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Research report

Brain network function during shifts in learning strategies in portal hypertension animals

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ABSTRACT

Patients with minimal hepatic encephalopathy exhibit early impairments in their ability to shift attentional set. We employed a task-switching protocol to evaluate brain network changes. Strategy switching requires the modification of both the relevant stimulus dimension and the required memory system. Rats were trained in an allocentric (A) and a cue-guided (C) task using a four-arm maze. To examine priming, we changed the order in which the tasks were presented. Five groups of animals were used: a SHAM (sham-operated) A–C group ($n = 10$), a SHAM C–A group ($n = 8$), a PH (portal hypertension) A–C group ($n = 8$), PH C–A group ($n = 8$), and a naïve group ($n = 10$). The triple portal vein ligation method was used to create an animal model of the early evolutive phase of PH. The animals were tested in the four-arm radial water maze in a single 10-trial session each day for six days (three days for the allocentric task and three days for the cue-guided task). The metabolic activities of the brains were studied with cytochrome oxidase histochemistry, and brain network changes were assessed with principal component analysis. The behavioural results revealed significant increases in the numbers of correct choices across training days in all groups studied, and facilitation of the acquisition of the second task was present in the C–A groups. Moreover, different brain network activities were found; in the experimental groups, the performance of A–C switch involved the prefrontal cortex, and the key structures involved in the C–A switch in the other groups were the dentate gyrus of the dorsal hippocampus and the basolateral and central amygdala. These networks have a common nucleus of structures (i.e., the parietal cortex and the dorsal and ventral striatum), whereas other structures were specifically involved in each type of strategy, suggesting that these regions are part of both circuits and may interact with one another during learning.

1. Introduction

Behavioural flexibility refers to the ability to learn a new strategy while inhibiting the execution of a previous strategy under changing environmental contingencies (Ragozzino et al., 1999). Behavioural flexibility is the general concept behind switching tasks that require modifying both the relevant stimulus dimension (e.g., from allocentric to cued-learning and vice versa) and the required memory system (White and McDonald, 2002). In this regard, different brain regions have traditionally been considered to mediate allocentric- and cued-learning. The hippocampus and the striatum (STR) have conventionally been considered to be key brain regions that are associated with independent memory systems. The hippocampus has been linked to spatial strategies (Bartsch et al., 2010) that require the development of a spatial cognitive map from an internal representation of the relationships between distal landmarks (de Bruin et al., 2001). In contrast, the striatum is involved in the acquisition of stimulus–response (S-R) memories (Fidalgo et al., 2011). Furthermore, a parallel coding by hippocampus and striatum related to behaviours relevant to a particular association (stimulus–stimulus or stimulus-reward) or a specific stimulus has been reported (Mizumori et al., 2004; Yin and Knowlton, 2004). More specifically, the striatum appears to be necessary for the formation of associations between cues and rewarded responses (cued-learning) (Miyoshi et al., 2012). This type of flexibility requires the reassignment of brain systems to perform the task correctly and is mediated by the prefrontal cortex in humans and non-human primates (Owen et al., 1990; Dias et al., 1997).

Over the years, studies have shown that different learning strategies require different neural networks that may could

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partially overlap (Rubio et al., 2012). Indeed, it is well known that the prelimbic area is involved in strategy shifting and that the infralimbic region is involved in the formation of new choice patterns. Furthermore, the interaction between these structures and the striatum during the execution of dynamic behaviour has been shown (Ragozzino, 2007). Indeed, dorsomedial striatal inactivation impairs shifts between visual cues and an egocentric response strategies (Ragozzino et al., 2002) that are revealed when the prelimbic area is inhibited. Furthermore, it has been reported that the hippocampus and prefrontal cortex operate in parallel during the acquisition of spatial information (Rogers and Kesner, 2006), which highlights the existence of interactions between learning and memory structures.

Therefore, it is not difficult to understand that damage to one of these structures could cause impairments of cognitive flexibility similar to those that are present in numerous human conditions, including Huntington' disease, Alzheimer' disease (Menzie et al., 2012), and hepatic encephalopathy (HE) (Amodio et al., 2005).

The treatment of HE patients is not uniform and requires precise development of several new tools that enable the assessment of cognitive performance (Córdoba, 2011). Because HE is a continuum, understanding the early stages, known as minimal HE (mHE), is necessary from a preventive point of view. Several studies of animal models of mHE have shown delays in the acquisition of reference memory (Arias et al., 2012) and deficits in spatial working memory (Méndez et al., 2008). Thus, we used a model of triple stenosing ligation of the portal vein (Aller et al., 2005) to create a set of neuropsychological methods that can provide a more objective assessment and be useful in researching the effect of new treatments.

