Disturbance of Retention of Memory After Focal Cerebral Ischemia in Rats

Makoto Hirakawa, MD; Akira Tamura, MD, DMSc; Hiroshi Nagashima, MD; Hitoshi Nakayama, MD, DMSc; Keiji Sano, MD, DMSc

Background and Purpose The purpose of this study was to investigate the behavioral changes, in particular retention of memory, after focal cerebral ischemia in rats.

Methods Ischemia was produced by permanent occlusion of the left middle cerebral artery (MCA). For quantitative behavioral analysis, one-trial passive avoidance response and active avoidance response with the discrete lever-press avoidance procedure were observed. One group of animals was trained once to learn the passive avoidance task 1 day before surgery. The response latency was examined 4 and 14 days after surgery. The second group was trained to learn the active avoidance task for 2 weeks before surgery. The avoidance rate was examined 3 and 14 days after surgery.

Results The MCA-occluded group showed significant failure of memory retention in both of these tasks (P<.01). The nonoperated group and sham-operated group showed no definite memory failure.

Conclusions Retention of memory in the passive avoidance response and the active avoidance response was disturbed after left MCA occlusion in the rat. These results strongly suggest that this model can be used to assess memory disturbance after focal cerebral ischemia. (Stroke. 1994;25:2471-2475.)

Key Words • behavior, animal • cerebral ischemia • memory • rats

Materials and Methods This study was approved by the Animal Research Committee of our university.

Surgical Procedure The left MCAs of rats were occluded at their proximal portion. The method of left MCA occlusion, which was originally developed by Tamura and coworkers, was slightly modified to conduct long-term experiments. In brief, each animal was placed in the lateral position, and a linear skin and muscle incision was made between the left eyeball and the left external ear. After the temporalis muscle was retracted on either side of the midline of the muscle without removal of the muscle, zygomatic arch, and eyeball, a small burr hole was opened in a basal surface of the temporal bone between the orbital fissure and the foramen ovale (Fig 1). The left MCA was permanently occluded with a bipolar electrocoagulator and severed with microscissors. The animals whose MCAs were only exposed without occlusion served as the sham-operated group. Animals that underwent anesthesia but not surgery were used as the nonoperated control group. The anesthesia was induced with 4% halothane and maintained with 2% halothane in a mixture of 70% nitrogen and 30% oxygen. The three groups were subjected to the same duration and degree of anesthesia. The mean operative time was 10 minutes, and the duration of anesthesia was 15 minutes.

Passive Avoidance Response Sprague-Dawley rats (age, 10 to 12 weeks; body weight, 270 to 300 g) were used. The training was carried out according to the one-trial step-through procedure.4,9,10 The rat was placed in an illuminated safe compartment (40×25×25 cm) with a hole in the wall through which the rat could enter into a dark
Avoidance session, consisting of 30 minutes of training per day, the starting point of the 25-second intertrial interval. One avoid the electric shock, and the trial program was returned to the warning period of 5 seconds. Visual and auditory stimuli (lighting with a 30-W pilot lamp and a pure tone of 600 Hz at 60 dB, respectively) were used as the warning signals. After 4 and 14 days postoperatively, the test trials for evaluation of memory retention ability were carried out according to the discrete lever-press avoidance procedure (GT 7721, O'Hara & Co Ltd). Once the four paws were on the grid, a scrambled foot-shock (0.8 mA) was delivered to the grid. The rat could escape from the shock only by stepping back into the safe, illuminated compartment. On the following day, the animal was anesthetized and randomly assigned to one of the three experimental groups: 11 rats received only anesthesia, 21 rats underwent MCA occlusion surgery, and 16 rats were subjected to sham operation. The test trials for evaluation of memory retention ability were carried out according to the discrete lever-press avoidance procedure (GT 7721, O'Hara & Co Ltd). The temporal components of the avoidance schedule were an intertrial interval of 25 seconds and a warning period of 5 seconds. Visual and auditory stimuli (lighting with a 30-W pilot lamp and a pure tone of 600 Hz at 60 dB, respectively) were used as the warning signals. After this conditioned stimulus, a shock was given in the form of an electric current of 0.2 mA during the training session. If the animal completed a lever-pressing action within the warning period, the warning signals ceased, the animal was able to avoid the electric shock, and the trial program was returned to the starting point of the 25-second intertrial interval. One avoidance session, consisting of 30 minutes of training per day, was conducted every other day during the training period. The index of the discrete avoidance behavior was indicated by the avoidance rate, which was expressed as the percentage of correct avoidance responses based on the number of trials. After 11 sessions of routine training procedure, more than 70% of the animals showed an avoidance rate of higher than 60%. At that time, only the animals whose average avoidance rate of the last three sessions exceeded 60% were selected as trained rats. These rats were randomly assigned to three groups and received the following treatments 3 days after the end of training: 3 rats received only anesthesia, 15 rats underwent MCA occlusion surgery, and 8 rats were subjected to sham operation.

