allowed 3 weeks to recover from surgery before tests began. To control for the temporal effects of the lesions, a group of rats ($n = 6$) receiving large (7 mg/ml solution) or small (1.75 mg/ml solution) colchicine lesions was sacrificed 1 month after the lesion.

2.2. Object recognition tests

All behavioural experiments were carried out in a sound- and light-attenuated behavioural room adjacent to the animal housing facility. The behaviour was video-tracked and recorded on DVD for blind analysis. For the object recognition test and the object-place test, we used an open field (45 cm × 45 cm) with 20 cm high white Perspex walls. Conspicuous posters were placed in the lateral walls. In the simple test of object recognition, based of the natural tendency of the rat to investigate novel objects, exploration of the novel object is assumed to indicate memory of the familiar object [8]. The rats were first habituated to the open field for 30 min during 3 consecutive days. On day 4, two identical objects were placed diagonally across the field. All objects used were small rubber toys of different colours and shapes. The rats were placed in the open field and were allowed to explore the objects during 3 min. The rats were then returned to a holding cage for 15 min. A 1 min discrimination test followed in which a novel object replaced one of the objects presented previously. For the video analysis, an area whose boundaries extended the perimeter of the target objects by 1 cm was drawn on the screen. The time during which the snout of the rat was within the boundaries of the area was measured. Data were expressed a percent time of exploration for each object [i.e., percent exploration of object A = exploration of object A/(exploration of object A + exploration of object B) × 100].

In the object-place test, the exploration of an object which has been displaced from a previous position is held to reflect memory of the object and the position it occupied in space [9]. For the object-place test, the rats were again habituated to the open field in the presence of the same spatial cues for 30 min during 3 consecutive days. On day 4, rats were exposed to the open field in the presence of two novel objects and were allowed to explore them for 5 min. The rats were then returned to a holding cage for 5 min. In the discrimination test, one of the objects was moved to a different location within the arena. Rats were allowed to investigate the objects for 1 min. The video analysis and quantification was performed as indicated previously for the object discrimination test.

2.3. Unconditioned place discrimination

We conducted an unconditioned place discrimination task in the elevated T-maze [28]. The T-maze was made of white Perspex. The T-maze runway was 80 cm long and 10 cm wide, with two 30 cm long side arms of equal width. The walls of the maze were 10 cm high. A guillotine door was used to open the start box and two additional doors were used to block access to the arms, as needed. The T-maze was placed in the same location in which the object recognition and object-place recognition had been performed, maintaining the position of extra-maze cues. Habituation sessions were performed during 3 consecutive days, with rats being allowed to freely explore the maze for 15 min. On day 4, rats were placed on the start box for 10 s, the door was open and access was given to one of the arms while blocking access to the other. The rats were given 3 min to explore the maze in such conditions. The arm that was blocked in the sample run was randomly counterbalanced between rats. After the 3 min sample run, rats were put in a holding cage for 90 s and immediately placed back in the start box. Access was given to both arms of the maze in the test run. The behaviour was recorded on DVD for 3 min and the time spent by the rats in each of the arms was measured. The data were expressed as percent time spent in each arm [i.e., percent time in arm A = percent time in arm A/(percent time in arm A + percent time in arm B) × 100]. To provide a measure of general motor activity the number of arm entries was counted.