The Effects on Spatial Memory after Cervical Lymphatic Trunk Blockage in Rats

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Abstract **Objective** To explore the effect on spatial memory after cervical lymphatic trunk blockage in rats. **Methods** 24 male Wistar rats were randomly divided into the model and sham-surgery groups. All the rats were trained with information swim(IS) and choice swim (CS). The former represents spatial reference memory, and the later examined spatial working memory. The model rats were blocked in cervical lymphatic trunk as lymphostatic encephalopathy (LSEP) models. Acquired spatial memories were tested during 6-8 days and 15-17 days after operation. **Results** The latency of information swim of the 6-8 days model rats was significantly longer than that of the sham's (P<0.01), but there was no significant difference of choice swim between both groups(P>0.19). **Conclusion** Lymphostatic encephalopahy can damage spatial reference memory, but has no impact on working memory of the rats.

Key Words lymphostatic encephalopathy; spatial reference memory; spatial working memory; cervical lymphatic blockage

Spatial memory can be damaged in many brain diseases.Lymphostatic encephalopathy (LSEP) is cephalic hyperproteinemia caused by the brain lymph drainage blockage, accompanied with quantities of clinical manifestations. Of which, memory decrease is a common clinical manifestion. Many animal models have been studied in LSEP effects to brain functions.[1, 2], but few researches were reported about the effects to spatial memory.Spatial memory was classified as spatial reference memory and spatial working memory. Markowska has designed the delayed nonmathing to position task (DNMPT) in the water maze, which can test spatial reference memory and spatial working memory in the same condition[3]. So it is comparable of the effects to both memory of various factors. To explore the effect to spatial memory, cervical lymphatic trunk was blocked in rats to make the model of lymphostatic encephalopathy. Water maze task can test spatial reference memory and spatial working memory.

MATERIALS AND METHODS

Experimental animals

24 male Wistar rats(2~3months). After formation of spatial memory, they were randomly divided into 2 groups: model group, whose cervical lymph nodes were removed and lymphatic trunk was blocked; sham-surgery groups, who were under sham surgery.

Principle of experimental design

For adaptive training , one session consisting of nine trials was conducted each day for 3 days;task training 10 days, 9times/day; 3days break; cervical lymph nodes were removed and bilateral cervical lymph trunks were blocked; spatial memory tests were conducted 6~8 days (when edema was significant) and 15~17 days (when edema disappear) after surgery. The procedure is the same as preoperation.

Non matching–to–position task

Water maze was taken by Markowska. Briefly, the tank was 1.8 m in diameter and filled with water at a temperature of 24.6℃. The collapsible escape platform (Lucite, 10 ×10cm) was either 1 cm below the surface of the water for the regular training trials (platform trials) to allow the animal to climb onto it or beneath the surface of the water and thus unavailable to the rat during the variable-interval probe trials. A Plexiglas T-shaped partition was inserted in the water tank (1.3m in diameter) to create a start section and two choice sections. A sliding panel either remained centered, allowing access to both choice sections, or was moved to one side to block one section. A collapsible escape platform ($10cm^2$) was located in each one of the two choice sections. In the raised position, the top of the escape platform was 1 cm below the surface of the water, and in the lowered position, the top of the platform was 19 cm below the surface of the water. Only one platform was

available (raised position) for the rat for each trial. To train the rat to swim and climb onto the platform, a straight swim procedure (one session, 10 trials) was conducted. A second shaping procedure, using the "T" partition, trains the rat to swim to the platform located in either choice section of the water maze (2 consecutive d, nine trials/d), with starting point at the entrance to the choice section (day 1) or in the start section (day 2). For training, each trial consisted of two parts: an information Swim (IS) and a choice swim (CS). For IS, one choice section was open; the platform was located in the open section in its raised position. The rat was allowed 60 sec to locate the platform. After 10 sec on the platform, the rat was placed in a holding cage for 1 min. For the CS, both choice sections were open, but only the platform in the section that was closed previously was available to the rat. If the rat entered the incorrect section, the sliding door was closed, confining the rat for 30 sec. After punishment, the sliding door was opened, and the rat was allowed to locate the platform in the correct section. One session consisted of nine trials (intertrial interval was 10 min). And choice accuracy is the comparion between the times of straight swim to platform and total swim times.

