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DIFFERENTIAL CONTRIBUTION OF THE HIPPOCAMPUS IN TWO DIFFERENT DEMANDING TASKS AT EARLY STAGES OF HEPATIC ENCEPHALOPATHY

N. ARIAS,* M. MÉNDEZ AND J. L. ARIAS

Laboratorio de Neurociencias, Departamento de Psicología, Universidad de Oviedo, Plaza Feijoo s/n, 33003 Oviedo, Spain

INEUROPA, Instituto de Neurociencias del Principado de Asturias, Spain

Abstract—The hippocampus has been established as a site of plasticity during the acquisition of spatial memory. The memory for spatial locations is impaired in patients who develop hepatic encephalopathy (HE). We wondered how the hippocampus can manage different hippocampal-dependent tasks in a type B model of the early evolutive phases of HE induced by triple portal vein ligation. We used a one-trial object-place recognition task that involves making judgements about whether a stimulus was encountered before in that location and a reversal learning task performed in the Morris water maze that involves reward-guided behavior and decision making. Our behavioral results showed impairments in the acquisition of both tasks by the portal hypertension group compared with the sham-operated group. To label brain areas related to these tasks, we marked the expression of the c-Fos protein and revealed high c-Fos immunoreactivity in cornu ammonis 1 (CA1), cornu ammonis 3 (CA3) and entorhinal (Ent) cortex of the PH group compared with the SHAM group in the object-place recognition task and a decrease in c-Fos-positive cells in the reversal task in the CA1, CA3, dentate gyrus (DG), cingulate (CG), prelimbic (PL), and infralimbic (IL) cortices in the PH group compared with the SHAM group. In conclusion, the study corroborated the pivotal role of the hippocampus in spatial memory deficits found in the early stages of type B HE and noted its differential contribution in each of the tasks.

Key words: one-trial object-place recognition task, reversal learning, c-Fos immunoreactivity, hepatic encephalopathy, rat.

INTRODUCTION

The hippocampus has been established as a site of plasticity during the acquisition of spatial memory and it is modified upon retrieval (Li et al., 2013). The existence of individual place cells that are active during the initial approximation to the environment and during the ongoing dimensions of the rat's purposive behavior (Ainge et al., 2007) indicates that the hippocampus may mediate the spatial and episodic dimensions of the animal's experience (Ainge et al., 2007). The implication of the hippocampus in spatial memory has been revealed through numerous studies of working and reference memory tasks in a radial-arm maze (Olton and Papas, 1979; Poirier et al., 2008), a Morris water maze (Morris et al., 1982; Méndez et al., 2010; Arias et al., 2012) and even during spontaneous associative object-location and object-context information (Save et al., 1992; Dix and Aggleton, 1999), indicating hippocampal involvement in the integration of multimodal information (Rajji et al., 2006; Langston and Wood, 2010).

However, there is a different involvement of the hippocampal subfields during these spatial memory tasks. The dentate gyrus (DG) plays an important role in spatial navigation (Derrick, 2007; Kesner, 2007), pattern separation (Leutgeb et al., 2007; Clelland et al., 2009), context-related functions (Lee and Kesner, 2004; Hernandez-Rabaza et al., 2008) and spatial information encoding (Lee and Kesner, 2004). Projections between the DG, CA3 and the hippocampal field cornu ammonis 1 (CA1) have been broadly demonstrated. The CA1 is critical for the consolidation and retrieval of spatial information (Rolls and Kesner, 2006; Vago et al., 2007), whereas the cornu ammonis 3 (CA3) has been more associated with the process of encoding (Hunsaker et al., 2008). This region also enables rapid associations between spatial locations or places and objects (Rolls, 2013).

The memory for spatial locations can be operationalized by stimuli (e.g., objects, platforms) encountered by animals and the occasion (contextual or temporal) in which the event took place (Eacott and Norman, 2004; Babb and Crystal, 2006). Impairments to this memory have been shown in numerous neurodegenerative diseases such as Korsakoff's syndrome (Kessels and Kopelman, 2012), Alzheimer's disease and in patients who develop hepatic encephalopathy (HE) Felipo et al., 2012. HE can arise across a spectrum of

*Correspondence to: N. Arias, Laboratorio de Neurociencias, Departamento de Psicología, Universidad de Oviedo, Plaza Feijoo s/n, CP: 33003 Oviedo, Asturias, Spain. Tel: +34-985103212.
E-mail address: ariasnatalia@uniovi.es (N. Arias).

Abbreviations: ANOVA, analysis of variance; CA1, cornu ammonis 1; CA3, cornu ammonis 3; CG, cingulate cortex; DG, dentate gyrus; HE, hepatic encephalopathy; IL, infralimbic cortex; PBS, phosphate-buffered saline; PL, prelimbic cortex; PP, portal pressure; Prh, perirhinal cortex.

