

EFFECTS OF 6-OHDA INFUSION IN THE PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS ON THE LEARNING AND MEMORY PROCESSES IN RATS

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Abstract: Male Wistar rats were subjected to right-unilateral 6-hydroxydopamine (6-OHDA) 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. Lesion of the PVN significantly decreased the spontaneous alternation percentage in Y-maze task, in a dose-dependent manner, suggesting effects on spatial memory, especially on short-term memory. 6-OHDA significantly increased the number of working memory errors and the number of reference memory errors in radial 8 arm-maze task, in a dose-dependent manner, suggesting effects on spatial memory formation. In elevated plus maze measuring anxiety, 6-OHDA significantly diminished anxiety state, in a dose-dependent manner. In summary, we provided that the PVN have a facilitatory effect on learning and memory processes.

INTRODUCTION

The hypothalamo-pituitary-adrenocortical (HPA) axis consists of corticotropin releasing factor-41 (CRH-41) containing neurons in the paraventricular nucleus of the hypothalamus (PVN), which send their axons to the median eminence (ME), and in response to stressful stimuli, CRH-41 is realised into the portal circulation causing increased adrenocorticotrophic hormone (ACTH) and consequently adrenocortical secretion. The PVN also contributes to learning, memory, as well as having a role in stress, pain, and immune responses (Marquez et al., 2004). PVN lesion by means of 6-OHDA induced norepinephrine (NE) depletion in the hypothalamic level. The NE and serotonin (5-HT) depletion in the hypothalamus inhibited HPA responses (Feldman and Weidenfeld, 1995). The stimulatory effects of these neurotransmitters on the HPA axis at the hypothalamic levels have been documented by *in vitro* and *in vivo* studies. Thus, intraventricular administration of NE caused an elevation in portal blood CRH-41 levels (Plotsky, 1997) and NE injection into the PVN produced a rise in plasma corticosterone (CS) levels (Saphier and Feldman, 1989). It has also been demonstrated that both NE and 5-HT stimulate the realised of CRH from the hypothalamus (Kageyama et al., 1998), and depletion of this neurotransmitters in the hypothalamus by specific neurotoxins blocked the increase in HPA axis activity in response to stressful stimuli (Feldman and Weidenfeld, 1996). In the present study we evaluated the effects of PVN lesion in 6-OHDA treated rats on learning and memory processes.

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, 6-hydroxydopamine (6-OHDA) (Sigma) was dissolved in 0.9% NaCl containing 0.1% ascorbic acid to avoid oxidation. Vehicle and 6-OHDA (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 6-OHDA 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 6-OHDA-induced PVN lesion on learning and memory

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

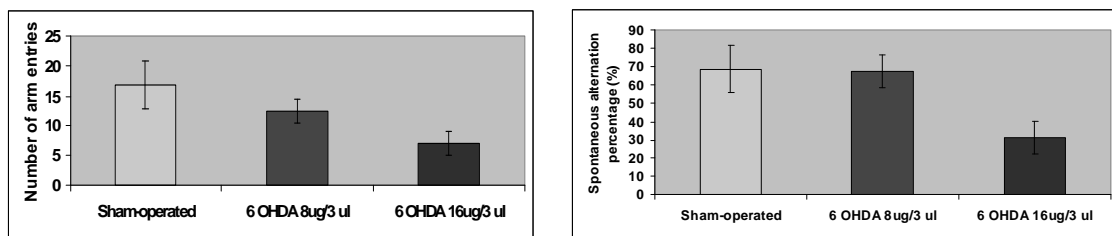


Fig. 1. Alterations of number of arm entries (left) and percentage of spontaneous alternation (%) (right) induced by PVN lesion with 6-OHDA. Data are presented as the mean \pm SEM; (n=6) * $p < 0.007$ vs. control group

