SPATIAL MEMORY DEFICITS INDUCED BY SYSTEMIC LIPOPOLYSACCHARIDE ADMINISTRATION

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Abstract: Stimulation of the immune system has been found to enhance, impair, or have no effect on various learning and memory tasks. The effects of systemic endotoxin treatment with lipopolysaccharide (LPS, 250 µg/kg in saline) were examined. Rats given LPS display spatial memory deficits in Y-maze and radial arm-maze tasks. Taken together, these results indicate that LPS treatment specifically impaired spatial learning performances in rats.

INTRODUCTION

Lipopolysaccharide (LPS) is a Gram-negative bacterial endotoxin and a potent microglial cell activator (Kim et al., 2000; Lieberman et al., 1989). LPS intranigral injection induces damage in nigrostriatal dopaminergic system and Parkinsonian-like symptoms in experimental animals (Hererra et al., 2000). Moreover, the blood-brain barrier can become leaky as a result of sepsis (Brandtzaeg et al., 1989), allowing LPS to enter the cerebrospinal fluid. These findings suggest that LPS exposure might also be a potential factor in the pathogenesis of Parkinson’s disease (PD).

Systemic administration of LPS causes an activation of phagocytes resulting in the release of pro-inflammatory cytokines, such as interleukin-1 beta (IL-1β), tumor necrosis factor alpha (TNF-α), and interleukin-6 (IL-6). After release, the cytokines target specific sites within the nervous system resulting in a set of physiological and behavioral symptoms known as “sickness behaviors” (Larson et al., 2002). These behaviors include decreased locomotion, decreased grooming, fever, hypersomnia, decreases in social interactions and decrease in food and water intake and are considered to be a part of the specific adaptive response that allows an organism to cope with invading pathogens (Hart, 1988). Moreover, numerous studies have supported the fact that LPS-induced immune activation impairs attention, but have a little effect on short-term memory (Holden et al., 2008), reduces avoidance learning (Kohman et al., 2008), deteriorates spatial learning performance (Arai et al., 2001), exacerbates local inflammatory response and increases neuronal death during chronic neurodegeneration (Cunningham et al., 2005).

The aim of the present study was to investigate the effects of bacterial endotoxin on spatial memory performance in Y-maze and radial arm-maze tasks following endotoxin injections in normal rats.

MATERIALS 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). This study was approved by the local Ethic Committee and also, efforts were made to minimize animal suffering and to reduce the number of animals used.

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. Spontaneous alternation behavior is considered to reflect spatial working memory, which is a form of short-term memory (Hritcu and Nabeshima, 2009).

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 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, the animals were shaped for 4 days to run to the end of the arms and consume the bait. The bait was initially available throughout the maze, but gradually was restricted to the food cup. 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 where within an arm. Measures was 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). The time taken to consume all five baits was also recorded. 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). Each animal was subjected to one trial each day.

Entries to repeat, choice accuracy was measured by entries to repeat, which was the number of arms entered until a repeat entry was made in the same arm in working or reference type memory, respectively

Drug administration

LPS from *Escherichia coli* serotype 0111:B4, (250 µg/kg, Sigma, dissolved in physiological saline 0.9% NaCl solution) was intraperitonealy (i.p.) injected (1 ml/kg b.w.) in LPS-treated rats for a period of 7 continuous days during radial arm-maze task and then animals were trained on the Y-maze task. Furthermore, animals were given LPS injections during training in Y-maze task. In radial arm-maze and Y-maze tasks, rats were treated with LPS 60 min before placement in the training session. Control animals received i.p. an equal volume of physiological saline (1 ml/kg b.w.) during radial arm-maze and Y-maze tasks.

Statistical analysis

Results were expressed as mean ± 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 DISSCUSIONS

1. Effect of chronic LPS administration on learning and memory

In Y-maze task, we observed a significant impairment of spatial memory in LPS-treated rats, indicated by a decrease of spontaneous alternations percentage ($p<0.05$) (Fig. 1B) compared to control group. This effect could be attributed to decreased motor activity, because the number of arm entries was significantly changed ($p<0.01$) (Fig. 1A).

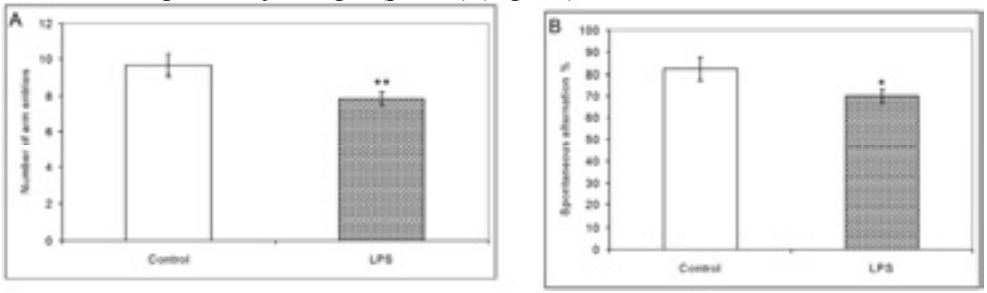

Fig. 1. Effects of LPS treatment on number of arm entries (A) and on spontaneous alternation % (B) in Y-maze task. Data are means ± S.E.M. (n=10 per group). **p<0.01, *p<0.05 vs. control group.

