

EFFECT OF SEROTONIN DEPLETION IN THE PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS ON THE BEHAVIORAL RESPONSES IN THE RAT

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Abstract: Male Wistar rats were subjected to right-unilateral 5,7-dihydroxytryptamine (5,7-DHT) lesion (8μg/3μl and 16μg/3μl) of the paraventricular nucleus of the hypothalamus (PVN) or were sham-operated, and their ability to acquire the operant task was studied by means of Y-maze and radial 8 arm-maze tasks. Serotonin depletion in the PVN non-significantly increased the spontaneous alternation percentage in Y-maze task. 5,7-DHT non-significantly increased the number of working memory errors and significantly increased the number of reference memory errors in radial 8 arm-maze task, suggesting effects on spatial memory formation, especially on long-term memory. In elevated plus maze measuring anxiety, 5,7-DHT significantly diminished anxiety state. In summary, we provided that the serotonin level in the PVN influences learning and memory processes.

INTRODUCTION

Interactions between serotonin and corticotropin-releasing factor (CRF) have been demonstrated by various studies in different parts of the brain. Both are activated by stressful stimuli. Additionally, serotonergic fibres directly synapse with the parvocellular division of the paraventricular nucleus (PVN) that mainly synthesizes CRF. The functional impact of this serotonin-CRF interaction on CRF-induced responses remains unclear. CRF is a neuropeptide particularly synthesized in the parvocellular division of the PVN. CRF, a 41-residue peptide, is a principal activator of adrenocorticotrophic hormone (ACTH) secretion by the anterior pituitary (Dunn and Berridge, 1990). Activation of this axis, also called the “stress axis” or hypothalamic-pituitary adrenal axis (HPA), results in glucocorticoid release into the systemic circulation. Glucocorticoids are in turn considered to be key players in the organism’s response to stress (Herman et al., 1996). However, the role of CRF in stress is not limited to endocrine responses. CRF containing neurons are found throughout the limbic and other parts of the brain, and central CRF has behavioral and autonomic effects that suggest it plays an important role in the coordinated physiological and behavioral responses to stressful stimuli (Larsen et al., 1996). Serotonergic (5HT) systems are also activated by stressful stimuli. Their role is less well-defined, but, in general, reducing serotonin levels or activity increases the responsiveness to stress, though the literature is not unanimous; the HPA axis becomes hyperactive, behavioral responses are accentuated, and animals may overreact to demanding situations (sometimes referred to as ‘impulsiveness’). CRF producing neurons of the PVN receive a direct (though modest) serotonergic innervation from the B7–B9 cell groups (Larsen et al., 1996). Electrical stimulation of the dorsal raphe nucleus resulted in excitation of PVN neurons that was blocked by parachlorophenylalanine, a serotonin depleting drug (Larsen et al., 1996).

The present study investigated the effect of serotonin depletion using the serotonergic neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) in the paraventricular nucleus to determine whether low serotonin induced changes of the behavioral responses in rats.

MATERIALS AND METHODS

Animals

Male Wistar rats weighing 200-250 g at the start of the experiment were used. The animals were housed in a temperature- and light-controlled room (22 °C, a 12-h cycle starting at 08:00 h) and were fed and allowed to drink water ad libitum. Rats were treated in accordance with the guidelines of animal bioethics from the Act on Animal Experimentation and Animal Health and Welfare Act from Romania and all procedures were in compliance with the European Council Directive of 24 November 1986 (86/609/EEC).

