change in the liver does not cause prolonged clearance of barbiturates from the blood. This prolonged sleeping time is not obtained with similar doses of Tech. DDD in the rat although a *slight* lowering of tolerance to anoxia may result.

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## Learning Deficit in Mature Rats Recovered from Early Post-Natal Infection With West Nile Virus. (24003)

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It has been established that certain viruses, which cause disease of the central nervous system (CNS) of man, can also cause a fatal CNS infection in rats, provided young enough animals are tested. A uniformly fatal encephalitis occurs in 7-day-old rats following either intracerebral injection or intranasal instillation of either St. Louis encephalitis virus (SLE) or Japanese B encephalitis virus (JBE). However, at 12 days of age rats manifest a high degree of resistance to both viruses and at 21 days of age are completely resistant, as far as clinically apparent infection is concerned(1,2). The resistance of older rats is not absolute, however, since, as has been demonstrated in the case of SLE, an inapparent infection occurs in 21-day-old rats (unpublished data). Furthermore, it has been observed that an occasional 12-day-old rat may develop a clinically apparent infection with JBE and then recover(2). When our studies were undertaken, the age-susceptibility pattern of the rat to West Nile virus (WN), a virus closely related to both SLE and JBE was being studied. It was found that the rat reacts to intracerebrally injected WN in the same manner that it does to JBE in that 7-day-old rats develop a uniformly fatal encephalitis while, in 12-day-old rats, some may succumb but most survive without evidence of CNS involvement although an occasional animal manifests signs indicating CNS dysfunction with subsequent recovery. On the other hand, rats 3 to 4 weeks of age never develop a clinically apparent infection. Although rats which have resisted or recovered from infection with above viruses do not subsequently show obvious abnormalities it seemed reasonable to assume that the viruses may have caused some damage to the CNS which could be reflected in subsequent behavior of the animal, especially behavior near the upper limit of complexity. To test this hypothesis one behavioral pattern, the learning of a moderately complex maze, was investigated. Rats which had received WN intracerebrally and rats which had received no virus, but whose brains had been similarly traumatized, were studied in a 14 choice-point modified Stone Multiple T-maze and the re-

sults compared.

*Materials and methods.* A single suspension of WN was used and was prepared by homogenizing WN-infected mouse brains of 3- to 4-week-old Rockland Swiss mice in a Waring blendor in sufficient inactivated, undiluted rabbit serum to make a 10% (W/V) suspension of virus-infected brains. The resulting homogenate was centrifuged 5 minutes at 3000 rpm and the supernatant fluid distributed in 2.2 ml amounts in screw-capped vials. The vials containing the virus were rolled in a dry-ice-alcohol bath and, after freezing, the virus was stored in a dry-ice cabinet. When needed, the virus was thawed by placing the virus-containing-vial in 37°C water bath. The mouse intracerebral $LD_{50}$ titer(3) of the virus at the time it was used in the experiments reported here was $1 \times 10^{-7.7}$. A 10% (W/V) suspension of normal mouse brain (NMB), injected into control rats, was prepared in the same manner as described for preparation of the virus suspension. Sprague-Dawley rats, born on 2 consecutive days in animal quarters at the Medical Center, were injected intracerebrally with either 0.05 ml of the 10% suspension of WN or 0.05 ml of the 10% suspension of NMB, when they were 12 days old. Injections were made into the right cerebral hemisphere, using 0.25 ml syringe and 27 gauge ⅛ inch needle. No anesthetic was used. Some animals in each litter received WN and part NMB. Following inoculation, the animals were observed at least once, and frequently twice, daily for 21 days. They remained with the mother until they were 21 days old at which time they were weaned and sex determined. Males and females were kept in separate cages. When animals were 107 and 108 days old they were transferred from the Medical Center to North Little Rock Veterans Administration Hospital where one of us (ODM) conducted the maze studies. Prior to being transferred the rats were numbered by the method used by Walker(4). During maze learning studies the rats were tested by number and the experimenter had no knowledge of previous history of the animals. After approximately 1 month in their new quarters the animals were introduced to the maze. Before beginning any learning trials, the rats were placed in the goal box twice daily for 30 minutes on 5 consecutive days. Since thirst was to be used as motivation for running the maze, water was available to rats during 30 minute intervals spent in the goal box. After this period of orientation the animals were tested in the maze, one learning trial/day, for 8 consecutive days. The learning trials were then interrupted for 20 days after which they were resumed and continued for 7 consecutive days for a total of 15 trials. The 20-day interval was introduced to determine whether animals would forget, during this period, what had been learned during the first 8 trials. The time/trial and number of errors (number of blind alleys entered)/trial/rat were recorded. Test and control animals were deprived of water for 23½ hours prior to a learning trial.