Surgery

Rats were anesthized with 1% pentobarbitol and fixed , disinfected. Longitudinal incision was made in the median line of neck. All superficial lymph nodes were separated and removed, lymph vessels were ligated and stump was electically scorched.Profound cervical lymph nodes were separated beside the jugular vein, and cervical lymphatic trunk was ligated. Skin was sutured. In sham-surgery groups skin was incised and then sutured, without any management of lymph node and vessels.

Statistical analysis

All data were analyzed with SAS5.03 software and expressed as ±s. Mean comparisons were performed using two-tailed paired Student's t test.

RESULTS

Presurgical spatial test

Information swim time is to test spatial reference memory. In the presurgical training period, information swim time of the rats decreases gradually with the increase of training days, the 1st day: 18.84±5.27s, the 10th :4.62±0.98s, there is significant difference between them(P<0.01).The 8th to 10th day, model group 5.56± 0.85s, sham surgery group 5.37±0.79s, no significant difference is seen between them(P>0.21).

Choice swim time is the period to find the hidden platform. Choice swim time decreases gradually with the increase of training days, the 1st day 8.24±2.13s, the 10th day 3.67±0.98s, there is significant difference between them(P<0.01). The 8th to 10th day, model group 3.81±0.64s, sham surgery group 4.19±0.98s, no significant difference is seen between them(P<0.13).

Memory test 6~8days after surgery

Information swim time of LSEP rats prolongs significantly.Model group 8.86±1.01s, sham surgery group 5.52±0.82s, there is significant difference between them (P<0.01). Information swim time of model group and sham surgery group(6~8days after surgery) are shown as table 1

Table 1 Information swim time after surgery ($\bar{x}$±s, seconds)

Groups	6 days	7 days	8 days
Model group	8.24±0.66	10.45±0.24	7.65±0.77
Sham surgery group	5.85±0.46	5.68±0.83	5.45±0.62

Choice swim time Model group 4.21±0.52s, sham surgery group 4.19±0.32s, no significant. difference is seen between them (P>0.19)

Table 2 Information swim time after surgery ($\bar{x}$±s, seconds)

Groups	15 days	16days	17days
Model group	5.75±0.53	5.82±0.42	5.51±0.37
Sham surgery group	5.67±0.84	5.91±0.34	5.46±0.56

Choice swim time Model group 4.12±0.23s, sham surgery group 4.28±0.25s, there is no significant difference between them (P>0.15)

Spatial memory test 15~17days after surgery

Information swim time model group 5.81 ±0.43s, sham surgery group 5.54 ±0.51s, no significant difference is seen between them (P>0.12). Information swim time of model group, sham surgery group(15~17days after surgery) is shown as table 2

DISCUSSION

Changes of spatial reference memory in LSEP Memory mailnly consists of three parts: acquisition, consolidation and retrieval. In this experiment, it was proved that spatial reference memory of rats declines in LSEP, that's to say, retrograde memory declines. It has been proved by Smith and Scheff et al[6, 7] that damages to cortex will hurt spatial referenc memory of rats and mice; Chen et al[8, 18] proved that after bilateral lesions of hippocampus, mentaintion of spatial reference memory would deline. All these results were similar to our experiment. In this experiment, there was no significant difference in information swim time between sham surgery group and model group 15~17days after surgery, which proves spatial reference memory of rats recovers. Spatial working memory is intact in LSEP models 6~8days and 15~17days after surgery, there is no significant difference in choice swim time between model group and sham surgery group, which proved that spatial working memory is intact. Hamm et al[9] think that spatial working memory will be hurt after brain damages , which is different to our study. It's possibly because:① the intervals between acquisition and consolidation were different in these two studies. In Hamm's experiment, the intervals were 4 minutes. So the longer intervals added more complexity, since the animals should take more time to keep memory[10]; ②task difficulty is different in these two water maze.Although spatial working memory is tested in water maze, the position of platform and entrance to water of rats changes frequently in Hamm's experiment.