### Statistical Analysis

The results are expressed as mean±SD. The Kruskal-Wallis test followed by post hoc analysis was used for the statistical analysis for the passive avoidance tests. To analyze the data of the active avoidance tests, two-way ANOVA was carried out. Because of the use of multiple comparison, Bonferroni's correction principle was applied.

### Results

#### Passive Avoidance Response

Five MCA-occluded rats and one sham-operated rat died during the 14-day examination period after treatment and were eliminated from the analysis. Fig 2 shows the latency period of the step-through procedure in the passive avoidance task for each group. During the training period, the average latency period of the step-through procedure was similar among all three groups; the mean of all animals before surgery was 19.5±2.7 seconds. On the fourth day after treatment, the mean latency periods for MCA-occluded (n=16), sham-operated (n=15), and nonoperated (n=11) groups were 124.3±190.3 seconds, 436.7±216.5 seconds, and 438.4±193.5 seconds, respectively. The mean latency periods on the 14th day were 176.1±169.6 seconds, 489.1±193.2 seconds, and 521.5±175.0 seconds, respectively. The Kruskal-Wallis test followed by post hoc analysis with the Mann-Whitney U test showed no significant difference in the latency periods between the sham and normal groups. Therefore, the latency period of the MCA-occluded group was compared with that of the sham and normal groups. The analysis disclosed the significantly shorter latency period in the MCA-occluded group (P<.001 at any time point).

**Fig 1.** Drawings show surgical procedure of middle cerebral artery (MCA) occlusion in rats. A low-power view (top) shows the skin incision and muscle incision (broken line). A high-power view (bottom) shows the site of the small craniectomy performed between the orbital fissure and the foramen ovale after retraction of the temporalis muscle on either side without removal of the muscle, zygomatic arch, and eyeball.

**Fig 2.** Line graph shows latency period of step-through procedure in passive avoidance task in normal, sham-operated, and middle cerebral artery (MCA)-occluded rats. Each point represents the mean±SD. ***P<.001 compared with normal and sham groups.
Discussion

For the evaluation of behavioral changes in small animals such as rats and mice, the following methods have been used: (1) passive avoidance response, (2) active avoidance response, and (3) the maze test. In this study we used passive avoidance response and active avoidance response to evaluate the preservation of memory after MCA occlusion.

The passive avoidance procedure is a quick and simple task to administer. Therefore, this procedure is widely used to measure cognitive alterations after drug administration, lesions, and behavioral manipulations. Behavioral changes measured with this procedure after focal cerebral ischemia in the rat have already been evaluated and reported. In these studies the latency periods of passive avoidance response were evaluated in animals that were trained postoperatively. Therefore, acquisition and reacquisition of memory were significantly disturbed in the MCA-occluded animals, and retention of memory seemed to be impaired in these rats. However, two different aspects of memory, acquisition and retention, were not separately considered in these studies. It is quite natural to expect that the impairment of memory acquisition caused by MCA occlusion markedly affects the preservation of memory. In the present study we focused on the retention of memory by training the animals before surgery. The results disclosed a significant impairment of the retention of the ability to perform the passive avoidance task in the MCA-occluded animals, while animals in the sham-operated and nonoperated groups showed marked hesitation toward entering the dark compartment after only one-trial learning before surgery. However, it is not always clear what this simple procedure measures.

The active avoidance response is a kind of operant behavior that may be manifested in certain human behaviors. In the active avoidance response experiment, a preliminary study disclosed that Fischer-344 rats had a much higher avoidance rate than Sprague-Dawley rats, whose mean rate was less than 40%. Kuribara et al reported interstrain differences in the acquisition of conditioned avoidance responses in the rat, and Kuribara also showed that Fischer-strain rats can successfully perform active avoidance tasks. It was reported that MCA occlusion in the Fischer-344 rat showed the most reproducible infarct volume among various strains tested. In our preliminary study, the atrophic ratio of this strain, which was expressed as the percentage of the left hemispheric area divided by the right hemispheric area in five coronal sections, was 61.5% ± 11.3% (n = 18) at 8 weeks after MCA occlusion. Therefore, we used Fischer-344 rats in this study. The avoidance rates in the sham-operated group on day 3 and day 14 did not significantly differ from the preoperative rate. However, the avoidance rate in the MCA-occluded group remained low even 14 days after surgery. Thus, a significant impairment of the retention of the ability to perform the active avoidance task was also observed in the MCA-occluded animals.

