

SEROTONIN DEPLETION AND SCHIZOPHRENIA: EFFECTS ON LEARNING AND MEMORY PROCESSES

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Abstract:

5,7-dihydroxytryptamine (5,7-DHT) (150 μ g; 4.5 μ l/ventricle), a serotonergic neurotoxin, significantly decreased spontaneous alternation in Y-maze task and working memory in radial arm-maze task, suggesting effects on short-term memory, without affecting long-term memory, explored by reference memory in radial arm-maze task. Parachlorophenylalanine (PCPA) (3 days treatment 200 μ g, i.c.v.), a serotonin synthesis inhibitor, significantly decreased spontaneous alternation in Y-maze task, suggesting effects on short-term memory. These results suggest that serotonin, among other neurotransmitters, plays an important role in cognitive functions, especially in short-term memory, with relevance for treatment of cognitive impairment in schizophrenia.

Key words: serotonin, schizophrenia, memory processes

Introduction

Schizophrenia is a chronic and severe psychiatric disorder, affecting approximately 1% of the population world-wide (1) and placing a heavy burden on society, both in terms of emotional suffering and economic loss. In schizophrenia, positive symptoms include delusions, hallucinations and thought disorder, whereas negative symptoms include affective flattening, alogia and emotional and social withdrawal (2).

Cognitive impairment is regarded as the third key symptom group, including deficits in language and executive function, attention, verbal and visual learning and memory, and working memory (3). Antipsychotic drugs have been standard therapy in psychiatry; however, they have important limitations including resistance in some patients and serious side effects. It has been generally accepted that a major component of the mechanism by which antipsychotic drugs achieve

their effectiveness (decrease positive symptoms) is mediated by dopamine D2 receptor blockade (4). Typical antipsychotics, such as haloperidol, bind primarily to dopamine receptors and there is an apparent positive correlation between the affinity of typical antipsychotics for dopamine D2 receptors and their clinical dose (5). There are several drawbacks associated with the use of typical antipsychotic drugs.

Up to 30% of patients do not respond to treatment and these drugs produce undesirable extrapyramidal side effects and hyperprolactinaemia. Atypical antipsychotic drugs, such as clozapine, interact with a number of neurotransmitter and receptor systems through binding at multiple receptor sites (6). Whether atypical antipsychotic drugs are superior compared to typical antipsychotic drugs in terms of positive and negative symptom reduction, or extrapyramidal and endocrine side-effect profile, is still under intense debate (6). Cognitive symptoms tend to be only mildly improved by atypical antipsychotics, yet cognitive variables are more predictive of functional outcome in schizophrenia than other symptom domains (7).

In the present study we examined the effects of altered brain serotonin activity in rats on behavioral responses by means of Y-maze and radial arm-maze tasks.

MATERIAL AND METHODS

Animals

20 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 (45 mg/kg b.w., i.p., Sigma) anesthesia. Rats were mounted in the stereotaxic apparatus with the nose oriented 11° below horizontal zero plane.

Injections into the lateral ventricle were performed by stereotaxic microinjections through a 10µl Hamilton syringe at the following coordinates: 0.5 mm posterior to bregma; 1.3 mm lateral to the midline; 4.3 mm ventral to the surface of the cortex, with the incisor bar set at 3.3 mm beneath the level of the interaural line (8). After each injection, the needle was left in situ for 5 min, retracted 2 mm, and a second delay of 4 min was allowed before complete retraction.

All rats sustaining a 5,7-DHT lesion were pretreated with desipramine (25 mg/kg, i.p. in saline, Sigma) 20 min before anesthesia in order to protect noradrenergic system. Rats were injected (4.5 µl/ ventricle) with 150 µg/free base of 5,7-DHT (creatine sulfate salt; 338µg dissolved in 20 µl of physiological saline containing 0.2 mg/ml ascorbic acid, Sigma). The sham-operated control rats were injected with saline.

Learning and memory tests began 2 weeks after the operation.