Neurosurgery

All surgical procedures were conducted under aseptic conditions, under sodium pentobarbital (45mg/kg b.w., i.p., SIGMA) anesthesia. Rats were mounted in the stereotaxic apparatus with the nose oriented 11° below horizontal zero plane. The neurotoxin, 5,7-dihydroxytryptamine (5,7-DHT) (Sigma) was dissolved in 0.9% NaCl containing 0.1% ascorbic acid to avoid oxidation. Vehicle and 5,7-DHT (8μg/3μl and 16μg/3μl) were injected into the PVN. Rats were pretreated with desipramine (25mg/kg b.w., i.p.) (Sigma), 30 minutes prior to 5,7-DHT injection. Stereotaxic coordinates of the PVN with the bregma suture as zero reference point (Paxinos and Watson, 2005) were: 0.2 mm anterior to bregma;

0.3 mm to the midline; 7.5 mm ventral to the surface of the cortex. Learning and memory tests began 2 weeks after the neurosurgery.

Y-maze task

Short-term memory was assessed by spontaneous alternation behavior in the Y-maze task. The Y-maze used in the present study consisted of three arms (35 cm long, 25 cm high and 10 cm wide) and an equilateral triangular central area. The rat was placed at the end of one arm and allowed to move freely through the maze for 8 min. The time limit in Y-maze test was 8 min., and every session was stopped after 8 min. An arm entry was counted when the hind paws of the rat were completely within the arm. Spontaneous alternation behavior was defined as entry into all three arms on consecutive choices. The number of maximum spontaneous alternation behaviors was then the total number of arms entered minus 2 and percent spontaneous alternation was calculated as (actual alternations/maximum alternations) X 100 (Hritcu et al., 2007). Spontaneous alternation behavior is considered to reflect spatial working memory, which is a form of short-term memory.

Radial 8 arm-maze task

The radial arm-maze used in the present study consisted of 8 arms, numbered from 1 to 8 (48 x 12 cm), extending radially from a central area (32 cm in diameter). The apparatus was placed 40 cm above the floor, and surrounded by various extra maze cues placed at the same position during the study. At the end of each arm there was a food cup that had a single 50 mg food pellet. Prior to the performance of the maze task, the animals were kept on restricted diet and body weight was maintained of 85% of their free-feeding weight over a week period, with water being available ad libitum. Before the actual training began, three or four rats were simultaneously placed in the radial maze and allowed to explore for 5 minutes and take food freely. The food was initially available throughout the maze, but was gradually restricted to the food cup. The animals were shaped for 4 days to run to the end of the arms and consume the bait. To evaluate basal activity of rats in radial 8 arm-maze, the rats were given 5 consecutive training trials per day to run to the end of the arms and consume the bait. The training trial continued until all the 5 baits had been consumed or until all 5 minutes has elapsed. Criterion performance was defined as consumption of all 5 baits or until 5 minutes had elapsed. After adaptation all rats were trained with 1 trial per day. Briefly, each animal was placed individually in the center of the maze and subjected to working and reference memory tasks, in which same 5 arms (no. 1, 2, 4, 5, and 7), were baited for each daily training trial. The other 3 arms (no. 3, 6, 8) were never baited. An arm entry was counted when all four limbs of the rat were within an arm. Measures was made of the number of working memory errors (entering an arm containing food, but previously entered), reference memory errors (entering an arm that was not baited). Reference memory is regarded as a long-term memory for information that remains constant over repeated trials (memory for the positions of baited arms), whereas working memory is considered a short-time memory in which the information to be remembered changes in every trial (memory for the positions of arms that had already been visited in each trial) (Hritcu et al., 2007).

Elevated plus maze

Behavior in the elevated plus maze is also utilized to assess exploration, anxiety, and motor behavior. The elevated plus maze consists of four arms, 49 cm long and 10 cm wide, elevated 50 cm off the ground. Two arms were enclosed by walls 30 cm high and the other two arms were exposed. As per previous methods, rats were placed at the juncture of the open and closed arms and the amount of time spent on the open arms was recorded during a 5-min test. Time spent on the open arms is an index of anxiety.

Statistical analysis

Results were expressed as mean \pm S.E.M. The results were analyzed statistically by means of the Student's "t" test (T-test: Paired Two Sample for Means). $p < 0.05$ was taken as the criterion for significance.

