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Serotonin depletion of supramammillary/posterior hypothalamus nuclei produces place learning deficiencies and alters the concomitant hippocampal theta activity in rats

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ABSTRACT

Hippocampal theta activity is important for the acquisition of spatial information and is strongly influenced and regulated by extra-hippocampal inputs from the synchronising ascending system (SAS), which includes the supramammillary nucleus (SUMn) and the posterior hypothalamic nucleus (PHn). Together these nuclei play an important role in controlling the frequency encoding of theta activity and are innervated by serotonin synapses, which also regulate theta activity and learning abilities. The participation of the SUMn in place learning and modulation of hippocampal theta activity were recently shown; thus, we questioned whether serotonin acting on SUMn/PHn could modulate place learning ability and concurrent hippocampal theta activity. The serotonergic terminals of the SUMn/PHn in rats were lesioned through 5,7-dihydroxytryptamine (5,7-DHT) infusion, and hippocampal theta activity during the Morris water maze test was recorded. Rats in the vehicle group learned the task efficiently and showed learning-related theta changes in the CA1 and dentate gyrus regions throughout the training. The 5-HT-depleted rats were deficient in the Morris water maze task and showed theta activity in the CA1 and dentate gyrus that were unrelated to the processing of learning. We conclude that serotonin can regulate the hippocampal theta activity acting on the SUMn/PHn relay of the SAS and that the influence of 5-HT in these nuclei is required for the learning-related changes in hippocampal theta activity that underlie the successful resolution of the Morris water maze task.

1. Introduction

Hippocampal theta activity is characterised by a slow, almost-sinusoidal rhythmic activity pattern with frequencies of 4–12 Hz that are associated with voluntary behaviours (Bland, 1986; Buhot et al., 1995; Vanderwolf, 1988) and is important for learning and spatial memory (2004; Eichenbaum et al., 1992; McNaughton et al., 2006, Olvera-Cortes et al., 2002).

Hippocampal theta activity is regulated by extra-hippocampal inputs, that form part of the synchronising ascending system (SAS), a multi-synaptic circuit that includes structures from the brainstem, caudal diencephalon (posterior hypothalamic nucleus, PHn and supramammillary nucleus, SUMn), and septum (Vertes and Kocsis, 1997). Collateral projections of SUMn neurons that reach the medial septum/vertical limb of the diagonal band of Broca (MS/vBDB) and the hippocampal formation (Vertes and McKenna, 2000), together with projections of the PHn to the medial septum (Vertes, 1992) play an important role in controlling the frequency encoding of theta activity (Kirk and McNaughton, 1993). Briefly, electrical stimulation or the application of carbachol in the PHn produces continuous

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theta activity in the hippocampus, together with the activation of theta-ON cells in the MS/vBDB (Bland et al., 1990; 1994; Oddie et al., 1994; Smythe et al., 1991); in contrast, the electrolytic lesion of the PHn attenuates the hippocampal theta activity and reduces the theta frequency (Robinson and Whishaw, 1974). Additionally, the injection of procaine into the PHn blocks the hippocampal theta activity generated by reticular stimulation (Oddie, et al., 1994) and the septal theta-related phasic cell discharge (Bland, et al., 1994). Similarly, the SUMn stimulation produces hippocampal theta activity (Oddie, et al., 1994; Vertes, 1981), whereas the injection of procaine into several SUMn sub-regions in anesthetised rats reduces the frequency and amplitude of theta activity generated by the stimulation of the *reticular pontis oralis* nucleus (Kirk and McNaughton, 1993).

In spite of the influence of the SUM/PHn on hippocampal electrical activity, few studies have evaluated the participation of the SUM and any of the PHn, in memory. Functionally, the inactivation or pharmacological manipulation of the SUMn alters place-learning ability and concurrent hippocampal theta activity. Briefly, the reversible inactivation of the SUMn by the infusion of lidocaine impairs both the consolidation of reference memory and the consolidation and retrieval of working memory, evaluated in the Morris water maze (Shahidi et al., 2004). In addition, the injection of chlordiazepoxide into the SUMn produces a modest impairment in a one-day place-learning test in the Morris maze, and is associated with a decrease in the hippocampal theta frequency (0.35–0.5 Hz) (Pan and McNaughton, 1997). Thus, the SUMn participates in associative memory processing possibly by modulating hippocampal excitability.

In contrast, serotonin (5-HT) desynchronises hippocampal theta activity. The stimulation of the medial raphe nucleus desynchronises the hippocampal EEG (Assaf and Miller, 1978; Kitchigina et al., 1999; Vertes and Martin, 1988; Vinogradova et al., 1999), and lesions to the medial raphe nucleus resulted in the presence of persistent and continuous trains of theta activity (Maru et al., 1979; Vertes, 1986; Vinogradova et al., 1999). Additionally, 5-HT modulates learning and memory processes. Reductions of cerebral or hippocampal 5-HT have produced both learning enhancements or deficiencies or have had no effect on learning tests (1990; Adams et al., 2008, Altman et al., 1984; Anguiano-Rodriguez et al., 2007; Buhot et al., 2000; Riedel et al., 1999; Volpe et al., 1992), whereas an increase in 5-HT activity resulted in deficiencies in learning tests (Buhot et al., 1995; Carli et al., 1995; Riedel et al., 2002). However, it is still not known whether a relationship exists between serotonin manipulations, learning and hippocampal theta activity.