PCPA, which blocks the synthesis of serotonin, was administered i.c.v. by freehand through a plastic (silastic) cannula (0.9 mm outer diameter), implanted stereotaxically in the left cerebral ventricle at the following coordinates: 0.5 mm posterior to bregma; 1.3 mm lateral to the midline; 4.3 mm ventral to the surface of the cortex (8). The cannula was positioned with acrylic dental cement and secured by one stainless steel screw. After surgery the rats were isolated in separate cage and protected with large spectrum antibiotic (9). Five to seven days after surgery, the cannula was connected to a Hamilton syringe and 200µg PCPA/5µl (Sigma) was administered at a rate of 1µl/min for three consecutive days, to desipramine pretreated rats. Before withdrawal, the syringe was left in place for an additional 3 min to minimize dragging the injected solution. The sham-operated control rats were injected with saline.

Learning and memory test started, after 3 consecutive days of PCPA treatment.

The location of the i.c.v. cannulas in both 5,7-DHT and PCPA lesioned rats was verified by injecting a dye (trypan blue) through each cannula at the end of the experiment. Brains were removed and cut with a scalpel, and the spread of the dye within the ventricles was examined. All cannulas were found to be in the right position. Only the animals in which the placement and integrity of the cannula was verified were included in the behavioral data analysis.

Y-maze task

The 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 (10). Spontaneous alternation behavior is considered to reflect spatial working memory, which is a form of short-term memory.

Radial 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 in 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 5 minutes to 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. Rats were trained continually until reaching a criterion of at most 1 error per trial for 5 consecutive training trials. 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. The training trial continued until all 5 baits had been consumed or until 5 minutes had elapsed. An arm entry was counted when all four limbs of the rat were within an arm. Measures were made of the number of working memory errors (entering an arm containing food, but previously entered), and reference memory errors (entering an arm that was not baited) (10).

Statistical analysis

The results are expressed as means $\pm$ S.E.M. The results were analyzed using Student's t-test. Values of $p < 0.05$ were regarded as significant.

RESULTS

Effect of 5,7-DHT lesion on learning and memory

5,7-DHT (150 μ g, 4.5 μ l/ventricle) treatment evidenced a significant impairment of short-term memory, explored by means of Y-maze task, indicated by a decrease of spontaneous alternation percentage ($p < 0.0001$) (Fig. 1B). This effect could not be attributed to decreased motor activity, because the number of arms entries was not significantly changed ($p > 0.05$) (Fig. 1A).

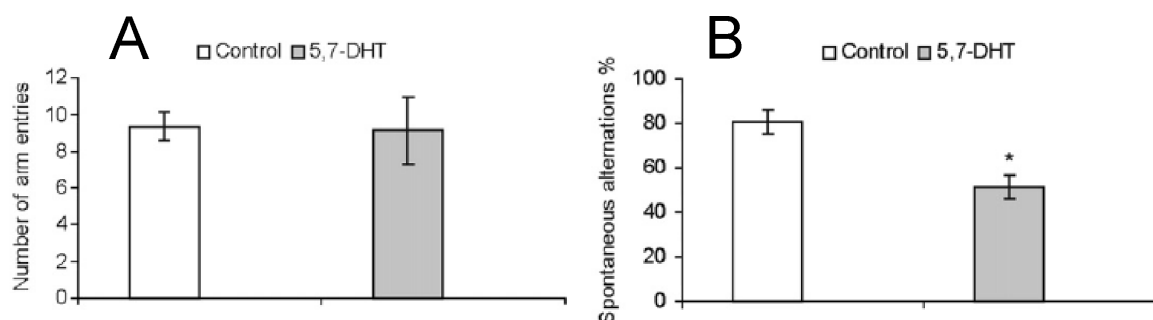


Fig. 1. Alterations of number of arm entries (A) and spontaneous alternation % (B) in Y-maze task induced by serotonergic depletion with 5,7-DHT. Values are means $\pm$ S.E.M (n=10 animals per group), * $p < 0.0001$ vs. Control group.

Serotonin depletion with 5,7-DHT impaired significantly short-term memory, tested by means of working memory errors ($p < 0.01$) (Fig. 2A) during 12 days training in radial arm-maze task. Regarding long-term memory, explored by means of reference memory errors ($p > 0.05$) (Fig. 2B), was unimpaired during 12 days training of rats with 5,7-DHT lesions in radial arm-maze task.