RESULTS AND DISCUSSIONS

1. Effects of 5,7-DHT-induced serotonin depletion in PVN on learning and memory

Experimental data were registered 2 weeks after neurosurgery. Lesion of the PVH evidenced non-significant changes of short-term memory, explored by means of Y-maze task, indicated by spontaneous alternation percentage (Fig.1). The motor activity significantly decreased, in a dose-dependent manner, because the number of arm entries was significantly changed (Fig. 1) compared to the sham-operated (SO) rats.

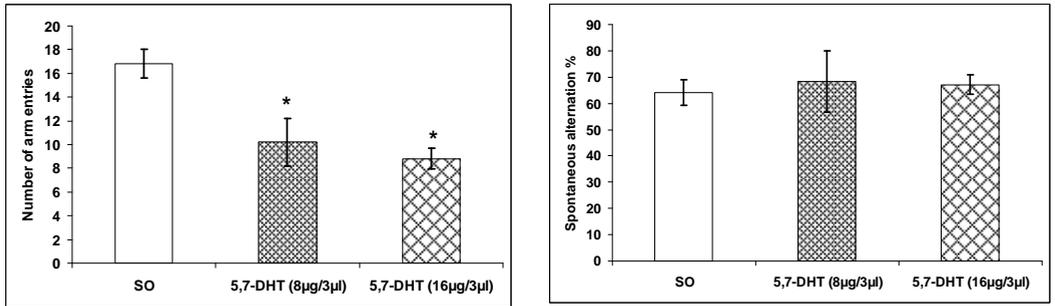


Fig. 1. Alterations of number of arm entries (left) and percentage of spontaneous alternation (%) (right) induced by serotonin depletion in PVN with 5,7-DHT. Data are presented as the mean \pm SEM; (n=6) *p<0.004 vs. SO group

5,7-DHT lesion of the PVN induced non-significant increase of the number of working memory errors (Fig. 2) and significant increase of the number of the reference memory errors (Fig. 3), explored by means of radial arm-maze, suggesting significant effects on reference memory.

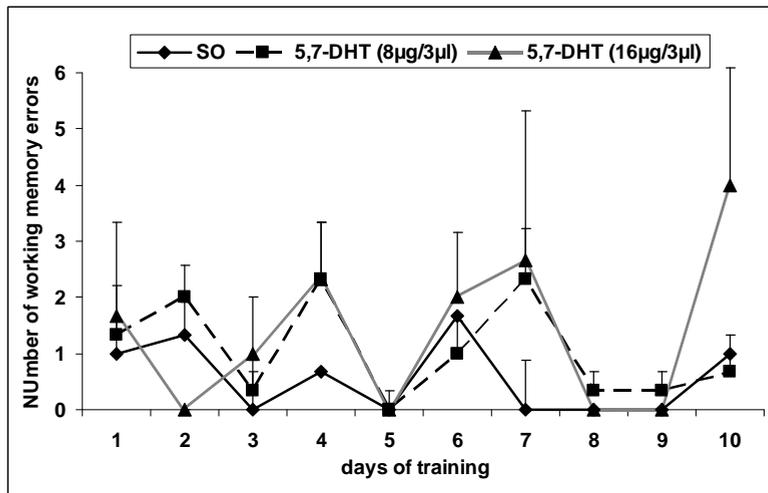


Fig. 2. Effect of 5,7-DHT-induced serotonin depletion in PVN on the number of working memory errors during 10 consecutive days training. Data are presented as the mean \pm SEM; (n=6).

