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Research Report

Finding the place without the whole: Timeline involvement of brain regions

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ABSTRACT

Mastering the Morris water maze (MWM) requires the animal to consolidate, retain and retrieve spatial localizations of relevant visual cues. However, it is necessary to investigate whether a reorganization of the neural networks takes place when part of the spatial information is removed. We conducted four experiments using the MWM. A classical reference memory procedure was performed over five training days, RM5 ($n=7$), and eight days, RM8 ($n=7$), with the whole room and all the spatial cues presented. Another group of animals were trained in the same protocol, but they received an additional day of training with only partial cues, PC ($n=8$). Finally, a third group of animals performed the classical task, followed by an overtraining with partial cues for four more days, OPC ($n=8$). After completing these tasks, cytochrome c-oxidase activity (CO) in several brain limbic system structures was compared between groups. In addition, c-Fos positive cells were measured in the RM5, RM8, PC and OPC groups. No significant differences were found among the four groups in escape latencies or time spent in the target quadrant. CO revealed involvement of the prefrontal and parietal cortices, dorsal and ventral striatum, CA1 and CA3 subfields of the dorsal hippocampus, basolateral and lateral amygdala, and mammillary nuclei in the PC group, compared to the RM group. In the OPC group, involvement of the ventral striatum and anteroventral thalamus and the absence of amygdala involvement were revealed, compared to the PC group. C-Fos results highlighted the role of the prefrontal cortex, dorsal striatum, anterodorsal thalamus and CA3 in the PC group, compared to the OPC, RM5 and RM8 groups. The animals were able to find the escape platform even when only a portion of the space where the cues were placed was available. Although the groups did not differ behaviorally, energetic brain metabolism and immediate early gene expression revealed the engagement of different neural structures in the groups that received more training without the entire surrounding space.

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1. Introduction

A wide variety of experimental procedures has been developed to test mnemonic processes in rodents. Among them, the Morris water maze (MWM) has been the most widely used (Morris, 1984) to measure spatial learning and memory processes in rodents. To master this task, the animal has to successfully navigate and locate a hidden platform to escape from the water. This has to be done by using spatial localizations of relevant visual cues that need to be consolidated, retained and retrieved in order to be used.

There are several theories about how the animals are able to identify the position of the goal using distal landmarks. Some authors propose that this skill is performed using a cognitive map that contains information about the position of the goal relative to the landmarks and the spatial relationship between the landmarks (Tolman, 1948). Other authors support a different theory, claiming that animals identify the location of the goal by taking a mental snapshot of some of the landmarks (Sheynikhovich et al., 2009). Finally, it has been proposed that in order to find the goal, the animals make an association between the goal and the surrounding cues (Pearce, 2009). In making these associations, the hippocampus has traditionally been involved in spatial information processing. Indeed, specific hippocampal involvement in learning allocentric spatial relationships has been shown in animal lesion studies (O'Keefe and Nadel, 1978; Morris et al., 1982). In addition, different studies have revealed that lesions in the hippocampus produce deficits in spatial learning and memory (Holdstock et al., 2000; Smith and Milner, 1981). Specifically, different sub-regions of the hippocampus make differential contributions to these spatial processes: CA1 appears to encode goal locations and the successful retrieval of goal associated spatial memories; CA3 provides invariant representations of the whole environment, independently of the task demand (Dupret et al., 2010); and the dentate gyrus has been broadly studied in spatial memories (Reagh and Yassa, 2014; Arias et al., 2014).

Moreover, the hippocampus has been found to work in parallel with the striatum. Whereas the hippocampus can transform cortical representations according to detected changes in the expected spatial context, the striatum updates cortical representations based on the most recent reinforcement consequences of previously learned sensory/motor associations (Mizumori et al., 2009). Ferretti et al. (2010) demonstrated the role of the ventral striatum in facilitating the proper flow of information or, alternatively, in inducing the plasticity needed for the long-term stabilization of critical information necessary for spatial navigation. In fact, the nucleus accumbens has converging inputs from the amygdala, the hippocampal formation, and the prefrontal cortex, brain regions involved in many aspects of the learning process, such as stimulus evaluation, spatial navigation and planning, respectively (Fidalgo et al., 2014).

Nevertheless, when a memory is acquired, the initial dependence on the hippocampus is replaced by a progressive strengthening of cortical connections that provide support for storage and retrieval (Frankland and Bontempi, 2005). Along these lines, the medial prefrontal cortex and parietal and parahippocampal cortices have been involved in goal-directed learning (Yin et al., 2005; Corbit and Balleine, 2003) and in tasks that require visual stimulus information processing and are thought to comprise a spatial/contextual information pathway (Reagh and Yassa, 2014). Finally, the mammillary bodies have been widely and independently associated with spatial navigation performance (Santín et al., 2003; Mendez-Lopez et al., 2009a, 2009b; Conejo et al., 2010; Vann, 2010; Loureiro et al., 2012).

However, despite this knowledge, it is unknown to what extent the first exposure to a partial cue environment is different from a full cue exposure, how overtraining with partial cues affects learning, and what brain changes underlie these processes.

For this reason, in this study we explore brain changes after the performance of the MWM task with a complete set of cues, after additional training when only a portion of the space where the spatial cues are placed is available, and after overtraining under this latter condition. We also assess the brain energetic metabolism and the immediate early gene expression in the brain structures related to performing these tasks, as several key molecular events that are crucial for the expression of neural plasticity, such as alterations in gene expression and protein synthesis, are required in the early stages of spatial memory formation and form the basis for long-term structural modifications (Ferretti et al., 2010; Bozon et al., 2002; Balderas et al., 2008).

2. Results

2.1. Behavioral results

We compare performance of the groups submitted to the classical reference memory procedure during five and eight days (RM5 and RM8, respectively), the group that was trained in the same reference memory protocol receiving an additional day of training with partial cues (PC) and the group that was overtrained with partial cues (OPC) after the classical spatial memory task. The two-way repeated-measures ANOVA of the average session latencies across groups from day 2 to 6, revealed significant differences across days ($F_{(4,149)}=36.060$, $p<0.001$). Post-hoc analysis showed differences between day 2 and the other days, between day 3 and days 4, 5 and 6, and between days 4 and 5 ($p<0.05$). No interaction effects (group × day) ($F_{(12,149)}=1.148$, $p=0.330$) or group differences ($F_{(3,149)}=0.345$, $p=0.793$) were found, suggesting that the escape response was the same on these days in all the groups.

ANOVA on day 5 revealed differences between groups ($F_{(3,29)}=3.657$, $p=0.025$) showing the effects of cue removal with increase latencies in the animal trained in a reference memory protocol on four consecutive training days and an additional day of training with partial cues (PC) and animals which performed the classical task on four consecutive days followed by an overtraining with partial cues for four more days (OPC groups) compared to rats tested on a classical reference memory task for five days (RM5) and eight days (RM8 groups).

Moreover, ANOVA in the OPC group showed no differences from days 6 to 9 ($F_{(3,31)}=3.073$, $p=0.050$) highlighting the acquisition of the behavioral criterion (Fig. 1).