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

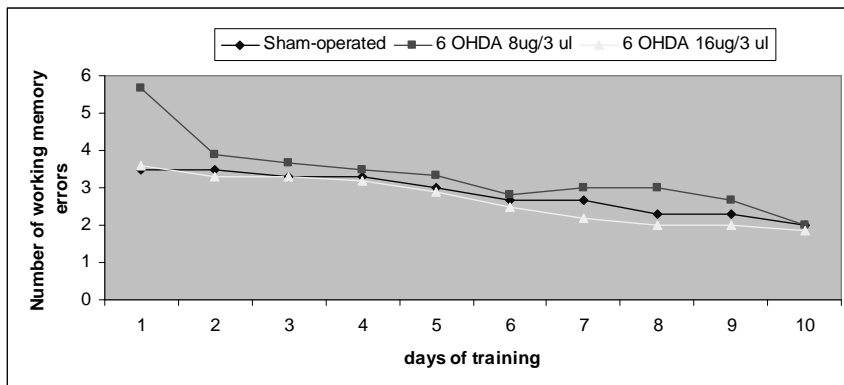


Fig. 2. Effect of 6-OHDA-induced PVN lesion on the number of working memory errors during 10 consecutive days training. Data are presented as the mean \pm SEM; (n=6).

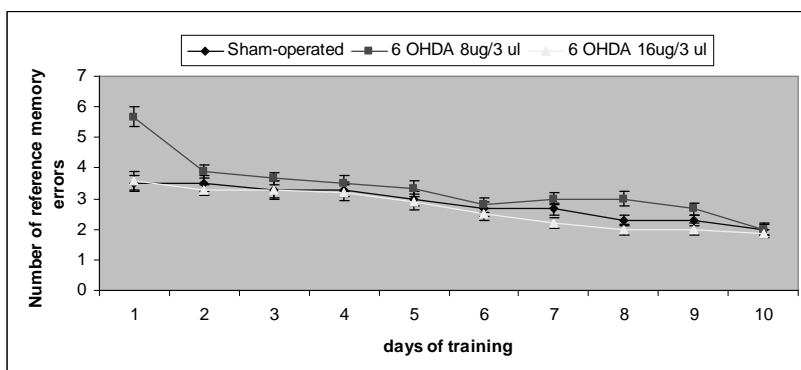


Fig. 3. Effect of 6-OHDA-induced PVN lesion on the number of reference memory errors during 10 consecutive days training. Data are presented as the mean \pm SEM; (n=6).

In the elevated plus maze task (Fig. 4), sham-operated rats spent less time on the open arms than 6-OHDA lesioned rats, suggesting that 6-OHDA significantly diminished anxiety state, in a dose-dependent manner.

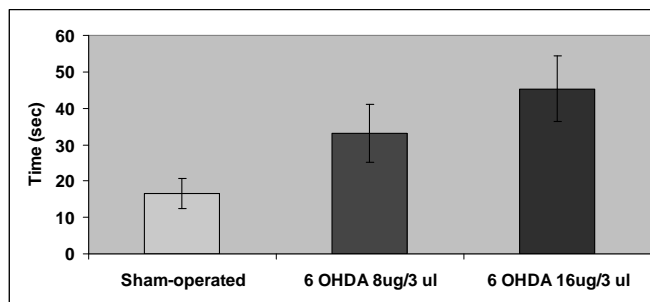


Fig. 4. Effect of 6-OHDA-induced PVN lesion on the time spent in the open arms in the elevated plus maze. Data are presented as the mean \pm SEM; (n=6).

Previous studies have demonstrated that NE depletion in the hypothalamus by 6-OHDA injection into the PVN caused a differential inhibitory effect on the HPA axis response to various stress stimuli (Feldman et al., 1996; Gaillet et al., 1991). In our study we assessed the effect of 6-OHDA (8 μ g/3 μ l and 16 μ g/3 μ l) on the PVN-induced memory impairment in rats. So, the facilitatory effect of NE on the HPA axis depends on the presence of this neurotransmitter in the PVN, and their depletion prevent the adrenocortical response. 6-OHDA – induced NE depletion on the hypothalamic level affect the memory performance in Y-maze and radial arm-maze, and significantly diminished anxiety state, in a dose-dependent manner.

CONCLUSIONS

On the basis of our results obtained by PVN lesion with 6-OHDA we can conclude that PVN facilitate retention of working and reference memory, which are a form of short-term and long-term memory, respectively.

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