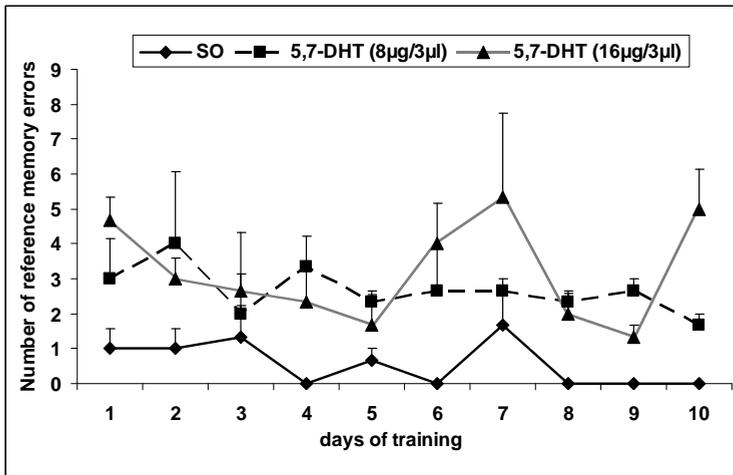


Fig. 3. Effect of 5,7-DHT-induced serotonin depletion in PVN on the number of reference memory errors during 10 consecutive days training. Data are presented as the mean \pm SEM; (n=6) *p<0.0004 vs. SO group

In the elevated plus maze task (Fig. 4), sham-operated rats spent less time on the open arms than 5,7-DHT (8µg/3µl) treated rats, suggesting that serotonin depletion in the PVN significantly diminished anxiety state.

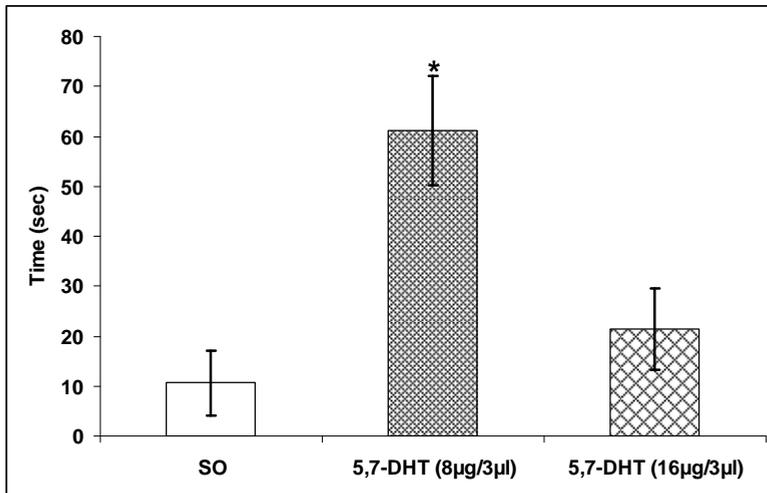


Fig. 4. Effect of 5,7-DHT-induced serotonin depletion in PVN on the time spent in the open arms in the elevated plus maze. Data are presented as the mean \pm SEM; (n=6) *p<0.0003 vs. SO group

Previous studies have demonstrated that serotonin depletion in the hypothalamus by 5,7-DHT injection into the PVN caused a differential excitatory effect on the HPA axis response to various stress stimuli (Larsen et al., 1996). In our study we assessed the effect of 5,7-DHT (8µg/3µl and 16µg/3µl) on the PVN-induced long-term memory impairment in rats. 5,7-DHT –

induced serotonin depletion on the hypothalamic level affect the memory performance in radial arm-maze, and significantly diminished anxiety state.

CONCLUSIONS

On the basis of our results obtained by serotonin depletion in PVN with 5,7-DHT we can conclude that PVN facilitate retention of reference memory, which are long-term memory.

REFERENCES

- Dunn, A.J., Berridge, C.W.**, 1990, *Brain Res. Rev.*; 15: 71-100
Herman, J.P. et al., 1996, *Crit. Rev. Neurobiol.*; 10: 371-394
Hritcu, L. et al., 2007, *Physiology and Behavior*, 91(5):652-657
Larsen, P. et al., 1996, *Neuroscience*; 70: 963-988
Paxinos, G., Watson, C., 2005, *Academic Press, Sidney*

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