The Disturbance of Water-Maze Task Performance in Mice with EMC-D Virus Infection

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ABSTRACT. The present study was performed to examine the influence of EMC-D virus on water-maze task performance in DBA/2 mice inoculated with 10^3 PFU/ml and BALB/c mice inoculated with 10^5 PFU/ml of EMC-D virus. In both strains, the latency to accomplish the task was shortened during successive 8 days of testing. However, the shortening of latency in the inoculated group was significantly smaller than that in the control group. Pathological studies showed that in both studies *St. pyramidale hippocampi* was commonly degenerated, but the degeneration of the spinal cord was shown only in DBA/2 mice. Therefore, the disturbance of water-maze task performance in mice infected with EMC-D virus might be partially due to the dysfunction of memory for working process or cognition of orientation other than underlying decreased ability in locomotion.—**KEY WORDS:** EMC-D virus, strain difference, water-maze task.

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EMC-D is a highly diabetogenic variant of encephalomyocarditis (EMC) virus [1, 2, 4]. Previous studies have demonstrated that DBA/2 mice, a highly susceptible strain to EMC virus-induced diabetes, show the focal degeneration of restrictive regions in the brain, such as *St. pyramidale hippocampi*, and in the spinal cord such as *Cornu ventrale*, *Funiculus ventralis* and *Funiculus lateralis* [4]. These pathological changes have been first noticed on 6th day postinoculation when hind-limb paralysis concurred [4]. However, such pathogenicity of EMC-D virus was variable among strains of mice; for instance, BALB/c mice exhibited only minimum degeneration limited to the hippocampus in the brain even if the dose of EMC-D inoculated was increased as much as 100 times in DBA/2 mice. In addition, BALB/c mice hardly show the hind-limb paralysis.

Although the effect of EMC-D virus on behavior in mice was poorly understood, it is conceivable that dysfunction of the hippocampus and *Nuc. amygdaloideus* might influence on space memory or memory of working process [3, 5]. In order to test this hypothesis, the possible disturbance of cognition of orientation or memory of working process as well as disorder of swimming performance was examined by the method of “water-maze task”.

Sixteen male DBA/2 mice (8-week-old) and twelve male BALB/c mice (8-week-old) were used in this study. A day before the testing, EMC-D virus was intraperitoneally inoculated to 10 DBA/2 mice and 6 BALB/c mice at a dose of 10^3 PFU/ml and 10^5 PFU/ml, respectively. Other 6 DBA/2 mice and 6 BALB/c mice were injected with 0.1 ml of saline as controls.

The apparatus for water-maze is presented in Fig. 1. It was a large round pool made of acrylic resin with a size of 62 cm in diameter and 15.5 cm in height. This pool was filled with water at a height of 10 cm above the base and was placed on a metal frame support so that the water level was 75 cm above floor level. The entire apparatus was placed in the center of a laboratory room with a size of 1.5 (W) × 1.5 (L) × 2 (H) m. The inner surface of this room was surrounded with black walls and lightened by 40 w fluorescent light. A white column platform (5 cm in diameter, 9.5 cm in height), which did not protrude above the water surface, was set in a constant position, 9.3 cm

Fig. 1. The schematic illustration of the apparatus for the water-maze.

from the edge of the pool, so that the mice could escape from the water onto it. Water was colored white by 250 ml of milk in order to hide the platform. This water was changed group by group and the whole unit was disinfected with 5% hypochlorous acid solution. The position of a observer in the room was constant through all testings.

At the start of the trial, the mouse was seized by its tail, and placed in the water facing the platform at the opposite side of the platform. On releasing the mouse, the timing was simultaneously started and the pool was covered. If the mouse did not locate the platform within 60 sec, it was removed from the water and placed on the platform for 15 sec. When the mouse found the platform, the trial was ceased and the mouse was allowed to remain on the platform for 15 sec. The trial was repeated 3 times, at a 5 min-interval, a day for each animal, and continued for 8 consecutive days. The dependent variable for analysis was latency to find the platform.

Some other DBA/2 and BALB/c mice were assigned for histopathological examination on the central nervous

system on the 3rd and 6th day postinoculation. The brain and spinal cord were fixed in 10% neutral buffered formalin. Paraffin sections (4 micronmeters) were made and stained with hematoxylin and eosin.

Percent changes of the latency to find the platform in comparison with that on the first experimental day (D1) in DBA/2 mice with or without EMC virus inoculation are presented in Fig. 2. On the first day, mean latency was 50.1 sec in the infected group and 55.1 sec in the control group, respectively. In both groups, the latency decreased gradually as the day went by, whereas the infected group showed a longer latency than the control group on D3 ($p < 0.05$, non-paired t -test). Moreover, a significant decrease in latency ($p < 0.05$, paired t -test) was already seen on D3 in the control group, while in the infected group it was not observed till D5 postinoculation.

Similarly, percent changes of the latency to find the platform in BALB/c mice are compared between the infected and control groups in Fig. 3. On the first day, mean latency was 37.5 sec in the infected group and 39.3 sec in the control group, respectively. In both groups, the latency tended to decrease during the test period, but the decrement was milder than that in DBA/2 mice even though the dose of virus inoculated was 100 times as large as that in DBA/2 mice. The infected mice tended to show a longer latency than the control group on D4 and D6 ($0.1 < p < 0.2$ non-paired t -test). In the infected group, a significant decrease in latency was seen on D7 ($p < 0.05$ paired t -test) as compared with the latency on D1, while it was already apparent on D4 in the control group.

The histopathological examination showed that in both DBA/2 and BALB/c mice, *St. pyramidale hippocampi* was commonly degenerated, while the lesion of *Nuc. amigdaloides corticalis* and *St. granulosum cerebelli* was sometimes involved. In the spinal cord, the lesion was frequently found in *Funiculus ventralis* and *Funiculus lateralis* and *Cornu ventrale* in DBA/2 mice, while it was rarely found in BALB/c mice. These histopathological changes were characterized by pyknosis of neurons and demyelination of axons.

Less pathological changes in the spinal cord in BALB/c mice, in spite of a much higher dose of the virus inoculated seemed to be closely associated with the fact that BALB/c mice infected with the virus hardly showed the hind-limb paralysis during this study. Therefore, the tendency for elongated latency in the infected DBA/2 and BALB/c mice might be partially due to the dysfunction of memory for working process or orientation in the water-maze task other than underlying decreased ability in locomotion. In fact, the hippocampus and *Nuc. amygdala* have been thought to be the most important regions for space memory, which has been further elucidated by the removal of the hippocampus [5] and by the presence of "place unit" recorded from the hippocampus [3].

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Fig. 2. Percent changes of latency in the water-maze task in DBA/2 mice with or without infection of EMC-D virus during successive 8 days postinoculation. ○; control group ($n=6$), ●; infected group ($n=10$). Each value indicates the mean $\pm$ S.E. †; $p < 0.05$ (compared between control and infected groups), *; $p < 0.05$ (compared with the value of the first day postinoculation).

Fig. 3. Percent changes of latency in the water-maze task in BALB/c mice with or without infection of EMC-D virus during successive 8 days postinoculation. ○; control group ($n=6$), ●; infected group ($n=6$). Each value indicates the mean $\pm$ S.E. *; $p < 0.05$ (compared with the value of the first day postinoculation).

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