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clinical severity, encompassing subtle loss of cognitive function, lethargy, depressed consciousness and coma (Tranah et al., 2013). As the disease progresses, intellectual abilities deteriorate and patients show neuropsychological disturbances that affect orientation (Weissenborn et al., 2003). In liver cirrhosis, it is estimated that between 60% and 80% of patients suffer from minimal HE (Ortiz et al., 2005). Functional neuroimaging of the brain in these patients reveals alterations in the hippocampus and related regions that appear to be responsible for the clinical characteristics such as deficits in attention and spatial orientation (Weissenborn et al., 2004).

In the current study, we used a spatial task where the animals have to recognize that an object they had previously experienced had changed location (one-trial object-place recognition task), which implies making judgements about whether a stimulus was encountered before in that location and a spatial reversal learning task that assesses the ability to associate a reinforcement with a previously neutral stimulus in the environment and to subsequently change this association. This, in turn, involves reward-guided behavior and decision making (Rudebeck and Murray, 2008) in a model of the early evolutive phases of HE.

To label the hippocampal regions and other brain areas, we marked the expression of the c-Fos protein. The study of c-Fos can provide information about neuronal plasticity required for memory processes (Kaczmarek, 1993). Expression of the c-Fos protein is induced after learning and is indicative of a change in neuronal activity (Kaczmarek, 1993; Radulovic et al., 1998; Santín et al., 2003). This technique allows the simultaneous examination of the activity of populations of neurons in multiple brain regions (Wan et al., 1999). Because the hippocampus does not act as an isolated structure in spatial learning tasks, the following cerebral regions involved in the tasks were studied: the prefrontal cortex and the adjacent cortical areas within the temporal lobe, such as the entorhinal (Ent) cortex and perirhinal (Prh) cortex. These areas were chosen based on evidence from ablations, electrophysiological lesions or dysfunctions, suggesting that these structures might be involved in object-place and reversal spatial tasks (Zhu et al., 1995; Schoenbaum et al., 2003; Stalnaker et al., 2007; Méndez et al., 2010).

EXPERIMENTAL PROCEDURES

Animals

We used 30 male Wistar rats from the vivarium of the University of Oviedo. The rats weighed between 250 and 270 g at the beginning of the study. The rats were housed in groups of three to five, three weeks prior to the beginning of the experiments and maintained under standard laboratory conditions (20–22 °C, 65–70% relative humidity and a 12-h light/dark cycle (08.00–20.00/20.00–08.00)). Food and water were available *ad libitum* throughout the course of the experiments and sessions were performed during the light phase, between 9:00 a.m. and 13:00 p.m. All procedures were carried out according to the European Parliament and the Council of the European Union 2010/63/UE and were approved by the Oviedo University committee for animal studies. The animals were randomly distributed into two groups: portal hypertension (PH group, $n = 14$) and sham-operated (SHAM group, $n = 16$). Then, animals were distributed into a reversal task (SHAM group, $n = 10$ and PH group, $n = 9$) and a one-trial object-place recognition task (SHAM group, $n = 6$ and PH group, $n = 5$).

Procurement of experimental models

The experimental models that required surgery were anesthetised by an i.m. injection of ketamine (100 mg/kg) and xylazine (12 mg/kg). With regard to postsurgical care, rats were kept near a heat source (10–15 min) until they regained consciousness to prevent postoperative hypothermia. They were then introduced into individual polycarbonate cages for 15 days.

Portal hypertension. Portal hypertension was produced by triple stenosing ligation (Diéguez et al., 2002). A midline abdominal incision was performed and a section of the intestinal loops was gently shifted to the left and covered with saline-moistened gauze. The portal vein was isolated and three ligatures, fixed on a silastic guide, were performed in the superior, middle and inferior portions. The stenoses were calibrated by simultaneous ligation (4–0 silk) around the portal vein and a 20-gauge blunt-tipped needle. The abdominal incision was closed in two layers with catgut and 2–0 silk. The postoperative period started immediately after the intervention and lasted until the behavioral evaluation 45 days later.

Sham-operated. A bilateral subcostal laparotomy with prolongation to the xyphoid apophysis followed by isolation of the portal vein was performed. The operative field was irrigated with saline solution during the intervention, in the same manner as the portal hypertension surgeries. Finally, the laparotomy were closed by continuous suture on the two layers with catgut and 2–0 silk. The postoperative period started immediately after the intervention and lasted until the behavioral evaluation 45 days later.

Portal vein pressure measurement

To confirm the portal hypertension, splenic pulp pressure, an indirect measurement of portal pressure (PP), was measured using the method described by Aller et al. (2006) in 13 subjects randomly selected from the two groups (PH group, $n = 7$ and SHAM group, $n = 6$).

Apparatus

Object exploration was assessed in a square open field (100 × 100 × 40 (height) cm) with an open roof in a rectangular room with several distal cues and a proximal cue. A small circular white sticker was located on the east wall of the open field. The open field was composed of gray fiberglass and two diffuse white lights, located at the sides of the room, which provided an illumination density of approximately 50 lux at the