*Results.* Twenty-four rats which had recovered from infection with WN and 18 NMB controls were run in the maze. The maze-learning curves of virus and control groups appear in Fig. 1 and 2. Fig. 1 shows mean

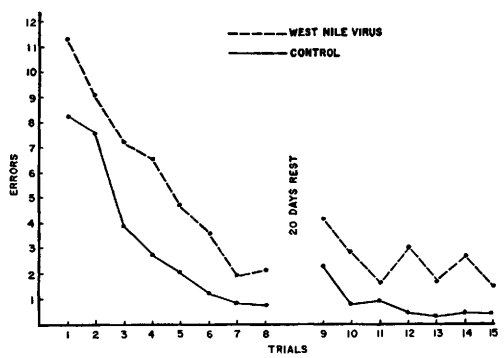


FIG. 1. Mean errors per trial.

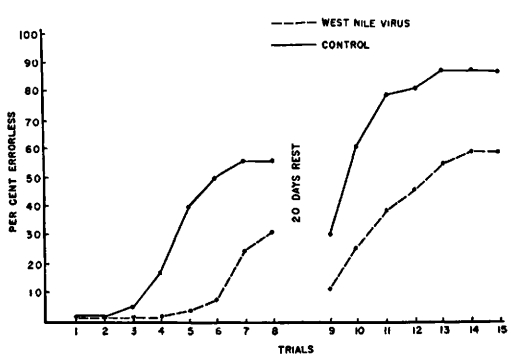


FIG. 2. % of rats having errorless run per trial.

TABLE I. Number of Rats Making Scores above and below Median.

| | West Nile | Controls | Total |
|---|---|---|---|
| Above median | 19 | 2 | 21 |
| Below " | 5 | 16 | 21 |
| Total | 24 | 18 | 42 |

errors trial for each group and it will be noted that while the virus-infected animals did learn they did so at a slower rate than the controls. During the 20-day period between trials 8 and 9 the absolute memory loss of test animals is not noticeably different or at least not greater than that for the control group as shown by performances on 9th trial. Thereafter their learning proceeded at a slower rate and their performance never approached the level attained by controls. The percentage of each group achieving an errorless performance on a given trial is shown in Fig. 2. There is a striking difference in learning plateaus reached by each group. It will be noted that more than 50% of the controls had mastered the maze by the 7th trial while less than 30% of virus-treated rats had done so by the 8th trial. By the 13th trial approximately 90% of controls had errorless runs whereas no more than 60% of the WN had learned the maze after 15 trials.

Table I shows distribution of virus-infected and control rats above and below the median number of errors for all 42 animals. It will be seen that, while there is some overlapping, most infected rats had scores above the median whereas, control animals, with 2 exceptions, had scores below.

The data were subjected to further analysis by the Mann-Whitney U Test(5) and yielded a P-value of less than 0.01. This is a non-parametric test appropriate for distributions which are slightly skewed.

*Discussion.* Our studies were initiated to determine whether WN, injected directly into brains of 12-day-old rats, would affect their ability to learn a multiple T-maze. The results obtained indicate that the virus did influence learning ability. The shapes of the learning curves of virus-treated and control rats are quite similar but show that the control group learned more rapidly than the test animals. Following a period of rest between trials the WN group appeared to have proportionately no more memory loss than the control group. From these studies the cause of this reduction in ability to learn is not apparent. However, in studies not reported here, it has been observed that WN virus increases at least 10,000 fold in the whole brains of 12-day-old rats between 7 and 96 hours following intracerebral injection of the virus. If it could be demonstrated that certain nerve cells were preferentially damaged during this multiplication an explanation for the learning deficit might be evident. The rats used in the maze experiments seemed to possess good sensory functions, although it is possible that there might have been a subtle generalized sensory dysfunction. If the defect is on a sensory basis, it would seem that more than one sensory function would have to be involved since Honzik(6) has shown that the deficit following obliteration of a single sensory function is minimal. Since a rather difficult learning procedure was used, it is perhaps quite probable that the cause of impairment is referable to higher centers rather than sensory, unless it can be demonstrated that neuronal damage is so extensive that it involves combinations of tactual, visual, kinesthetic or olfactory pathways.

*Summary.* Our data indicate that West Nile virus, injected intracerebrally into 12-day-old rats, affects their ability to learn a moderately complex maze. After 13 learning trials approximately 90% of control animals had learned the maze, while no more than 60% of virus-infected animals had errorless runs after 15 trials. The memory loss of virus-treated animals following a period of 20 days between runs was proportionately no greater than that for controls.

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