Possible mechanism to the memory damages of LSEP in LSEP, spatial reference memory and spatial working memory are tested in the same conditions. So the task conditions are not the cause of different effect to the two memories. Hippocampus plays the key role in spatial learning and memory [11] and damages to hippocampus can affect spatial working memory[12, 15, 16] and spatial reference memory[5,8]. But it is reported that other parts of brain take part in the regulation of spatial working memory[13]. In LSEP, edema was different in portions of the brain[4], so did the changes of the functions. Hippocampal formation is affected more by protein edema, and it is essential to the mentaintion of spatial reference memory[14,17]. Thus the spatial reference memory was significantly affected in model group. Besides hippocampus, working memory depends more on neocortex and somewhere, which are less affeceted by protein edema. So changes of spatial working memory were not significantly.

REFERENCES

1. Wang HJ, Zhang XT, Chen LB, et al. Effects of cervical lymph blockage of rats on morphology of brain, blood pressure and EEG. J Shandong Med Univ, 1995, 33(4):271-274.
2. Foldi M, Casley-Smith Jr. Lymphangiology. Stuttgart:F.K. Schattauer GmbH, 1983, 475-505.
3. Markowska AL, Price D, Koliatorsos VE. Selective effects of nerve growth factor on spatial memory as assessed by a delayed non mathing-to-position task in the water maze. J Neuroscience, 1996, 16(10):3541-3548.
4. Smith DH, Soares HD, Pierce JS, et al. A model of parasagittal controlled cortical impact in the mouse, cognitive and histopathologic effects, J Neurotrauma.1995, 12(2):169-178.
5. Scheff SW, Baldwin SA, Brown RW, et al.Morris water maze deficits in rats following traumatic brain injury lateral controlled cortical impact. J Neuroanatomy, 1997, 14(9):615-627.
6. Chen Y, Constantini S, Trembovler V, et al.An experimental model of closed head injury in mice:pathology, histopathology and cognitive deficit.J neurotrauma, 1996, 13(10):557-568.
7. Hamm RJ, Temple MD, Pike BR, et al.Working memory deficits following traumatic injury in the rat. J Neurotrauma, 1996, 13(6):317-323.
8. Santin LJ, Rubio S, Begega A, et al. Effects of mammillary body lesions on spatial reference and working memory tasks. Behav Brain Res, 1999, 102(1-2):137-150.
9. Elliot K, Whelan J.Functions of the septo-hippocampal system. Amsterdam:Esevier science, 1978, 327-49.
10. Aggleton JP, Keith AB, Rawlins JN, et al.Removal of the hippocampus and transetion of the fornix produce comparable deficits on delayed non mathing to position by rats. Behav Brain Res, 1992, 52(1):61-71.
11. Morris RG, Hagan JJ, Rawlins JN.Allocentric spatial learning by hippocampectomised rats: a further test of the "spatial mapping" and "working memory" theories of hippocampal function. Q J Exp Psychol B, 1986, 38(4):365-395.
12. Granon S, Poucet B.Medical prefrontal lesions in the rat and spatial navigation: evidence for impaired planning. Behav Neurosci, 1995, 109(3):474-484.
13. Sarihi A, Motamedi F, Naghdi N, et al. Lidocaine reversible inactivation of the median raphe nucleus has no effect on referenc memory but enhances working memory versions of the Morris water maze task. Behav Brain Res, 2000, 114(1-2):1-9.
14. Rashidy-Pour A. Motamedi F, Motahed-larijani Z. Effects of reversible inactivations of the medial septal area on reference and working memory versions of the Morris water maze.

Brain Res,1996, 709(1):131-140.
15. Astur RS, Taylor LB, Mamelak AN, et al. Humans with hippocampus damage display severe spatial memory impairments in a virtual Morris water task. Behav Brain Res, 2002, 132(1):77-84.
16. Jeltsch H, Bertrand F, Lazarus C, et al. Cognitive performances and locomotor activity following dentate granule cell damage in rats: role of lesion extent and type of memory tested. Neurobiol Learn Mem, 2001, 76(1): 81-105.
17. Simonova Z, Sterbova K, Brozek G, et al. Postnatal hypobaric hypoxia in rats impairs water maze learning and the morphology of neurones and macroglia in cortex and hippocampus. Behav Brain Res, 2003, 141(2):195-205.
18. Vann SD, Aggleton JP. Evidence of a spatial encoding deficit in rats with lesions of the mammillary bodies or mammillothalamic tract. J Neurosci, 2003, 23(8): 3506-14.