In radial arm-maze task, LPS-treated rats showed significant increase of both working memory errors ($p<0.05$) (Fig. 2A) and reference memory errors ($p<0.0001$) (Fig. 2B) compared to control rats, during 7 days training. Furthermore, spatial memory degradation after LPS

treatment resulted in significant decrease of entries to repeat ($p<0.0001$) (Fig. 2C) and increase of time taken to consume all five baits ($p<0.0001$) (Fig. 2D) compared to control rats.

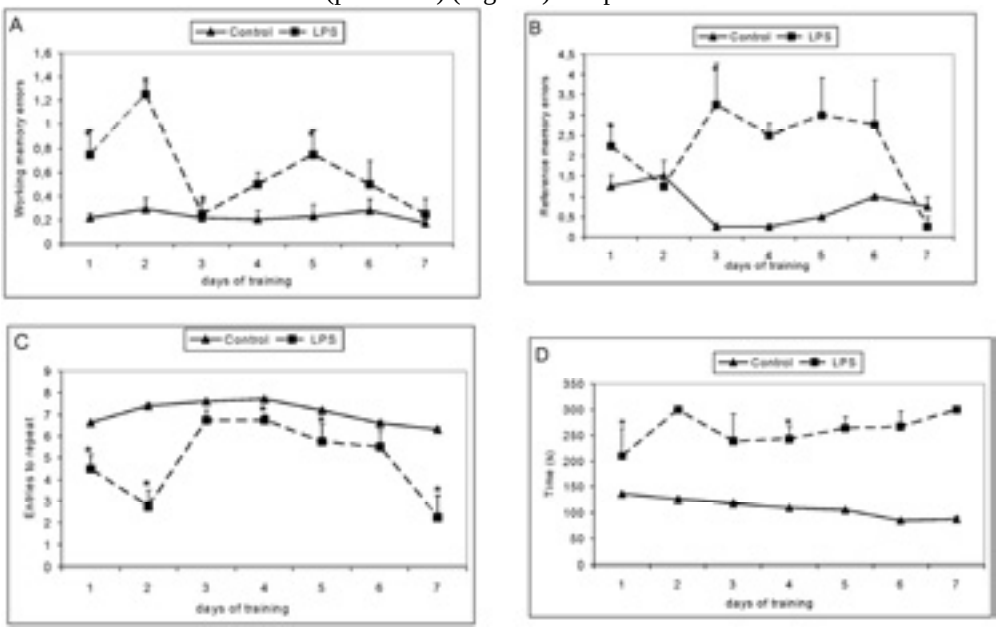

Fig. 2. Effects of LPS treatment on the number of working memory errors (A), the number of reference memory errors (B), entries to repeat (C) and time taken to consume all five baits (D) during 7 days training in radial arm-maze task. Values are means ± S.E.M. (n=10 animals per group).

Previous studies have been demonstrated that LPS endotoxin temporarily activates the immune system, stimulating cytokine production and in a correlated fashion to disrupt learning in some tasks (Pugh et al., 1998). Successful learning depends upon a variety of constituent cognitive processes, any of which might be the underlying cause for the disruption of learning. Previous studies noted that post-training consolidation is at least one such processes disrupted by LPS (Holden et al., 2008).

In our study, LPS-treated groups showed significant impairment of spatial memory (decrease of spontaneous alternations percentage in Y-maze task and increase of working memory errors and reference memory errors in radial arm-maze task) and decreased motor activity (decrease of number of arm entries in Y-maze task) compared to control rats. One of the sickness behavior caused by LPS treatment is decreased locomotion. There has been debate about the interpretation of results in various spatial memory paradigms such as water maze (Cunningham et al., 2008) for rats treated with LPS. It is possible that the deficit in memory performance observed in the present study is a result of a decrease in locomotor behavior rather than a cognitive impairment. These deficits along with the motor reducing effects of LPS could have caused an even greater motor impairment than LPS treatment alone which could have been interpreted as a deficit in memory observed in Y-maze.

Moreover, our data revealed that systemic administration of endotoxin resulted in behavioral deficits in radial arm-maze task, as shown by increased number of working memory

errors and reference memory errors in LPS-treated groups. Rapid development of tolerance to the effects of LPS can appear following 2-3 injections (Engeland et al., 2001). Since animals were given injections with LPS for a period of 7 continuous days, behavioral tolerance likely develops and decreased the effects of LPS.

CONCLUSIONS

On the basis of our results obtained by LPS administration, we can conclude that in the rats, LPS administration induced significant impairment in spatial memory performance in LPS-treated animals.

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