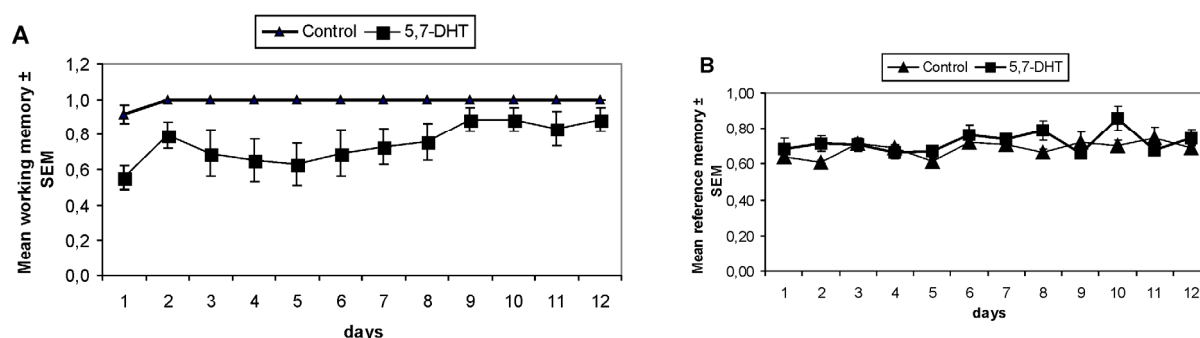


Fig. 2. Effects of serotonin depletion on working memory errors (A) and reference memory errors (B) during 12 days training in radial arm-maze test. Values are means $\pm$ S.E.M. (n=10 animals per group).

Effect of PCPA lesion on learning and memory

PCPA lesion of the serotonergic system, significantly impair short-term memory, explored by means of Y-maze task, indicated by a decrease of spontaneous alternation percentage ($p < 0.001$) (Fig. 3B). Regarding to motor function, PCPA lesion of the serotonergic system induced a significant decrease of locomotor activity ($p < 0.01$) (Fig. 3A).

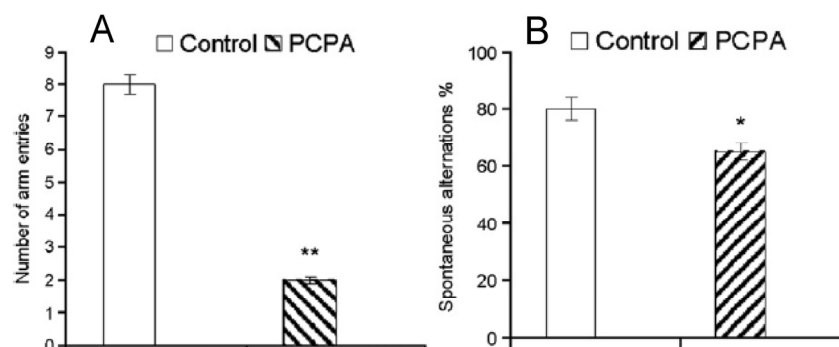


Fig. 3. Alterations of number of arm entries (A) and spontaneous alternation % (B) in Y-maze task induced by serotonergic depletion with PCPA. Values are means $\pm$ S.E.M (n=10 animals per group), * $p < 0.001$, ** $p < 0.01$ vs. Control group.

DISCUSSIONS

Previous studies have suggested the controversial role of serotonergic systems in learning processes. For example, serotonergic lesions have been reported to have no effect, facilitate or impair performance in various learning tasks (11).

Our results showed that serotonergic depletion by means of 5,7-DHT or PCPA induce a board spectrum of behavioral effects.

Brain serotonin depletion by means of 5,7-DHT induced a significant decrease in short-term memory (significant decrease of spontaneous alternation percentage in Y-maze test) and working memory (explored through the radial arm-maze test), without affecting significantly locomotion (non-significant decrease of number of arm entries in Y-maze test) and reference memory (explored through radial arm-maze task), compared to control rats.

PCPA, a serotonin synthesis inhibitor, significantly impair short-term memory, explored by means of Y-maze task (a significant decrease of spontaneous alternation percentage), which can be attributed to a significantly decrease of locomotor activity (a significant decrease of number of arm entries in Y-maze test) compared to control rats.

CONCLUSION

In conclusion, we found that the normal function of the serotonergic neuronal system is more important for the short-term memory and the working memory than for the long-term memory, since long-term memory was not affected in 5,7-DHT and PCPA treated rats. Consequently, pharmacological targets for improved treatment of cognitive impairment in schizophrenia are being strongly pursued (12).

These findings suggest that the serotonergic depletion contributes to the difficulty in modulation functions that might be essential for the short-term memory.

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