Recently, it was shown that significant, selective 5-HT depletion in the hippocampus that produced the prevalence of high-frequency theta activity (6.5–9.5 Hz) in CA1 facilitates spatial learning during the resolution of the Morris water maze (Gutierrez-Guzman et al., 2011). Because SUMn/PHn constitutes a critical nodal area in the encoding of the amplitude and/or frequency of theta activity (Bland et al., 1990, 1994; Kirk and McNaughton, 1993), SUMn contributes to associative memory modulation and both nuclei are innervated by serotonin (Vertes and Kocsis, 1997; Vertes and Martin, 1988), we questioned whether serotonin acting on SUMn/PHn could modulate place learning ability and concurrent hippocampal theta activity. Serotonergic terminals of the SUMn/PHn were lesioned in rats through a 5,7-dyhidroxytriptamine infusion, and hippocampal theta activity was recorded during performance of the Morris water maze test.

2. Materials and methods

2.1. Animals

Twenty-six Sprague–Dawley male rats weighing 350–450 g were used. All the experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80-23) and approved by the Research Ethics Committee of the Instituto Mexicano del Seguro Social. The rats were housed under standard conditions in the animal facility. The rats were assigned to the vehicle group or to the SUMn/PHn serotonin depleted group (5HT-D).

2.2. Surgery

For the implantation of the recording electrodes, rats were first given an injection of atropine sulphate (0.1 mg/kg i.p.), followed 10 min later by an injection of pentobarbital (30 mg/kg i.p.). A concentric bipolar electrode was chronically implanted into the CA1 field of the right hippocampus at the edge of the strata oriens and pyramidal layer, with the following coordinates: 4.0 mm posterior to the bregma, 2.2 mm lateral from the midline, and 2.6–2.7 mm dorsoventral from the cranial surface. A second electrode was placed into the dentate gyrus with the following coordinates: 3.5 mm posterior to the bregma, 1.5 mm lateral from the midline and 3.4 mm dorsoventral from the cranial surface (Paxinos and Watson, 1989). A third recording electrode was implanted into the SUMn with the following coordinates: 4.7 mm posterior to bregma, 0.2 mm lateral from the midline and 8.7 mm dorsoventral from the cranial surface (the data obtained from this electrode were not included in the present work).

The recording electrodes were made of nichrome wire with a diameter of 65 μm fastened inside a stainless steel # 25 calibre cannula isolated with epoxy resin, with a small surface exposed on the tip. A stainless steel scroll placed on the bone over the frontal cortex surface served as the ground. The implant was fixed with acrylic cement.

The 5HT-D group of rats received an injection of desipramine (30 mg/kg i.p., to protect noradrenergic terminals) 30 min before receiving 2 bilateral injections of 5,7-dihydroxytriptamine (5,7-DHT; 2 μg dissolved in 0.1 μl of 0.1% ascorbic acid in saline solution) at an infusion rate of 0.1 μl/min for 4 min. The injections were placed into the SUMn (4.7 mm posterior to the bregma, 0.2 mm bilateral from the midline, and 8.7 mm dorsoventral from the cranial surface) and into the PHn (3.7 posterior to the bregma, 0.3 mm bilateral from the midline, and 8.2 mm dorsoventral from the cranial surface). Vehicle rats received injections of the vehicle solution (0.4 μl, at a rate of 0.1 μl/min).

2.3. Behaviour

Rat training began 15 days after surgery. Behavioural tests were conducted using a swimming pool (1.4 m in diameter) filled with water maintained at 28–30 °C and dyed blue with gentian violet. A glass platform (12 cm × 12 cm) was placed inside the pool such that its surface was 1.5 cm below the water level and was maintained in a fixed position at the north quadrant of the pool. The pool was located in an experimental room (5 m × 5 m) with constant, stationary environmental extra-maze cues (e.g., laboratory equipment, experimenters).

The rats were trained for six consecutive days, undergoing four daily trials with inter-trial intervals of 2 min each. Each trial consisted of placing the animal in the water facing the pool wall and removing it 15 s after reaching the platform. If the rat failed to find the platform within 60 s, it was placed on the platform for 15 s. On the seventh day, the platform was removed from the pool and the animals were challenged with a single 30 s search trial (the probe trial). The maze was virtually divided into four equal sized quadrants identified by the cardinal points. For each trial, the rat was randomly placed at different quadrants of the pool and the position of the sunken platform remained constant during the training. The behavioural tests were video-recorded, the paths traced and the lengths estimated. The mean swim distances from the four daily trials were calculated from the latency and distance data. In the probe trial, the number of crosses