Yamamoto et al showed that the activity of the MCA-occluded animals did not significantly differ from that of the sham-operated and nonoperated animals. Meanwhile, neurological deficits including abnormal posture and hemiparesis were observed in the MCA-occluded rats during the first 4 weeks after occlusion. In the passive avoidance response study, the rats in the MCA-occluded group entered the dark room despite their hemiparesis, although the rats in the sham-operated and nonoperated groups approached the entrance but hesitated to enter the dark compartment and retreated. Based on these observations, the latency period in the MCA occlusion group may be overestimated but cannot be underestimated in the passive avoidance procedure. On the other hand, neurological deficits such as hemiparesis may affect the result of the active avoidance task, although most of the animals were able to walk and push the lever to avoid electric stimulation in the active avoidance response experiment. Our preliminary study showed that learning behavior for the active avoidance task was significantly disturbed for the entire 8-week period after preoperative training. However, to rule out this possi-
bility, we needed further observation results, ie, until the animals became free from palsy. In the formation and preservation of memory, the role of the hippocampus and its related structures, such as the amygdala and anterior temporal lobe, has generally attracted more attention than other structures. However, in the MCA occlusion model used in this study, the cerebral cortex and striatum perfused by the MCA become infarcted, but the hippocampus is spared. Although memory is distributed throughout various areas of the nervous system such that no single memory center exists and many parts of the nervous system participate in the representation of a single event, the most likely site of storage is the set of particular cortical processing systems that is engaged during the perception, processing, and analysis of the material being learned. Experimental studies in rats have shown that, in some cases, the neocortex itself must be a memory storage site.

Recent research on the possible relation between behavioral change and the neurochemical system has demonstrated the role of acetylcholine in memory function. The septohippocampal cholinergic system is generally considered to be the main center of the memory process. On the other hand, the basal nucleus projection to the cortex has attracted particular attention in relation to Alzheimer's disease. It is reported that excitotoxic lesions of the rat basal forebrain induced deficits in learning, attention, and passive avoidance. Thus, the basal forebrain may also be an important component of the neural circuitry involved in learning and memory. In the MCA occlusion model, the cholinergic basal forebrain complex is not involved in the infarcted area, but the cholinergic fibers from the basal nucleus to the cortex are injured. It was reported that the acetylcholinesterase-positive fiber density was significantly reduced in the cortex on the occluded side that the acetylcholinesterase-positive fiber density was significantly decreased. Based on the results cited above, the memory disturbance observed in this study is ascribed to the impairment of cerebral neocortex.

In conclusion, retention of memory in the passive avoidance response and the active avoidance response was disturbed after left MCA occlusion in the rat. Based on the results of the present study and those of other studies that used the same MCA occlusion model, it is suggested that this MCA occlusion model can serve as a reproducible quantitative model of long-term memory disturbance due to cerebral infarction.

Acknowledgments
This study was supported in part by a grant-in-aid for scientific research from the Ministry of Education, Science, and Culture of Japan and by research grants from the Japan Brain Foundation, the Eisai Pharmaceutical Company, and the Ono Pharmaceutical Company. We gratefully acknowledge the excellent technical assistance of Noriko Tomukai and Tomomi Iwasawa and the excellent secretarial assistance of Kimi Tatebe. We also gratefully acknowledge Dr Kiyoshi Takagi for his contribution to the statistical analysis and for reading the manuscript.

References
26. Paxinos G, Butcher LL. Organizational principles of the brain as revealed by choline acetyltransferase and acetylcholinesterase dis-
A reduced memory in passive and active avoidance responses is demonstrated 4 and 14 days after occlusion of the left middle cerebral artery in the rat. This is an extension of an earlier study from the same group, and the results are in agreement with the well-known clinical observation that memory disturbances are common in patients after hemispheric strokes.

Studies on behavior and functional outcome are important in experimental stroke research. Knowledge about the spontaneously occurring functional improvement in the strain and the method used in a particular laboratory is of importance when evaluating to what extent the outcome can be modified by pharmacological or other interventions. The correlation between neuropathology and behavior has been good in some studies but not in others and seems to vary with time and with the tests used.1,4

It would have been interesting to know if the memory failure correlated with the tissue loss in the individual rats. Different strains of rats and different surgical methods are currently used in experimental focal brain ischemia. To compare studies on behavioral outcome from different laboratories, it is desirable that some information is given about the extent of neuronal death in the tested rats.

Barbro B. Johansson, MD, PhD, Guest Editor
Department of Neurology
Lund University Hospital
Lund, Sweden

References
Disturbance of retention of memory after focal cerebral ischemia in rats.
M Hirakawa, A Tamura, H Nagashima, H Nakayama and K Sano

Stroke. 1994;25:2471-2475
doi: 10.1161/01.STR.25.12.2471

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1994 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/25/12/2471

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org/subscriptions/