To accomplish this goal, histochemical labelling of cytochrome c-oxidase (COx) was performed. COx is a mitochondrial enzyme that reflects changes in tissue metabolic capacity that are induced by the sustained energy requirements of skeletal muscle (Liu et al., 2014), heart diseases (Matam et al., 2014) and the nervous system associated with learning (Poremba et al., 1998) and spatial memory in the Morris water maze (MWM) (Conejo et al., 2007). The aim of this study was to examine the brain networks that are involved in the encoding and retrieval of spatial information required for these switching strategies and, specifically, to determine how the selected rat brain regions differed in their activation when the allocentric- and cued-task order was changed. During the *allocentric task*, the rats had to acquire long-term memories of the location of the submerged platform based on their orientation relative to the positions of distal stimuli in the experimental room. In the *cue-guided task*, the rats were trained to locate the platform based on a yellow globe that was situated 20 cm above the platform. This version of the task requires the execution of the same response (cue-guided), rather than navigation to the same place.

2. Materials and methods

2.1. Subjects

Forty-four adult male Wistar rats (250–300 g) from the animalarium of the University of Oviedo were housed four per cage in a temperature- and humidity-controlled vivarium under a 12-h light/dark cycle (lights on 8.00 A.M.). All experimental procedures were performed between 9.00 and 12.00 A.M. Experimental procedures were performed in accordance with the Directive of the European Commision (20120/63/EU) and Spanish legislation (R.D. 1201/2005) and approved by the local committee for animal studies.

The rats were randomly distributed into the following five groups: portal hypertension allocentric-cue guided (PH A–C group, $n = 8$), portal hypertension cue guided-allocentric (PH C–A group, $n = 8$), sham-operated allocentric-cue guided (SHAM A–C group, $n = 10$), sham-operated cue guided-allocentric (SHAM C–A group, $n = 8$), and a group of rats that received only gentle handling (naïve group, $n = 10$). The microsurgical procedures were carried out as described in Arias et al. (2012).

2.2. Behavioural procedures

The rats were trained in a black fibreglass radial arm water maze (RAWM, each arm was 80 cm × 12 cm) that was placed 50 cm above floor level. The maze had four arms in the shape of a cross. The water depth was 30 cm, and the temperature was 22 ± 2 °C. The RAWM was in the centre of a 16 m^2 lit room (two halogen lamps of 4000 l×) and was surrounded by panels on which the spatial clues were placed. The behaviour of the rat in the RAWM was recorded by a video camera (Sony V88E) connected to a computer equipped with the EthoVision Pro program (EthoVision 3.1; Noldus Information Technology, Leesburg, VA).

The learning protocol consisted of single 10 trial-sessions each day over 6 days in which the first 3 days were designated for the acquisition of one task, and the last 3 days were used for the acquisition of the other task (e.g., 3 days were allotted for the allocentric task, and the next 3 days were allotted for the cue-guided task). During the allocentric task the RAWM was surrounded by panels on which the spatial cues were placed. In the cue guided task the RAWM was surrounded by curtains and the only guide was a yellow globe situated 20 cm above the platform. In each trial, the rats were placed in the centre of the pool facing a different arm and allowed to swim to the platform. If the animal failed to find the platform, it was guided to the platform. Once the rat reached the platform, it remained there for 15 s. Between trials, the rat was placed in a small square box for 30 s. The platform was in the same arm throughout the entire 10 trial-session each day, but the location of the platform was changed each training day.

Every day at the end of each 10 trial-session, a probe test was applied in which the pool platform was removed, and the rat was introduced into the pool for 25 s. Immediately after the probe test, the rats were subjected to an additional trial in which the hidden platform was replaced to avoid any possible interference from the probe test.

All subjects in the experimental groups reached the learning criterion of choosing the correct arm in 70% of trials.

2.3. Cytochrome oxidase histochemistry

The protocol used was the same as that described by Arias et al. (2010). Ninety minutes after the last training day, brains were removed, frozen rapidly in N-methylbutane (Sigma–Aldrich, Madrid, Spain) and stored at −40 °C until processing for quantitative COx histochemistry. To quantify enzymatic activity and to control for staining variability across different baths, sets of tissue homogenate standards from Wistar rat brains were cut at different thicknesses (10, 30, 40 and 60 μm) and included with each bath of slides. Quantification of COx histochemical staining intensity was performed with densitometric analysis using a computer-assisted image analysis workstation (MCID, Interfocus Imaging Ltd., Linton, England) composed of a high-precision illuminator, a digital camera, and a computer with specific image analysis software (Leica Q-Win, Germany). The mean optical density (OD) of each region was measured in the bilateral structures using three consecutive sections in each subject. In each section, four non-overlapping readings were taken using a square-shaped sampling window that was adjusted for the size of each brain region. A total of twelve measurements were taken per region by an investigator who was blind to group membership. These measurements were averaged to